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Optical Alignment Procedure for the Analytical Ultracentrifuge

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INTRODUCTION

Many partial alignment procedures have appeared for the schlieren/interference optical system of the analytical ultracentrifuge (1–3). Each procedure for orienting isolated optical elements (or groups of elements) is directed toward specific applications and particular types of work. This paper presents a complete alignment procedure which utilizes new adjustable optical elements. It discusses the considerations underlying each individual alignment method and also presents a "troubleshooting" chart for evaluating major anomalies appearing in schlieren and interference patterns.

A great variety of alignment procedures could be developed, each offering advantages peculiar to itself. This specific procedure was selected because it provides three important advantages. First, in as many cases as possible, it relies on objective criteria to evaluate the correct position of a given optical element and thus keeps observer error to a minimum. Second, it provides optimum optical conditions for sedimentation equilibrium techniques, now more and more widely in use, yet without sacrificing the degree of optical accuracy necessary in sedimentation velocity work. And third, it makes use of the additional flexibility provided by adjustable optical elements, thus ensuring greater sensitivity. It should be noted that some individual procedures given below are adaptations of well-known optical methods, while others have been developed specifically for the analytical ultracentrifuge.

EXPERIMENTAL

The alignment procedure to be presented is applicable to an analytical ultracentrifuge[1] equipped with the combination schlieren/interference optical system. It is assumed that the machine is also equipped with the adjustable optical elements described below. These adjustable elements (push-pull slits, light-source mount, mask holder for condensing lens,

mirror mount, camera lens mount, and cylindrical lens mount)[1] have proved to be of great value in optical alignment, for together they provide a wider range of directional adjustments and permit the reproducible orientation of certain optical elements. Thus, these elements contribute to an over-all increase in the flexibility of the system as well as in the sensitivity and accuracy with which the system can be aligned. Of course, existing elements can be modified to provide the necessary range of adjustment. In such cases, the same procedure can be followed; only the mechanics of adjusting need be changed.

Push-Pull Slit Assembly

The push-pull jaws (see Fig. 1) replace the older light-source jaws and eliminate the need to rotate the source when changing optics, for this

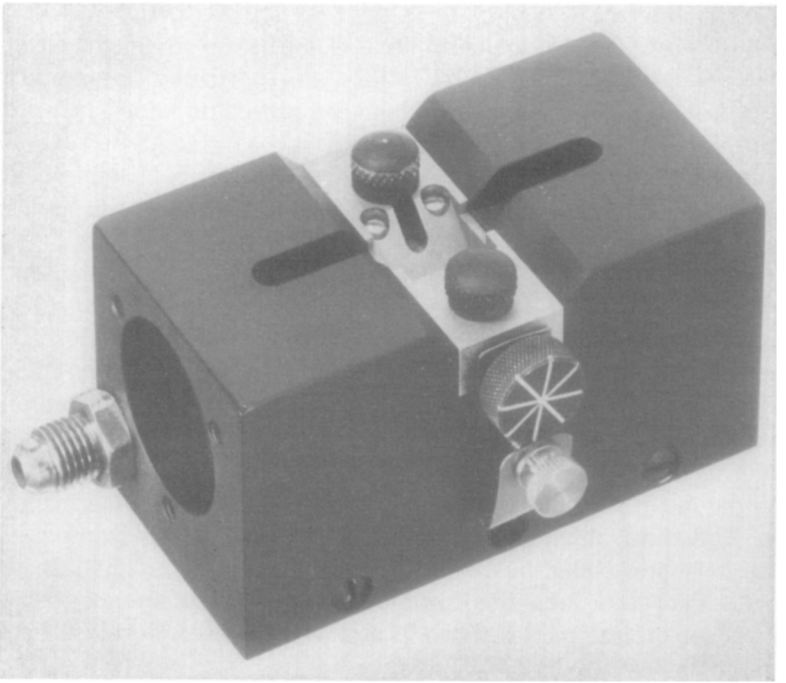

FIG. 1. Push-pull slit assembly.

new assembly incorporates an interference slit located perpendicularly to the schlieren slit formed by the jaws. Furthermore, since the interfer-

[1] Manufactured by the Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

ence jaws are perpendicular to the arc of the AH-6 lamp, they provide a short source that closely approaches a point source.

To Change Optics: To change from the schlieren to interference position, loosen the two black knurled screws above the jaws; slide both jaws as far to the front of the housing as possible; and tighten the rear knurled screw. Then to change from interference to schlieren, merely loosen the rear knurled screw and let the jaws slide toward the back of the housing until the back of the rear jaw is flush with the back of the housing; then tighten both knurled screws and adjust the slit width control as usual.

Note that the interference jaws are originally set with a 0.001-in. slit. If more light is required for a run, rotate the source to the interference position rather than merely changing the spacing between the jaws.

Adjustable Light-Source Mount

The adjustable light-source mount (see Fig. 2) provides a means of adjusting the light source front-to-rear of the machine for different rotor

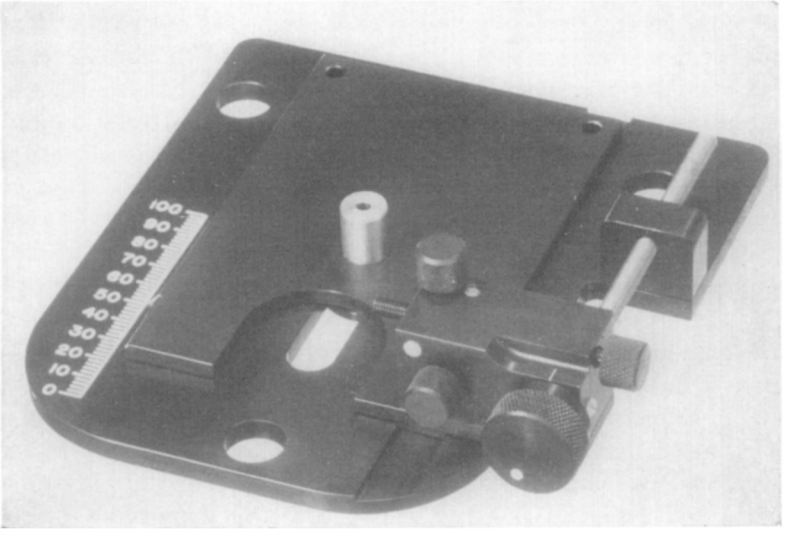

FIG. 2. Adjustable light-source mount.

speeds (4). The adjustable mount consists of a base plate installed in the conventional manner above the three light-source legs, a sliding plate which provides front-to-rear freedom for the source, a scale that indicates front-rear position of the sliding plate, a pivot pin that receives the light-source housing, and adjustment knobs that control the position of the housing.

The sliding plate is positioned on the base plate and is moved front-to-rear by the front-rear adjustment knob. When the knob is turned clockwise, the plate moves to the rear of the assembly; and when the knob is turned counterclockwise, the plate moves to the front of the assembly. As the sliding plate travels back and forth on the base plate, a pin on the side of the plate moves along the scale etched onto the base plate. This scale thus permits reproducible front-rear orientation of the sliding plate and thus of the light-source housing which is mounted on the plate.

A pivot pin in the center of the adjustable mount receives the lamp housing. As discussed previously, the housing is moved front-to-rear by the front-rear knob. Schlieren orientation of the housing is controlled by the schlieren positioning knob at the left of the front-rear knob; interference orientation is controlled by the interference positioning knob at the right of the light-source mount. When the housing has been properly positioned, it is then locked in place by the locking knob.

