UCRL-11623 Rev.

University of California

Ernest O. Lawrence Radiation Laboratory

ULTRACENTRIFUGE PHOTOELECTRIC SCANNER, MULTIPLE CELL

Berkeley, California

DISCLAIMER

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AEC Contract No. W-7405-eng-48

Sept. 28, 1965

ERRATA

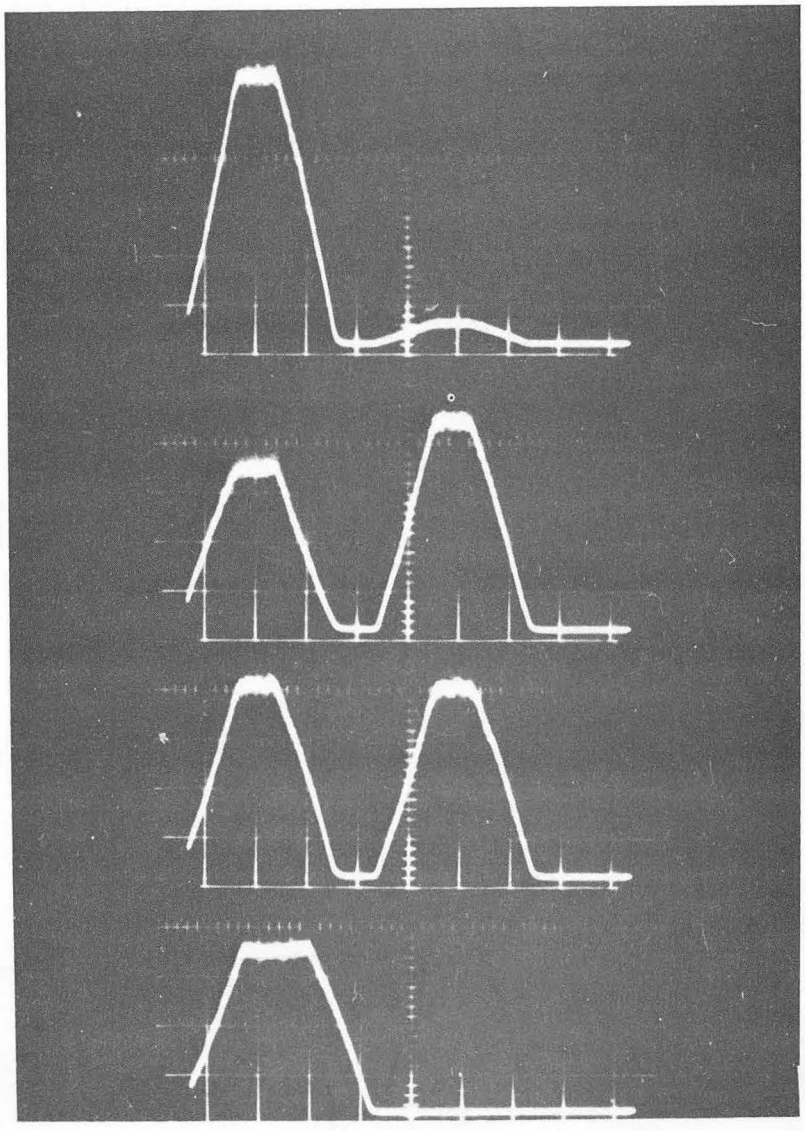
TO: All recipients of UCRL-11623 Rev.

FROM: Technical Information Division

Subject: UCRL-11623 Rev., ULTRACENTRIFUGE PHOTOELECTRIC SCANNER, by Kenneth W. Lamers, dated August 2, 1965

