Published in final edited form as:

Colloids Surf A Physicochem Eng Asp. 2012 March 5; 397: 59–62. doi:10.1016/j.colsurfa.2012.01.029.

Aligning pitch for measurements of the shape of captive bubbles

Hamed Khoojinian, Jim P. Goodarzi, and Stephen B. Hall*

Departments of Biochemistry & Molecular Biology, Medicine, and Physiology & Pharmacology, Oregon Health & Science University, Portland, OR 97239-3098, United States

Abstract

The first step required for the determination of surface tension from the shape of a captive bubble is the correct alignment of both the solid support against which the bubble floats and the camera used to record its profile. The solid support should be perpendicular to the gravitationally vertical axis. The camera used to visualize the bubble must be aligned to its axis of symmetry. Alignment of roll for both the camera and solid support is straightforward. For well-collimated light, yaw is unimportant. We show here how to align pitch, first adjusting the camera relative to the gravitational vertical, and then adjusting an agarose dome used as a ceiling above captive bubbles within the visual frame of reference.

Keywords

Axisymmetric fluid-fluid interface; Capillarity; Drop-shape analysis; Profile analysis; Surface tension; Tensiometry

1. Introduction

Determinations of surface tension from the shape of captive bubbles [1–6] require accurate measurements of their profiles. The first step in this process is the correct alignment of both the solid structure that provides a ceiling against which the bubble floats, and of the optical system used to record the profile. The mechanical equilibrium between surface tension and the difference in pressures across the fluid–fluid interface determines the axisymmetric shape of the bubble within the gravitational frame of reference, regardless of these alignments. If the solid support is not parallel to the gravitationally horizontal plane, however, the fluid–solid interface will deviate from axisymmetry. This deviation can produce errors particularly for methods that rely on measuring the height of points on the interface. Misalignment of the camera relative to the bubble can produce skewed images that, if uncorrected, would lead to inaccurate assessment of the interfacial shape. Accurate measurements therefore require alignment of the solid support relative to the gravitational vertical, and of the camera relative to the bubble's axis of symmetry. The simplest, practical approach is to align the camera in the gravitational frame of reference, and then adjust the solid support within the visual field.

Alignments can be specified as rotational adjustments of roll, pitch, and yaw within orthogonal planes relative to the gravitational frame of reference (Fig. S1). Yaw is least

© 2012 Elsevier B.V. All rights reserved.

*Corresponding author at: Pulmonary & Critical Care Medicine, Mail Code UHN-67, Oregon Health & Science University, Portland, OR 97239-3098, United States. Tel.: +1 503 494 6667. sbh@ohsu.edu (S.B. Hall).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.colsurfa.2012.01.029.

important. Because of their symmetry, bubbles have profiles that are independent of the angle within the horizontal plane from which they are viewed. Confining chambers that are not axisymmetric may produce optical distortions that depend on yaw, but particularly with well-collimated light, that alignment generally has limited significance.

Roll of a camera in the vertical plane perpendicular to the optical axis (Fig. S1) does affect the imaged profile. Roll, however, is easily set by adjusting the camera such that a plumb line is vertical within the visual field [7]. This approach has the advantage over the use of a spirit-level to position the case of a camera that it avoids unwarranted assumptions concerning the orientation of the detector within the case. Setting roll of the solid support to be horizontal within the visual field is also straightforward, and misalignments are easily recognized. Any residual deviations between the frames of reference for the camera and the axis of symmetry can be corrected mathematically [8].

Setting of pitch in the vertical plane that includes the optical axis (Fig. S1) has received less attention. The changes produced by incorrect pitch are subtle because the imaged silhouette remains axisymmetric. Mathematical corrections for misalignment are unavailable. Here we present a simple protocol for aligning pitch of the camera relative to the gravitational vertical. We then illustrate how to align the pitch of the solid support with a ceiling commonly used for captive bubbles.

2. Materials and methods

Our studies visualize captive bubbles floating below a solid support with a charge-coupled device (CCD) camera. The chamber that contains the bubbles consisted of an optical cuvette with a 1-cm pathlength, the bottom of which is filled with agarose molded to have a conical indentation of $<10\ \mu\text{m}$ that maintains the bubble centered within the cell. In the current experiments, the cuvette was filled with buffer, sealed, inverted, and mounted on an optical rail supported by a universal ball-joint that allowed manual adjustments of its orientations. The CCD camera (TM-7EX, Pulnix, Inc., Sunnyvale, CA) with a 50 mm lens (Tamron, Commack, NY) was supported by superimposed x - y - z (OptoSigma, Santa Ana, CA) and tilt-tip (Edmund Optics, Barrington, NJ) stages to optimize adjustments of position and orientation. Reflective plates visualized by the camera were sheets of silicon nitride (NSG grade, 0.01" thickness, roughness $\sim 16\ \mu\text{inches}$, from BAE Systems, Vista, CA). Orientation of the tilt-tip stage and reflective plates was calibrated using a graduated spirit-level (McMaster-Carr, Elmhurst, IL). The light-source was a spotlight produced with a 14 watt green light-emitting diode (enLux Lighting, Tempe, AZ). Images were captured via frame grabber to computer using programs written in the graphical user-interface LabVIEW (National Instruments, Austin, TX).