Adjustable Mask Holder

The adjustable mask holder permits accurate alignment of the light-limiting masks for the condensing lens. Four masks are presently available: a schlieren mask with a single aperture, an interference offset mask, and two interference symmetrical masks. In the interference offset mask, one aperture is located on a radius from the center of rotation and the second aperture is displaced laterally. When this mask is aligned properly, the centered slit will lie along a radius of the cell. This arrangement ensures that deviations arising in the solution side of a double sector cell will not be cut off by the slits in the mask. This mask is generally used for standard interference runs as well as for schlieren/interference runs. In the interference symmetrical masks, both apertures are located symmetrically about a radius from the center of rotation. These masks, which differ only in slit width, were originally used in differential sedimentation (2) when comparing conjugate levels in a double sector cell. [The symmetrical mask with the extremely fine slits can be used for special applications involving the white light fringe (5).] The symmetrical masks should also be used in cases in which there are appreciable refractive index gradients in the solvent, as with organic solvents, for example. The use of a symmetrical mask ensures that comparisons are made between levels in the cell at which the refractive index difference is due only to contributions of the solute.

The mask holder (see Fig. 3) consists of two aluminum rings, with the upper ring permanently fastened to the bottom of the upper chamber plate. Two locating pins extend from the upper ring and position the lower ring above the rotor support fork. The bottom section of the lower

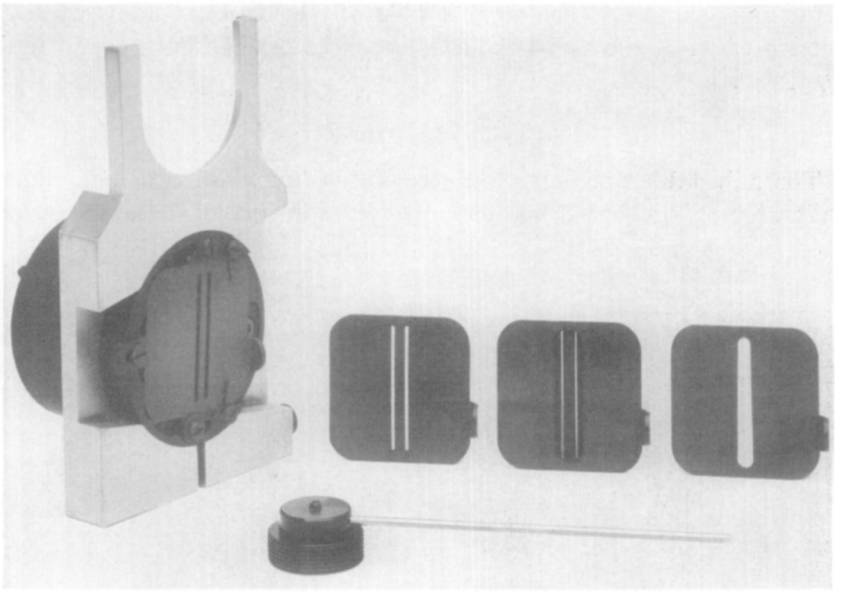

FIG. 3. Adjustable mask holder.

ring, which protrudes slightly from the support fork, has a rectangular indent that receives the mask and two spring clips that hold the mask in place. After the mask is installed, it is aligned by rotating the bottom section of the lower ring, with fine rotational adjustments provided by the fine adjustment screw. Once the mask is in the aligned position, it is locked in place by tightening two screws (with washers) in the rotatable section.

Note that, if the fine adjustment screw is screwed completely in, the mask cannot be rotated. And as the adjustment screw is screwed in, the bottom section will rotate by itself; but, when the screw is screwed out, the bottom section will not move. It must be rotated manually in either direction. However, it is recommended that the rotatable section be guided manually even when the fine adjustment screw is to be screwed in.

Also incorporated in the rotatable section is an adjustable stop which controls the right-left orientation of the mask. This stop is to be used when the drive has been replaced and the mask holder is no longer oriented to the proper axis. In such a case, the adjustable stop can be used to reorient the mask so that the apertures will remain on a radius of the rotor.

When Aligning a Mask: When aligning the mask, first loosen the two screws (with washers) in the lower ring so the mask can be rotated. Use the aligning tool (provided with the mask holder) to align the mask on

a radius of the rotor. The mask is aligned relative to a radius when the outside edges of the tool handle are parallel to the inside edges of the apertures.

Adjustable Mirror Mount

The adjustable mirror mount (see Fig. 4) provides two independent, calibrated adjustments: one for horizontal adjustment of the mirror and

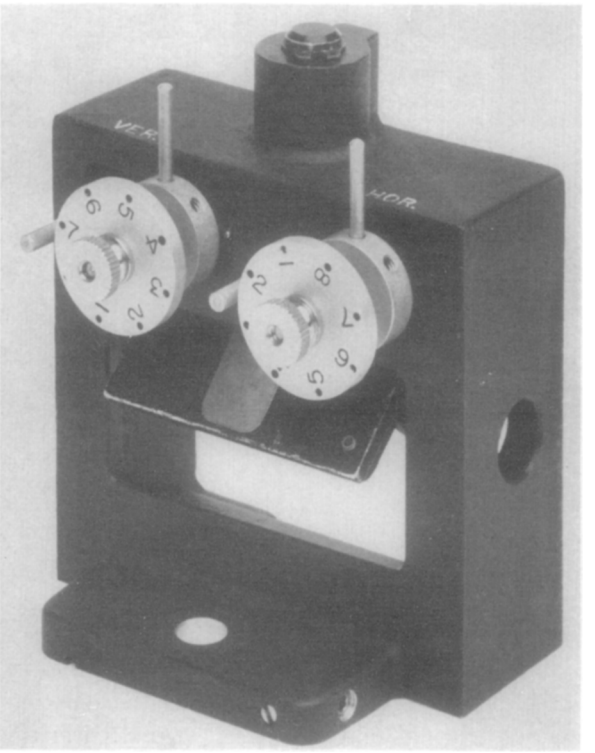

FIG. 4. Adjustable mirror mount.

one for vertical adjustment. The mirror is cemented to a mounting plate which, in turn, is held against a fixture. The mounting plate includes a hole in each of its four corners. Two of these holes accept aligning pins from the fixture; the remaining holes accept screws that hold the plate against the fixture. By means of rods along the center of its horizontal axis, the mount fixture is held in a yoke. Because of the manner in which the rods are positioned, the fixture is able to rotate about its horizontal axis; in addition, the yoke is able to turn about its central vertical axis. Thus, the mirror is rotated horizontally by means of the fixture and

vertically by means of the yoke. A positioning screw, located at the front of the mount to the left of the mirror, adjusts the position of the mount on the optical track. This adjustment permits the images on the optical track to be raised or lowered without changing the angle of the reflected light as it leaves the mirror.

Rotation of the mirror is controlled by two adjustment knobs, labelled "Hor." and "Ver.," behind the mount. Each knob is incremented from 1 to 8; a complete turn of the knob will move the image at the plate approximately 1 in. vertically or horizontally. Behind each adjustment knob is a locking bar which locks the knob in position after the adjustment has been made. When the locking bar points up, the knob is locked. To unlock the knob, push the bar 90° to the left so that it is horizontal and rests on the pins protruding from the back of the mount.

Adjustable Camera Lens Mount

The adjustable camera lens mount (see Fig. 5) permits four-way orientation of the camera lens. This mount can be rotated about its vertical and horizontal axes and can be adjusted in both the vertical and

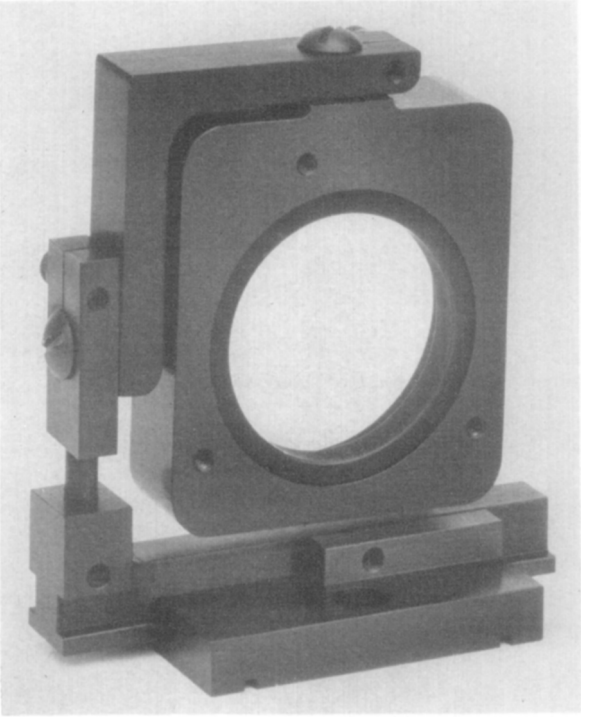

FIG. 5. Adjustable camera lens mount.

horizontal planes. These adjustments are controlled by four setscrews as shown in Fig. 5. When the camera lens is aligned, the vertical axis adjustment is made in conjunction with the horizontal plane adjustment and the horizontal axis adjustment in conjunction with the vertical plane.

Adjustable Cylindrical Lens Mount

The adjustable cylindrical lens mount (see Fig. 6) provides for both the rotational and the vertical adjustment of the cylindrical lens. A scale

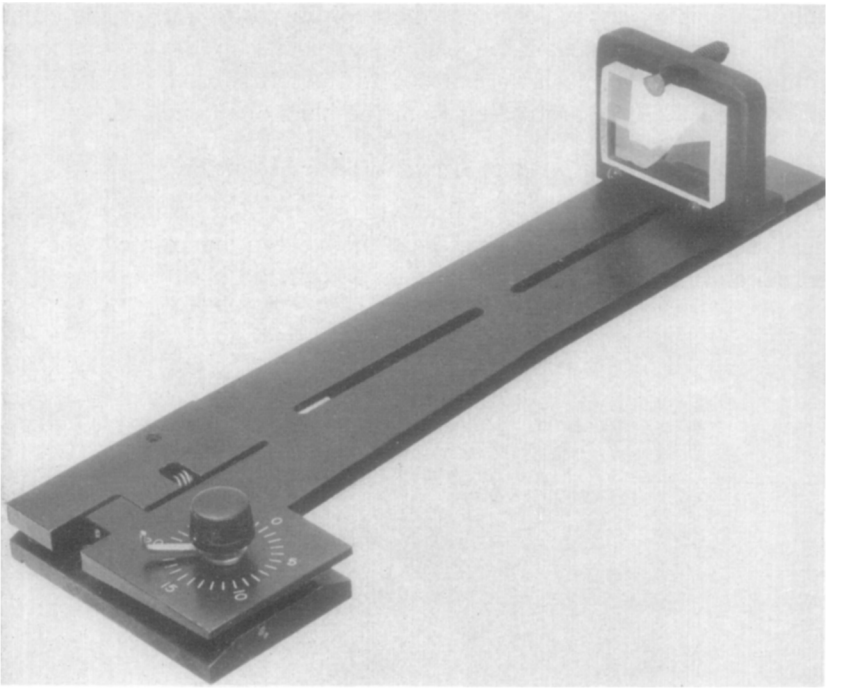

FIG. 6. Adjustable cylindrical lens mount.

on the mount permits reproducible rotational adjustments that are independent of any vertical adjustment. Rotational position of the lens holder is controlled by the knob on the scale; once the final adjustment is made, the rotational adjustment knob is locked in position by turning the locking bar clockwise. The knob can be unlocked by rotating the bar counterclockwise. Vertical adjustment is made using the Allen setscrew at the left of the rotational adjustment knob.

DISCUSSION

The following discussion describes each individual method to be performed in the alignment procedure. It presents the practical considera-

tions underlying each method as well as the theoretical basis for the method. Further theoretical considerations can be found under *Additional Comments.*

1. Adjust the height of the light source.

Comments: The procedure to be presented will position the slit of the light-source at the focal plane of the collimating lens. This adjustment keeps possible light deviations to a minimum, because it ensures that light from any point between the jaws will enter the chamber as a beam of parallel rays. An approximation of the focal plane is satisfactory, since a source positioned exactly at the focal plane could still not remedy the more significant deviations caused by the finite width of the slit. For example, if a source is positioned 1 mm from the focal plane of the lens, deviations from the norm (vertical center of a 12-mm cell) will be only 2×10^{-6} cm, while deviations resulting from the finite width of the slit will be 5×10^{-5} cm. It can thus be seen that, if the procedure to be presented for adjusting light-source height is performed correctly, deviations resulting from both the finite width of the slit and from an inexact position of the source at the focal plane will be beyond the measurable sensitivity of the system.

Principle of the method: Rays of light leaving a slit source in the focal plane of a collimating lens will, by definition, form a parallel beam after refraction through the lens. If this beam now falls upon a plane mirror, it will be reflected as a parallel beam of light; and, if this reflected beam then passes back through the lens, an image of the slit source will fall on the plane containing the object slit source (6). The image and object will lie in the same plane if, but only if, the object is in the focal plane of the lens. Thus, when the light source is properly positioned at the focal plane of the collimating lens, the observer will note that the source and the image of the source lie in the same vertical plane, whereas, if the vertical plane of the image does not correspond to the vertical plane of the object, then the source is readjusted until this correspondence is achieved.

2. Position the light source on the axis of the cell.

Comments: The procedure to be presented will position the light-source slit at such a point that light will enter the cell parallel to a meniscus or boundary. The necessity of having this condition met (when determining meniscus position in Archibald work, for example) has been demonstrated (1). This method will position the source in such a manner that the deviation through a 12-mm cell will be ± 0.004 mm for a source that is ± 0.2 mm from its correct placement.

Principle of the method: To ensure that light rays enter the cell

parallel to a meniscus or boundary, it is necessary that light enter the cell parallel to the axis of rotation. To achieve this condition, the method utilizes a cell with an aluminized quartz window; it is assumed that the surface of this window is perpendicular to the rotor's axis of rotation. Here the source is moved front-to-rear until the light becomes perpendicularly incident to the aluminized window. And when this light is reflected back, it will fall between the jaws of the slit source (since the angle of incidence equals the angle of reflection).