3. Results

Our approach first aligned pitch of the camera relative to the gravitational vertical. We then adjusted the pitch of a solid support that is commonly used as the ceiling above captive bubbles.

3.1. Alignment of camera

To align the camera, we used a reflective plate that was set to the gravitationally horizontal plane with a spirit-level (Fig. 1). Trigonometric analysis showed that within the camera's frame of reference, the tilt (θ), location, and magnification of the camera determined the visualized length of the plate, which appeared in the image as a vertical displacement, ΔZ (Fig. 1). With images obtained at defined locations, ΔZ provided the basis for determining θ .

To measure ΔZ , two approaches visualized the two ends of the plate by different procedures. Each initially determined the apparent vertical displacement, Z_1 , of the closer end within the camera's frame of reference (Fig. 2A). Bringing that edge into focus with an open aperture to minimize the depth of field established a reproducible distance, x , between the camera and the edge (Fig. 1). Z_1 was given by the distance within the image between the vertical midpoint and the location of the edge (Fig. 2A).

The first approach (a) for determining ΔZ then moved the camera horizontally to bring the distant end of the plate into focus and establish its apparent vertical location, Z_2^a (Fig. 1). The apparent length of the plate was obtained from $\Delta Z^a = Z_1 - Z_2^a$. The actual vertical difference between the two ends, Δz , within the camera's frame of reference was related to pitch by $\Delta z = p \sin \theta$ for a plate of length p . Because the horizontal displacement, x , set by the focal plane, was identical for both edges, ΔZ^a was related to the actual Δz by the camera's magnification, s/x , where s is the distance between the lens and detector:

$$\Delta Z^a = \frac{s}{x} \cdot z_1 - \frac{s}{x} \cdot z_2 = \frac{s}{x} \cdot \Delta z = \frac{s}{x} \cdot p \cdot \sin \theta \quad (1)$$

If supplemented with the magnification of the camera and the length of the plate, Eq. (1) allowed direct calculation of θ from ΔZ^a . Leveling pitch involved adjustments of θ until the horizontally displaced measurements established that the two ends occurred at the same vertical location within the image, so that $\Delta Z^a = 0$.

The second approach (b) determined ΔZ without moving the camera horizontally by inserting a pinhole aperture immediately in front of the lens. With the increased depth of field, images showed the full length of the plate (Fig. 2B). A series of these images then provided the apparent length of the plate, ΔZ^b , at different heights, expressed by Z_1 . For a correctly pitched camera at the height of the plate, where $Z_1 = 0$, ΔZ^b would vanish. With the angle ψ defined in Fig. 1:

$$\begin{aligned} Z_1 &= \frac{s}{x} \cdot z_1, \\ Z_2^b &= s \cdot \tan \psi = s \cdot \left[\frac{z_1 - p \sin \theta}{x + p \cos \theta} \right] = Z_1 \cdot \left[\frac{x}{x + p \cos \theta} \right] - \left[\frac{s \cdot p \sin \theta}{x + p \cos \theta} \right] \\ \Delta Z^b &= Z_1 - Z_2^b = Z_1 \cdot \left[\frac{p \cos \theta}{(x + p \cos \theta)} \right] + \left[\frac{s \cdot p \sin \theta}{(x + p \cos \theta)} \right] \end{aligned} \quad (2)$$

Images obtained at different z_1 demonstrated the expected linear dependence of ΔZ^b on Z_1 (Fig. 2C). Extrapolations that passed through the origin indicated correct alignment of pitch ($\theta = 0$).