3. Adjust the front-surfaced mirror and position the camera and cylindrical lenses on the optic axis.

Comments: The procedure to be presented will help ensure the optimum experimental conditions required for sedimentation equilibrium work. Because the optical system of the analytical ultracentrifuge was originally designed for sedimentation velocity studies (where relatively large deviations through the cell are usual), certain optical elements were positioned off-axis in order to keep deviated light closer to the axis of the camera lens. In this way, light deviations through most boundaries were kept within the confines of the optical system. In equilibrium work, however, large deviations through the cell do not normally occur and it is advisable that the lenses share relatively the same axis so that the transmitted light will remain on-axis. This procedure minimizes the possibility of lens abberrations affecting the quality of the image at the photographic plate. It should be further noted that this procedure does not significantly affect the accuracy of sedimentation velocity results, for, in moving boundary studies, experimental inaccuracy when determining required parameters becomes the major limiting factor rather than minor optical orientation problems. In addition, any light deviations resulting from a steep gradient can be kept within the system by the use of negative wedge windows in the cell assembly.

Principle of the method: Both the camera and the cylindrical lenses are positioned so that their optic axes are coincident to that axis defined by the midpoints of the cell assembly and the photographic plate. To achieve this coincident relationship, the procedure relies in part on reflections from the glass to air interfaces of the lenses to give an indication of proper lens position. That is, when this reflected light falls on the same axis as the transmitted light, the optic axes of the lenses are coincident to the axis along which the incident light travels.

4. Install the ruled glass disk in the rotor.

Comments: As will be discussed later, the camera lens is focused on the midplane of the cell. The thickness of the disk and its position in the

holder are so chosen as to bring the lines on the ruled surface into focus on a plane corresponding to the midplane of a 12-mm cell filled with water and containing a quartz upper window. When the thickness of the centerpiece, the upper window, or the refractive index of the fluid is significantly altered, then the camera lens should be refocused. [Another method (1), useful when focusing the camera lens on a level other than the midplane of the 12-mm cell described above, is to focus the camera lens on a hair or other mark on the upper window and then the lower window. The lens is finally positioned between these two focus positions.] In general, for every 1-mm change in optical distance at the cell, the camera lens is moved 2 mm along the optical track. Since the accuracy of the camera lens method to be presented is ± 0.3 mm, no change in camera lens position should be necessary when the optical path added to the middle of the cell is ± 0.15 mm or less.

5. Focus the camera lens on the midplane of the cell.

Comments: Because the cell is a three-dimensional object (having appreciable thickness along the optical path) which will be focused on a two-dimensional photographic plate, some question arises concerning the plane to select for camera lens focus. Whatever level is selected, it should ensure that the cell is accurately imaged on the plate; that is, the plane selected should preserve as much as possible the point-to-point correspondence between levels in the cell and levels on the plate, but without sacrificing its ability to sense significant correspondence changes that will occur in time. The procedure to be presented will focus the camera lens on the midplane of the cell. As will be seen below, this plane is selected as a compromise, for, although it will ensure that true meniscus position can be determined during a run, it may also mean that certain rays appearing on the plate will not represent their true level in the cell.

Consider the most deflected ray passing through a symmetrical boundary; this ray should form the midpoint of the schlieren peak and should correspond to the level of the maximum gradient in the cell. In Fig. 7, for example, ray A enters the solution at the level of maximum dn/dx. If the camera lens is focused on the midplane of the cell ($x = a/2$), ray A will represent on the plate the level of maximum dn/dx in the cell. However, this ray cannot actually be considered the most deviated ray, since it passed through the maximum gradient region only during the first instant of its entry. After entry, it passed through levels of decreasing dn/dx. On the other hand, ray O, which entered slightly above the maximum gradient, can be considered the most deviated ray because the average of the gradient levels it traversed is higher than the average traversed by ray A. Thus ray O will actually form the top of the peak.

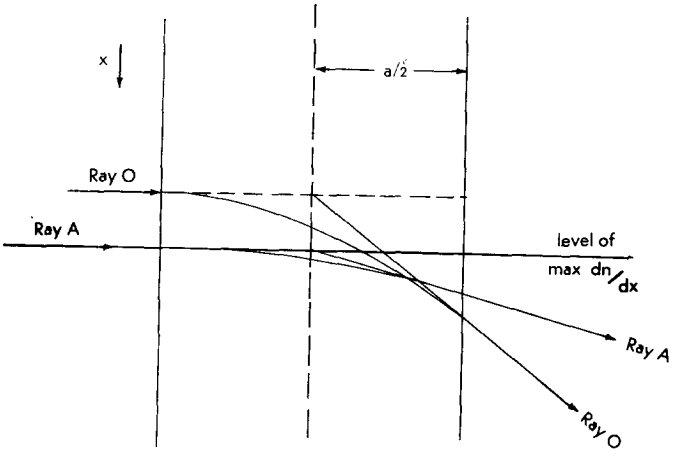

FIG. 7. Effect of camera lens focus on schlieren peak.

Now if the camera lens is focused on the midplane of the cell, ray A will form the midpoint of the peak, but ray O will form the top of the peak. Because the same ray (ray A) is not forming both the midpoint and the top of the peak, the top of the peak will be displaced. And, in this region, a point-to-point correspondence between levels on the plate and levels of the cell will not exist. This displacement phenomenon is called Weiner skewing (7).

To insure that rays A and O represent their true level in the cell and to keep Weiner skewing to a minimum, the camera lens must be focused on a cell level other than the midplane. Svensson (8) has shown that when the 2/3 level of the cell is selected for camera lens focus, Weiner skewing is minimized. He points out that, if the camera lens is focused on the midplane of the cell, the shift of the peak Δx is $a^2N'/6M$, where N' is the gradient, a is cell path length, and M is the refractive index of the solution at that level (8). Since a and M are constants for a particular run, the magnitude of Δx is dependent upon N'. Therefore, when the initial boundary is sharp, the displacement introduced by focusing on the midplane of the cell may be appreciable. However, as the boundary diffuses and the slope dn/dx decreases, Δx becomes negligible. In a sedimentation velocity run, the percentage error introduced by Δx, as compared with the total movement of the boundary through the cell, is small and may be neglected. Thus, a focus on the midplane is satisfactory. An additional reason for focusing on the midplane of the cell is illustrated in Fig. 8. The ray shown in Fig. 8 entered the solution just below the intersection of the meniscus and cell window. For this ray to represent

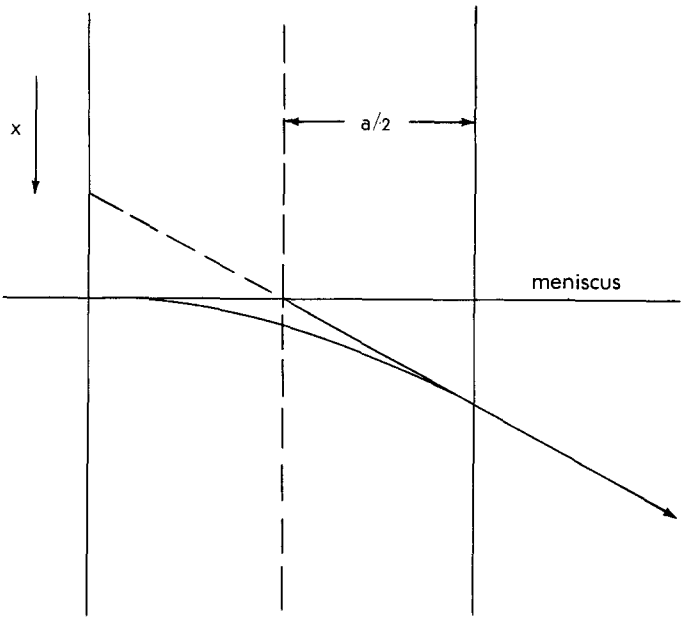

FIG. 8. Effect of camera lens focus on meniscus position.

the true meniscus position, the camera lens would have to be focused on the midplane of the cell ($x = a/2$). This consideration is critical in Archibald and equilibrium work, where it is necessary to determine the accurate position of the meniscus.

Yet, in certain instances, focusing on the midplane of the cell may produce a significant percentage error. In a synthetic boundary run with a low molecular weight material, for example, the error introduced by the apparent movement of the boundary due to the change in Δx may be quite large as compared to the small total true sedimentation of the material. And in this case, the 2/3 level of the cell should be chosen. In addition, for certain density gradient runs, it may be advantageous to focus on the 2/3 level or even on the 5/6 level (9).

Principle of the method: The procedure to be presented makes use of a phenomenon which occurs where the image of the phase plate intersects the image of the ruled glass disk. This phenomenon is similar to one which would occur if the middle of the camera lens were masked off. If this portion of the lens were masked off and the ruled glass disk placed in the rotor, a double image of each line would be observed on the plate for all camera lens positions other than the correct focus position. The separation of the lines of the double image would be dependent not only upon the degree to which the camera lens is out of focus but

also upon the width of the mask. For any given camera lens position, the wider the mask, the greater is the separation of the images; and conversely, the narrower the mask, the less is the separation of the images.

In the procedure to be presented, the schlieren analyzer is oriented at the 90.5° position. This positioning creates, in effect, a mask of continuous width placed over the middle of the camera lens; it will affect the image of the ruled glass disk only in that region containing the image of the analyzer element. In this region, the lines from the disk will be bent, rather than doubled, when the camera lens is out of focus. The degree of bending and direction of curvature are dependent upon the position of the camera lens with respect to the correct focus position. When the camera lens is at its correct position, no curvature of the lines will be noticeable in that region where the image of the lines intersects the image of the phase plate.

6. Position the schlieren analyzer at the focal plane of the condensing lens.

Comments: The procedure to be presented will place the schlieren analyzer at the focal plane of the condensing lens. This placement helps to ensure that the solvent and plateau regions in the cell appear flat when schlieren optics are used. It should be noted that this orientation of the analyzer can be accomplished far more critically than is required for schlieren work.

Principle of the method: This procedure is a modified version of the Foucault knife-edge test, utilizing the metallized line of the phase plate rather than the edge of a knife (10).

7. Focus the cylindrical lens on the plane of the schlieren analyzer.

Comments: The procedure to be presented will focus the cylindrical lens on the plane of the schlieren analyzer. For schlieren work, the cylindrical lens should be focused on the analyzer plane, while, for studies employing interference optics, the lens should be focused on the focal plane of the condensing lens. But since the schlieren analyzer is placed at the focal plane of the condensing lens, these two planes will be identical in a properly focused system. This condition may only persist, however, when the rotor is stationary for, during centrifugation, distortions are created that may place the source image past the plane of the schlieren analyzer. Experience indicates that the cylindrical lens can be defocused to quite a degree without affecting the sharpness of the interference fringes. But this same defocusing can significantly affect the sharpness of the metallized line. For these reasons, the cylindrical lens should be focused on the plane of the schlieren analyzer. Note also that

changes in camera lens position require refocusing of the cylindrical lens, but that changes in cylindrical lens position do not require refocusing of the camera lens.

Principle of the method: In this procedure the schlieren analyzer will be illuminated with diffuse light. The ground glass placed in front of the front-surfaced mirror will increase the angulation of the light as it leaves the image, thus decreasing the depth of focus and making it easier to determine the plane of intersection of the marginal rays. When the cylindrical lens is properly focused, the image of the phase plate will be sharp; the image will be fuzzy on either side of the correct focus position.

8. Adjust the rotational positions of the condensing lens mask and the cylindrical lens.

Comments: The condensing lens mask determines which arc of a rotor's revolution is to be viewed by the optical system. It is necessary that this mask be coincident to or symmetrical with a radius of the rotor (2). In accomplishing this condition, some investigators prefer to use gravity as a primary reference; others prefer the cylindrical lens, the photographic plate, or the upper lens mask. Each reference offers advantages peculiar to itself, yet the accuracy of the method is independent of the reference selected. The procedure to be presented chooses the upper lens mask as a reference and in this way ensures that light is kept on the optic axis of the condensing lens (see step *3*).

[A similar procedure can be used if the upper lens mask is placed on the collimating lens. This arrangement ensures that radial deviations in the cell are not cut off by the lens mask (11).]

Principle of the method: This procedure utilizes a double-sector centerpiece that has been modified to permit free communication between the sectors. The cell is filled with solution and centrifuged; boundaries are permitted to form. The interference system is then used to compare conjugate levels of the two sectors and form fringes. If either the mask or the cylindrical lens is misoriented with respect to a radius of the rotor, the optical system will compare nonconjugate levels in the cell, thus causing fringes to curve in the region of a boundary or steep gradient. The degree of curvature is dependent upon the steepness of the gradient and the extent to which the mask and cylindrical lens are misoriented.

9. Adjust the rotational position of the light source and the lateral position of the schlieren analyzer.

Comments: Rotational adjustment of the light source is important in both schlieren and interference optics. For maximum sharpness of interference fringes, the interference light-source slit must be parallel to the

axis of the cylindrical lens. And in schlieren optics, the rotational position of the light source with respect to the cylindrical lens is critical, if phase plate angles are to be meaningful. Furthermore, the light-source slit should be tangential to the cell contents along the rotor radius defined by the condensing lens mask. (It should be remembered that, in the previous step, the cylindrical lens was aligned parallel to this rotor radius.) Any deviation from perpendicularity along this line effectively increases the slit width with respect to the contents of the cell. (With the push-pull slit assembly, alignment of the schlieren light source also aligns the interference slits.) Lateral adjustment of the schlieren analyzer simply permits the use of very small phase plate angles.

Principle of the method: This procedure employs the metallized phase plate line to align the light source with respect to the schlieren analyzer. In step *8,* the latter element, rotated to 90°, was aligned with respect to a rotor radius. The shadow cast by the metallized line when the analyzer element is rotated to 0° will now be observed as it traverses the image of the light source. When the images of the source and line are parallel, it is an indication that the two optical elements are also parallel and that the light source is thus perpendicular to the proper rotor radius.

10. Adjust the height of the cylindrical lens.

Comments: The procedure to be presented uses the symmetrical mask to position the center of curvature of the cylindrical lens on the optic axis. This adjustment places the white light fringe in the center of the diffraction envelope and facilitates the determination of C_0. In equilibrium work, it is often necessary to determine the position of C_0 in the cell before molecular weight can be calculated. When interference optics are employed, it is more convenient to evaluate the C_0 position by using the white light or achromatic fringe as a reference, with the fringe located in the center of the diffraction envelope.

Principle of the method: Optical distance is defined as Σnl, where l is the physical distance through a medium of refractive index n. When the optical distances of two interfering rays are equal, the white light fringe (or zero order) is produced. Although the position of the diffraction envelope is essentially independent of the portion of the condensing lens traversed by the two rays, without a cell the position of the white light fringe becomes dependent upon the relative thickness of glass traversed by the two interfering rays. In this procedure, the cylindrical lens will be moved along its vertical plane until the two rays traverse the same thickness of glass, which will result in the white light fringe being positioned in the center of the diffraction envelope. Two different cylindrical lens height adjustments are required to achieve this effect, depending

on whether the offset of symmetrical condensing lens interference mask is used.