This second approach (b) was more sensitive than the first (a). The two apparent lengths of the plate could be expressed relative to each other:

$$\Delta Z^a = Z_1 - Z_2^a \quad (3)$$

$$\begin{aligned} \Delta Z^b &= Z_1 - Z_2^b = (Z_1 - Z_2^a) + (Z_2^a - Z_2^b) \\ &= \Delta Z^a + \left[\frac{s}{x} \cdot z_2 + \frac{s \cdot z_2}{x + p \cos \theta} \right] \\ &= \Delta Z^a + Z_2^a \cdot \left[\frac{p \cos \theta}{x + p \cos \theta} \right] \end{aligned} \quad (4)$$

Because all of the terms in the brackets are positive, $\Delta Z^b > \Delta Z^a$. For any particular plate, pitch, and camera, the visualized length of the plate would always be greater when measured

by viewing through the pinhole aperture than by moving the camera horizontally to focus on each end separately.

The requirement for multiple images with the camera in different positions could be avoided. Rather than moving the camera vertically, laminating plates having the same length but different widths produced a stairstep of reflective surfaces at different vertical displacements (Fig. 3A). A single pair of images (Fig. 3B and C) could determine ΔZ^b at several Z_1 (Fig. 3D) without moving the camera. This approach produced the most satisfying results.

3.2. Alignment of solid support

The solid support consisted of an agarose gel molded to form a concave ceiling with an indentation of $<10\ \mu\text{m}$ above an aqueous subphase. This dome provided a simple means for confining captive bubbles. Because the gel was partially transparent, the dome appeared within the image as a region with a gradation of grayscale (Fig. 4). The apparent thickness of the dome varied with pitch, passing through a minimum when viewed end-on. To align the solid support with the horizontal plane in the camera's frame of reference, adjustments simply pitched the dome to minimize its visualized thickness.

4. Discussion

This report describes methods for aligning the pitch of both the solid support used as a ceiling above captive bubbles and the camera used to image the bubble's profile. The importance of aligning the camera extends beyond captive bubbles to all experiments that analyze the shapes of bubbles and droplets. In contrast to roll, aligning pitch represents a subtler problem because the profiles remain axisymmetric. Mathematical formulae can correct for misalignment of roll [8], but not for pitch, which must be set correctly before obtaining images. Our approach represents an alternative to the prevalent use of a spirit-level to align the case of the camera. Because the orientation of the detector within the case is rarely specified, the procedure described here provides at least a necessary check on the alignment. The results with our particular camera (Fig. 3) demonstrate that misalignment of the case and detector represents more than a theoretical possibility.

Alignment of the solid support is important for a more restricted set of experiments. For bubbles and droplets that rest on an accessible surface, correct alignment is trivial, requiring only the same spirit-level used to orient the reflective surfaces in our experiments. The procedure remains important for supports with an inaccessible surface, such as the ceiling above captive bubbles.

The importance of correct pitch for the solid support depends on the methods used to analyze the shape of the bubble. Incorrect pitch obscures the location of the solid–fluid interface, but the fluid–fluid interface remains unaffected. Methods that find the Laplacian shape that best fits the bubble's profile can avoid the surface at the solid support because the rest of the interface contains sufficient information to determine surface tension. The faster methods, however, that analyze only a few points on the interface, such as calculations that use the height and maximum diameter [9], generally reference points on the fluid–fluid interface to their vertical displacement from the solid–fluid interface. Changing the pitch of the support by 0.4° with our system can shift the apparent location of the fluid–solid interface by 0.08 mm, causing a change in surface tension at 60 mN/m of 8 mN/m. Subtle misalignments of pitch can produce significant errors.

The pitch of both the camera and the solid support become particularly important at lower surface tensions. Pitch of the camera has no effect on the profile of a sphere, and relatively

little effect on the more spherical shapes at high surface tensions. For the flattened bubbles at low surface tensions, incorrect pitch distorts the profile. The low surface tensions also emphasize methods that require accurate pitch of the solid support. Calculations based on the height and diameter rather than the entire profile will always be faster, but at low surface tensions, the analysis that uses only the two dimensions is also more accurate [10]. Alignment of pitch is therefore particularly important at low surface tensions, both to avoid distorted profiles with the more flattened bubbles, and to accurately locate the solid–fluid interface as the basis for determining the bubble’s height.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

These studies were supported by the National Institutes of Health (HL54209 and HL60914).