ADDITIONAL COMMENTS

Light-Source Slit

Because the light-source slit is perpendicular to the meniscus, boundary, and cell bottom, a limitation is imposed on the accuracy of the system when interference optics are employed. This limitation is dependent upon the length of the slit used and becomes apparent when measurements are taken at the meniscus or cell bottom, for the longer the slit, the greater is the deviation of light through the cell, and the more closely the interference slit approaches a point source, the more closely does the system approach the ideal. When the push-pull interference slit assembly is used, the ideal slit length can be approached.

A further advantage of this slit assembly becomes apparent when cylindrical lens orientation is attempted. Due to the shortness of the slit, the slit's orientation with respect to the cylindrical lens is less critical than the orientation of a longer slit. Therefore, with this short slit the cylindrical lens may be rotated to a considerable degree before the interference fringes deteriorate to such an extent that rotational adjustment of the source becomes necessary. However, one disadvantage of this short slit is that certain phenomena which are normally averaged out when a long slit is used (i.e., oil droplets, dirt particles, and imperfections on chamber lenses) will be evident on the plate to a greater degree.

It is possible to determine theoretically an optimum slit width that will permit high fringe visibility and realistic exposure times. From Jenkins and White (12) the optimum source width for double slit interference is given by the expression $\epsilon = F\lambda/4d$, where ϵ = source width, f = focal length of the lens, and d = distance between interference slits. According to Born and Wolf (13), this expression corresponds to a fringe visibility as defined by Michelson ($V = I_{max} - I_{min}/I_{max} + I_{min}$) of 0.9. In a typical ultracentrifuge, $f = 58$ cm, $\lambda = 5461$ A, and $d = 0.4$ cm; therefore, $\epsilon = 58\ (5461 \times 10^{-8})/4(0.4) = 1.98 \times 10^{-3}$ cm. The slit width used in the push-pull assembly is 2.5×10^{-3} cm. This offers a fringe visibility of about 0.85.

Front-Surfaced Mirror

To keep the area occupied by the optical system to a minimum, the system is structured to form a right angle through the use of a front-surfaced mirror placed between the condensing lens and the schlieren analyzer. Adjustment of the mirror centers the image on the photo-

graphic plate and directs the light along the optic axis of the lens system. Vertical adjustment of the mirror merely raises or lowers the image at the plate. Horizontal adjustment, however, not only displaces the image laterally, but rotates it as well. Consequently, once the system has been aligned, any lateral movement of the mirror must be accompanied by an appropriate rotational adjustment of the cylindrical lens and also by an adjustment of the 90° position of the schlieren analyzer. Due to the relationship between image position and camera lens orientation, adjustment of the front-surfaced mirror should be rechecked after any future camera lens orientation.

Optical Lever Arm

Fringe spacing has been shown to equal $\lambda d/s$ (14). Since the fringe spacing on the photographic plate can be measured and since λ and s are known, then the optical lever arm d can be determined by the following relationship. Fringe spacing $= m\lambda d/s$, where m is the vertical magnification factor, s is the spacing between the slits, λ is the wavelength, and d is the optical lever arm. With the AH-6 lamp and the Kodak 77A Wratten filter, λ has been found to be 550 mμ (11). This shift of the mercury line from 546 mμ is due to the high operating pressure of the lamp which causes a change in the transition probabilities of the mercury atoms (15).

ALIGNMENT PROCEDURE

The instrument should be nominally aligned before the start of this procedure. Unless otherwise stated, each element should be in its approximate position and schlieren orientations and the schlieren light-limiting mask should be used. (Schlieren orientations: light source, oriented left-right; end trimmers, slightly over ends of slit assembly; light-source jaws, open ⅛ turn; phase plate, set at some angle other than 90°.) Also, unless otherwise stated, the chamber should be closed and evacuated during each step. Any extraneous elements installed for a particular step (an aligning target, for example) should be removed from the system when the step is completed.

1. Adjust the height of the light source.

Turn on the light source and tape the filter and dust cover to the bottom of the chamber hole that leads to the collimating lens. Then, using tape, mask off the center of the collimating lens; place a strip of tape, 1 cm in width, across the center of the lens with its edges parallel to the light-source slit. This mask will cause the reflected source image to be doubled everywhere but at the focal plane of the collimating lens. Now

place a quality front-surfaced mirror approximately 2 cm above the collimating lens. Displace the reflected slit image to the front of the light source by tilting the front edge of the mirror with a shim. Remove the left-hand screw from the shutter assembly and swing the assembly as far as possible to the right. Then evacuate the rotor chamber and remove the adjusting screw from the push-pull slit assembly. Holding a white card at the bottom of the jaws, in the plane of the slit, move the light source up and down by adjusting the three adjustable legs beneath the source assembly until a single sharp image is obtained. The source is now positioned in the focal plane of the collimating lens. The procedure can be made more sensitive if a portion of one of the reflected images is masked off and a magnifier of some type is used to view the image on the card.

Next, level the light source by placing a level on top of the lamp housing. Adjust the two back legs of the mount and then the front leg until the source is level.

The accuracy of this method is ± 0.5 mm.

2. Position the light source on the axis of the cell.

Assemble a cell having an aluminized quartz window[2] in the lower window holder. Place this empty cell and an appropriately weighted counterbalance in a rotor and run them at 7000 rpm (1). Check that the back of the rear push-pull jaw is flush with the back of the lamp housing; the slit assembly is now in the schlieren position. This position will serve as a reference when the jaws are either moved or removed. Now position the AH-6 lamp beneath the slit as follows: hold a piece of paper over the jaws and rotate the lamp until a single, bright band of light is visible on the paper. The arc of the lamp is now positioned between the light-source jaws.

Now to position the source so that light will enter the cell parallel to the meniscus, first move the end trimmers in an equal distance; then, as in step *1*, remove the left-hand screw from the shutter assembly and swing the assembly as far as possible to the right. With black tape (to prevent the transmitted light from interfering with the reflected image), tape off one side of a 3×5 white card along one edge. Now holding this card flat above the lamp housing, with the taped edge on the bottom and facing toward the rear of the instrument, move the entire source assembly around until the reflected source image falls on the card. Next, holding the card parallel to the slit, move the card across the jaws until its edge just begins to cut off the reflected image (i.e., it is just over the slit). Then holding both the card and housing firmly, simultaneously slide

[2] Available from Herron Optical Company, Los Angeles, California.

both toward you until the image just begins to travel off the card and the reflected image is roughly centered above the slit in the left-to-right direction. Then tighten the front leg on the light-source mount. Now rotate the light-source housing and pull back the front end trimmer. Rotate the back of the light-source plate from left-to-right so that the reflected image cuts the middle of the rear end trimmer; next, tighten the two back legs on the mount. The light source is now positioned so that light will enter the cell parallel to the meniscus.

The accuracy of this method is ± 0.2 mm.

3. Adjust the front-surfaced mirror and position the camera and cylindrical lenses on the optic axis.

Remove the camera and cylindrical lenses and lens mounts from the optical track. Then tape off a cell assembly in such a way that a square opening, about $\frac{1}{8} \times \frac{1}{8}$ inch, is centered on the cell and the rest of the cell is taped off. Install the cell and rotor in the chamber, with the opening centered rotationally below the condensing lens. Widen the light-source jaws and then move the end trimmers in an equal distance so that a small square source of light is formed. Now remove the 77A Wratten filter and place the aligning target in the slot in front of the camera. Then hold a hand mirror at a 45° angle at the entrance to the optical tube and, looking down the tube, adjust the front-surfaced mirror on the optical track so that the square of light seen on the target is centered left-to-right on the vertical line of the target and up-and-down on the second horizontal line from the bottom of the target.

Now place the camera lens and mount on the optical track near their correct focus position. Adjust the camera lens front-to-rear of the instrument so that the square of light seen on the target is centered on the target's vertical line. Then holding a piece of paper about 3 cm away from the side of the lens facing the camera, rotate the lens about its vertical axis and center any reflections off the lens in the main normal image observed on the paper. Then rotate the light source to the interference position. Adjust the vertical position of the camera lens so that the square of light is centered on the second horizontal line of the aligning target. Again holding the paper on the camera side of the lens, rotate the lens about its horizontal axis so that any reflections are centered in the main normal image observed on the paper. The camera lens is now positioned on the optic axis.

Place the cylindrical lens, lens holder and mount near their correct focus positions on the optical track. Adjust the height of the cylindrical lens so that the vertical position of the light image falls again on the second horizontal line of the target. The cylindrical lens is now posi-

tioned on the optic axis. (A more precise vertical adjustment of the cylindrical lens is made in step *10* below.)

To recheck lens orientation, remove the cylindrical and camera lenses and check the position of the square of light on the target. If the lenses are aligned properly, the position of the square of light on the target will not change.

The accuracy of this method is $\pm 0.07°$.

4. Install the ruled glass disk in the rotor.

Place the ruled disk, ruled surface down, in the cylindrical disk holder. Then place the holder in an Analytical-D rotor, with the lines on the disk perpendicular to the axis of rotation. This can be accomplished by rotating the holder until the lines are perpendicular to the scribe lines used to orient the cell in the rotor. Attach the rotor to the drive shaft and center the cell hole containing the ruled disk below the light-limiting condensing lens mask. Then, to obtain diffuse illumination, place a ground glass above the collimating lens and partially close the rotor chamber. If necessary, slightly rotate the disk holder or the rotor itself to obtain maximum sharpness of the lines as observed in the viewer.

5. Focus the camera lens on the midplane of the cell.

With the ground glass still in position over the collimating lens, close and evacuate the rotor chamber. Set the phase plate dial at 90.5°; then move the camera lens along the optical track in small increments about its optimum position, taking a photograph after each movement. Note that the image of a line from the disk appears to bend in the region where it intersects the schlieren analyzer element. As the camera lens is moved through its correct position, the line will become straight and will then begin to bend in the opposite direction. The camera lens is properly oriented when the image of the line is straight. (The photograph showing optimum position may also be used to determine the horizontal magnification factor of the system; see *Magnification Factors* below.)

The accuracy of this method is ± 0.3 mm.

6. Position the schlieren analyzer at the focal plane of the condensing lens.

Close the light-source jaws as far as possible and, if a horizontal striation is not present on the screen, introduce one by placing dust or a fine wire perpendicular to the source slit. Then rotate the schlieren analyzer from the 90° position to the 0° position while observing the image of the phase plate with respect to the horizontal striation. This

rotation should produce no tilt; but, if the image of the element does tilt, then move the analyzer along the optical track until the tilt is corrected. Note that phase plate angles between 10° and 0° offer the most precision. If at low angles the image of the schlieren analyzer element travels off the screen, set the front-to-rear position of the analyzer so that the metallized line of the phase plate just begins to touch the light-source image from the rear of the machine, as described in step *9* below.

The accuracy of this method is ±0.3 mm.

7. Focus the cylindrical lens on the plane of the schlieren analyzer.

Open the rotor chamber and place a ground glass in front of the front-surfaced mirror. Rotate the schlieren analyzer to 90°; then slide the cylindrical lens along the optical track in small increments, taking a photograph after each movement. Choose that position of the cylindrical lens which provides the sharpest focus of the analyzer element.

The accuracy of this method is ±0.3 mm.

8. Adjust the rotational positions of the condensing lens mask and the cylindrical lens.

Open the rotor chamber and place the interference symmetrical mask in the adjustable mask holder. Be sure the mask slides completely into the rectangular indent. Using the adjustable stop, adjust the left-right position of the mask so that it is centered in the lens opening. Loosen the two screws (with washers) in the bottom section of the lower ring so that the mask can be rotated. Then attach the aligning tool to the drive shaft coupling nut; the tool handle will be used to align the mask on a radius of the rotor. When the outside edges of the handle are parallel to the inside edges of the apertures, the mask is nominally aligned relative to a radius. Once the tool is installed, observe the shadow it casts on the mask. Turn the tool until the shadow of the handle falls between the apertures of the mask; then adjust the rotatable section of the holder until the edges of the shadow are parallel to the inside edges of the aperture. The mask is now nominally aligned; remove the aligning tool from the drive shaft coupling nut.

(Much time can be saved if the above procedure is performed carefully. The latter portion of step *8* will, however, act as a check on the above and will also provide the necessary fine adjustments.)

Once the mask is nominally aligned, rotate the cylindrical lens, as described below, so that its axis is parallel to the axis of the mask. First, with the chamber still open, rotate the schlieren analyzer to the 90° position and look into the viewer. Observe the interference fringes and

the image of the schlieren element; the image of the element should lie close to within the fringe pattern. If it does not, place a piece of thick glass at such an angle in front of the front-surfaced mirror that it will raise or lower the light-source image until the image falls closer to the element. While viewing the pattern, rotate the schlieren analyzer until the fringes and the metallized line are parallel (16). Leave the analyzer in this position. Then place a piece of ground glass over the collimating lens and rotate the cylindrical lens to obtain the sharpest image of the metallized line. After the sharpest image is obtained, remove the ground glass and close the chamber. Then rotate the light source for the sharpest Rayleigh fringes.

The condensing lens mask and the cylindrical lens have now been nominally aligned. For a check on the rotational position of these elements and for the required fine adjustments, proceed as described below.

Assemble a double sector cell with a centerpiece that has been modified to permit free communication between the two sectors. (Preferably, use a double sector centerpiece whose center rib has been removed; alternatively, use an interference synthetic boundary cell which has a scribe between the bottoms of the two sectors, or use a standard double sector centerpiece with two circular polyethylene gaskets on one face. In any case, it is essential that free communication exist between the two sectors.) Now insert 0.1 ml of FC-43 into each sector; next fill each sector half-full with ¼% serum albumin or any other solution which, at speeds below 20,000 rpm, will provide a concentration gradient at both ends of the solution column.

Place this cell and an appropriate counterbalance in the rotor, attach the rotor to the drive shaft, and operate the ultracentrifuge until concentration gradients are formed at the meniscus and cell bottom. Then, with the instrument still operating, rotate the cylindrical lens until fringes are obtained at the top of the solution column; the fringes at the bottom may still be tilted. After this rotational adjustment is completed, stop the instrument and remove the rotor. Place a piece of ground glass over the collimating lens and rotate the schlieren analyzer for maximum sharpness of the phase plate's metallized line. Afterward, remove the ground glass. Then, leaving the chamber open, use the viewer to observe the fringes and the element of the schlieren analyzer. The image of the element should lie close to the fringe pattern. If it does not, place a piece of thick glass at such an angle in front of the front-surfaced mirror that it will raise or lower the light-source image until the image falls closer to the element. Then rotate the rotatable section of the mask holder until the fringes and metallized line are parallel. Tighten the two screws (with washers) in the bottom of the lower ring.