References

1. Bashforth, F.; Adams, JC. An Attempt to Test the Theories of Capillary Action. University Press; Cambridge, England: 1883.
2. Padday JF. The profiles of axially symmetric menisci. *Philos T Roy Soc A*. 1971; 269:265–293.
3. Hartland, S.; Hartley, RW. Axisymmetric Fluid–Liquid Interfaces: Tables Giving the Shape of Sessile and Pendant Drops and External Menisci, with Examples of their Use. Elsevier Scientific Pub Co; Amsterdam; New York: 1976.
4. Adamson, AW.; Gast, AP. Physical Chemistry of Surfaces. Wiley; New York: 1997. p. 26-32.
5. Franses EI, Basaran OA, Chang C-H. Techniques to measure dynamic surface tension. *Curr Opin Colloid Interface Sci*. 1996; 1:296–303.
6. Zuo YY, Ding M, Li D, Neumann AW. Further development of Axisymmetric Drop Shape Analysis-Captive Bubble for pulmonary surfactant related studies. *Biochim Biophys Acta*. 2004; 1675:12–20. [PubMed: 15535963]
7. Thiessen DB, Chione DJ, McCreary CB, Krantz WB. Robust digital image analysis of pendant drop shapes. *J Colloid Interf Sci*. 1996; 177:658–665.
8. Song B, Springer J. Determination of interfacial tension from the profile of a pendant drop using computer-aided image processing. 1. Theoretical *J Colloid Interf Sci*. 1996; 184:64–76.
9. Malcolm JD, Elliott CD. Interfacial tension from height and diameter of a single profile drop or captive bubble. *Can J Chem Eng*. 1980; 58:151–153.
10. Schramm LL, Fisher DB, Schürch S, Cameron A. A captive drop instrument for surface or interfacial tension measurements at elevated temperatures and pressures. *Colloid Surface A Physicochem Eng Asp*. 1995; 94:145–159.

Fig. 1.

Dependence of the visualized height, ΔZ , of a leveled plate on the position and pitch of the camera. The schematic, drawn in the camera's frame of reference, shows the plate, with length p , leveled relative to the gravitationally horizontal plane, and the camera, pitched at an angle θ from the gravitational vertical. The actual vertical displacements, in the camera's frame of reference, of the front and back ends of the plate are z_1 and z_2 . The schematic shows the camera in two locations (gray and black), focused on the back (position a) and front (position b) edges of the plate. In each case, a distance s separates the detector (D) from the lens (L), which is located at a distance x from the edge. With the aperture fully open to minimize depth of field, images provide the apparent vertical displacement of the front (Z_1) and back (Z_2^a) edges from the center of the visual field. The vertical displacement of the distal end can also be determined from position b (Z_2^b) by inserting a pinhole aperture in front of the camera to increase the depth of field. The differences between the apparent vertical displacements for the two edges can then be obtained from images either at the two horizontally displaced locations ($\Delta Z^a = Z_1 - Z_2^a$) or at the single location with the two apertures ($\Delta Z^b = Z_1 - Z_2^b$). The angle ψ is defined in the figure.

Fig. 2.

Images of a leveled plate used to establish the pitch of the camera relative to the gravitational vertical. The camera and plate are arranged in configuration (b) shown in Fig. 1. Regions I, II, and III show the background light, the reflective surface (not evident in panel A), and the support below the front edge of the plate, respectively. (A) Image of the front edge of the plate, used to establish the imaged vertical displacement (Z_1) of the camera above the front end of the plate. The camera's aperture is opened fully to minimize the depth of field. (B) Image with a pinhole aperture placed immediately in front of the camera to maximize the depth of field. The light-source has a diffuser with circular markings to facilitate location of the plate's reflective surface. (C) Variation of ΔZ^b with Z_1 , obtained by moving the camera to different vertical locations. Relative pitch of the camera, determined with a graduated spirit-level, is expressed independent of any frame of reference.

Fig. 3.

Variation of ΔZ^b at different Z_1 , determined from images of a stepped plate. (A) The stepped configuration of reflective plates used to generate different values of Z_1 . (B) Image to measure values of Z_1 for the different levels, obtained with the camera's iris fully open to minimize depth of field. (C) Image to determine ΔZ^b , obtained after inserting a pinhole aperture to maximize the depth of field. (D) Variation of ΔZ^b with Z_1 , using values obtained from the images in panels B and C. Measurements were repeated with pitch leveled either according to a spirit-level placed on the case of the camera, or following adjustments so that linear extrapolation of the data passed through the origin.

Fig. 4.

The solid–liquid interface for an agarose solid support pitched at different angles above an aqueous subphase. The surface was formed by filling the bottom of a 1-cm pathlength optical cuvette with agarose, molding the agarose to have a slightly concave shape, filling the cuvette with buffer, and then sealing and inverting the chamber. After setting the pitch of the camera relative to the gravitational vertical, the cuvette containing the solid support was pitched through a range of $\sim 4^\circ$. Images show the liquid–solid interface with the top of the cuvette pitched to $\sim 2^\circ$ away from (panel A) and toward (C) the camera, respectively, and at the angle that minimized the visual thickness of the dome (B). Vertical arrows in each panel indicate the visualized thickness of the interface.