Now add enough ¼% serum albumin to fill completely the cell used above; centrifuge the cell. Then, using the rotational adjustment knob on the adjustable mount, rotate the cylindrical lens until the fringes seen in the viewer are straight at both the top and bottom of the cell. After this position has been determined, continue rotating the lens about its optimum visual position by turning the knob in increments of 10. After each adjustment, take a photograph; then, using a comparator, check the degree of fringe straightness present in each photograph. Lock the rotational adjustment knob at the position that produced the straightest fringe pattern. The cylindrical lens is now aligned rotationally.

Adjust the schlieren analyzer rotationally as follows. First, remove the cell and rotor from the chamber. Then, place the ground glass over the collimating lens and rotate the schlieren analyzer to obtain the maximum sharpness of the element as seen in the viewer. With an Allen wrench, set the phase plate dial so it reads 90°, remove the ground glass, and then rotate the condensing lens mask so that the fringes are parallel to the image of the analyzer element.

The accuracy of this method is ±0.1°.

9. Adjust the rotational position of the light source and the lateral position of the schlieren analyzer.

Rotate the schlieren analyzer to the 0° position; then rotate the light source until the slit image is parallel to the analyzer element. This parallel relationship can be evaluated by placing a white card at the entrance to the optical tube. Moving the analyzer front-to-rear of the instrument, watch the shadow cast on the card by the element as it cuts the light-source image. When the light source is parallel to the analyzer element, the source image will be darkened simultaneously along its full height.

Then set the front-to-rear position of the analyzer so that the metallized line just begins to touch the light-source image from the rear of the instrument.

The accuracy of this method is ±0.06°.

In order to obtain shorter exposure times in experiments using interference optics, the light source can be rotated 90° from the schlieren position and the interference light-source mask put in place of the light-source end trimmers. To adjust the rotational position for this case, with nothing in the chamber and the interference light-source mask removed, rotate the source to obtain the sharpest fringes as seen in the viewer. Look at the central band of fringes during this procedure and use neutral density filters or crossed polaroids to decrease the light density so that the image may be viewed with greater comfort. The accuracy of this method is ±0.08°.

10. Adjust the height of the cylindrical lens.

With no filter over the light source and the system in the interference position, look into the viewer at the central band of fringes. Look for orange light at either the top or the bottom of the pattern. Then lower or raise the cylindrical lens and observe the orange color shifting from top to bottom (or vice versa). Adjust the height of the lens so that the coloring at the top and bottom is either eliminated or made the same. Now, slightly adjust the height of the lens about this position, taking a photograph after each adjustment. Choose that position which shows three sharp fringes spaced symmetrically within the diffraction envelope.

The accuracy of this method is ± 0.2 fringes.

Magnification Factors

That photograph taken in step *5* which indicates the optimum focus position of the camera lens can be used to determine the horizontal magnification factor of the system. To obtain the vertical magnification factor of the system, first unscrew the two knurled screws that hold the phase plate to the analyzer and remove the phase plate. Then place a screw ring gasket and the ruled disk in the disk holder, with the ruled surface of the disk facing down. Make sure the lined rectangular area on the disk corresponds to the rectangular opening in the holder; then tighten the two screws that hold the disk in place. Install the disk holder on the schlieren analyzer (with the circular portion of the holder at the bottom of the analyzer) and tighten the knurled screws.

To ensure diffuse illumination, place a ground glass above the collimating lens. Then close the rotor chamber and turn the phase plate dial to the 0° position. Use the phase plate adjustment knob for the fine adjustments to ensure maximum sharpness of the image. Now, to determine the vertical magnification factor, take a photograph and measure the distance between the first and last lines appearing on the photograph. Then divide this distance by the distance between an equivalent number of lines on the disk itself. When replacing the phase plate, be sure to hold the phase plate upward against the pins on the analyzer before tightening the knurled screws.

(*Note:* The horizontal magnification factor is determined from the photograph taken in step *5* in the same manner as described above for the vertical magnification factor; however, the procedure for determining the former factor should be used *only* if the camera lens is focused on the midplane of the cell. If the camera lens is focused on some other level in the cell, then the magnification factor should be determined without the ground glass over the collimating lens.)

TROUBLESHOOTING

The following chart can be used to locate the cause and remedy of anomalies appearing in the schlieren or interference patterns.

Symptom	Probable cause	Remedy
Double meniscus with connected-channel double sector cell.	a. Offset mask used. b. Differential mask not aligned properly.	a. No remedy required. b. Refer to step *8*.
Fuzzy baseline.	a. Wide light-source slit. b. Cylindrical lens not focused properly. c. Double sector cell used.	a. Close jaws. b. Refer to step *7*. c. No remedy required.
Spurious sharp peaks moving in front of main peak.	a. Convection. b. Contaminating agents.	a. Check cell alignment. Mechanical vibration. Temperature gradient in cell—accelerate slowly. b. No remedy required.
Top or bottom of picture is cut off.	a. Light-source end trimmers in too far. b. Light-source position.	a. Move end trimmers back. b. Refer to step *2*.
Raised baseline.	a. Wedge effect of cell window. b. Hydrostatic compression of fluid in cell. c. Density gradient.	a. No remedy required. b. No remedy required. c. No remedy required.
Dark band through maximum region of peak or center of cell image.	a. Light being cut off by a lens element.	a. Use lower concentration. Wait for diffusion of peak. Use thinner cell. Use reverse wedge window.
Double baseline in spots. Varies with speed.	a. Window distortion.	a. Use new windows. Use sapphire windows.
Very wide meniscus.	a. Camera lens not focused properly. b. Cylindrical lens orientation.	a. Refer to step *5*. b. Refer to step *8*.
No meniscus or dark band in region of meniscus.	a. Camera lens not focused properly.	a. Refer to step *5*.
Double meniscus with single sector cell.	a. Light source off-axis. b. Offset mask on condensing lens.	a. Refer to step *2*. b. Use schlieren mask.

TROUBLESHOOTING (*Continued*)

Symptom	Probable cause	Remedy
Horizontal streaks.	a. Dust between light-source jaws. b. Etching of AH-6 lamp. c. Scratch or dirt on water jacket.	a. Blow air between jaws. b. Rotate or replace lamp. c. Take apart and clean or replace water jacket.
Horizontal streaks which change as phase plate is rotated.	a. Dirt on phase plate.	a. Clean phase plate.
Vertical streaks.	a. Dust, dirt, or oil on collimating or condensing lens.	a. Clean lenses.
Mottling effect in vertical area inside and outside of peak.	a. Dirt on front-surfaced mirror. b. Faulty mirror.	a. Clean mirror. b. Rotate or replace mirror.
Tilted baseline with just water in cell.	a. Cell window distortion. b. Nonparallel light in chamber. c. Schlieren analyzer position incorrect.	a. Use sapphire windows. b. Refer to step *1*. c. Refer to step *6*.
Rayleigh fringes fuzz at high speed.	a. Quartz distortion. b. Light source not oriented properly. c. Upper lens mask slits too wide. d. Sapphire distortion.	a. Use sapphire windows. b. Refer to step *8*. c. Use narrower slit upper lens mask. d. Use polarizer.

SUMMARY

A complete alignment procedure, utilizing new adjustable optical elements, is presented for the schlieren/interference optical system of the analytical ultracentrifuge. The practical and theoretical considerations underlying each individual alignment method are presented as well as a "troubleshooting" chart for evaluating and eliminating anomalies appearing in schlieren and interference patterns.

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