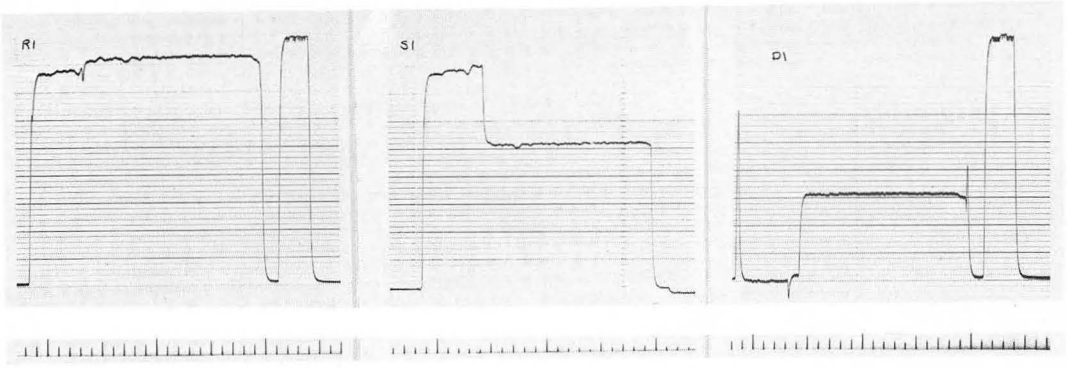
Please make the following corrections on subject report.

Figures 8, 11, 13, 14 were in error. Enclosed are the corrected Figs.



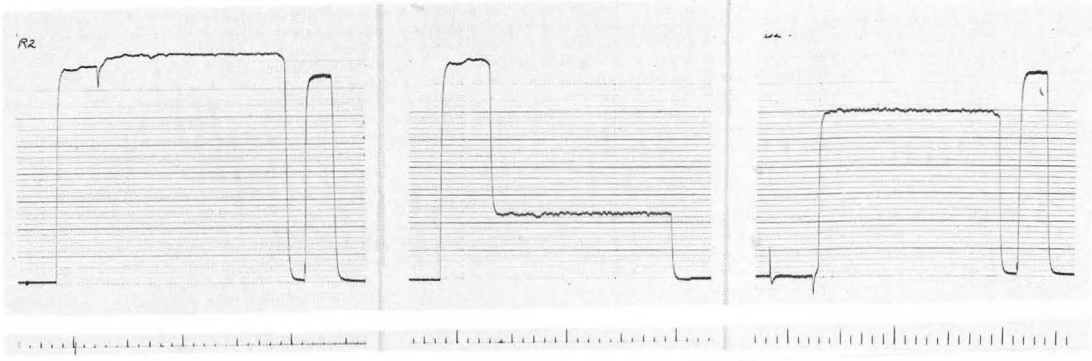
ZN-5098

Fig. 8



(a) (b) (c)

ZN-4904



(d) (e) (f)

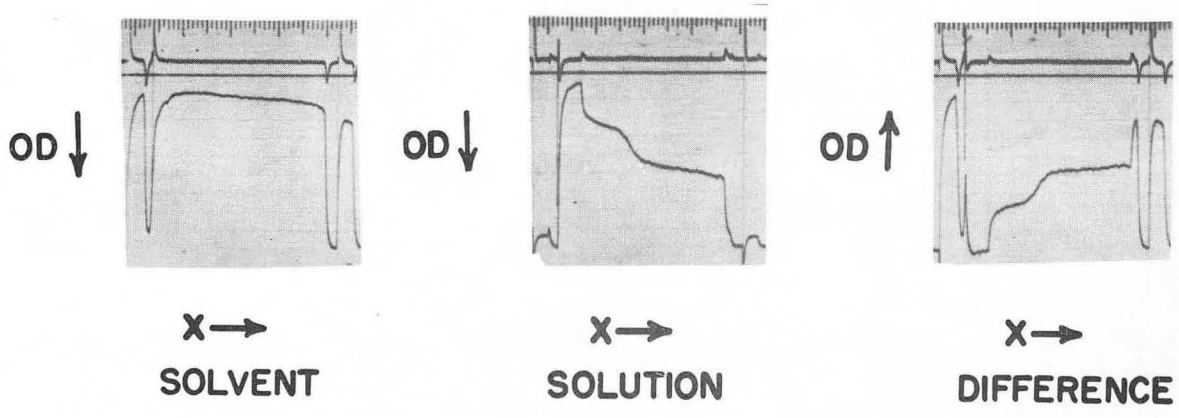
ZN-4905

Fig. 11

ALCOHOL DEHYDROGENASE PLUS DPNH

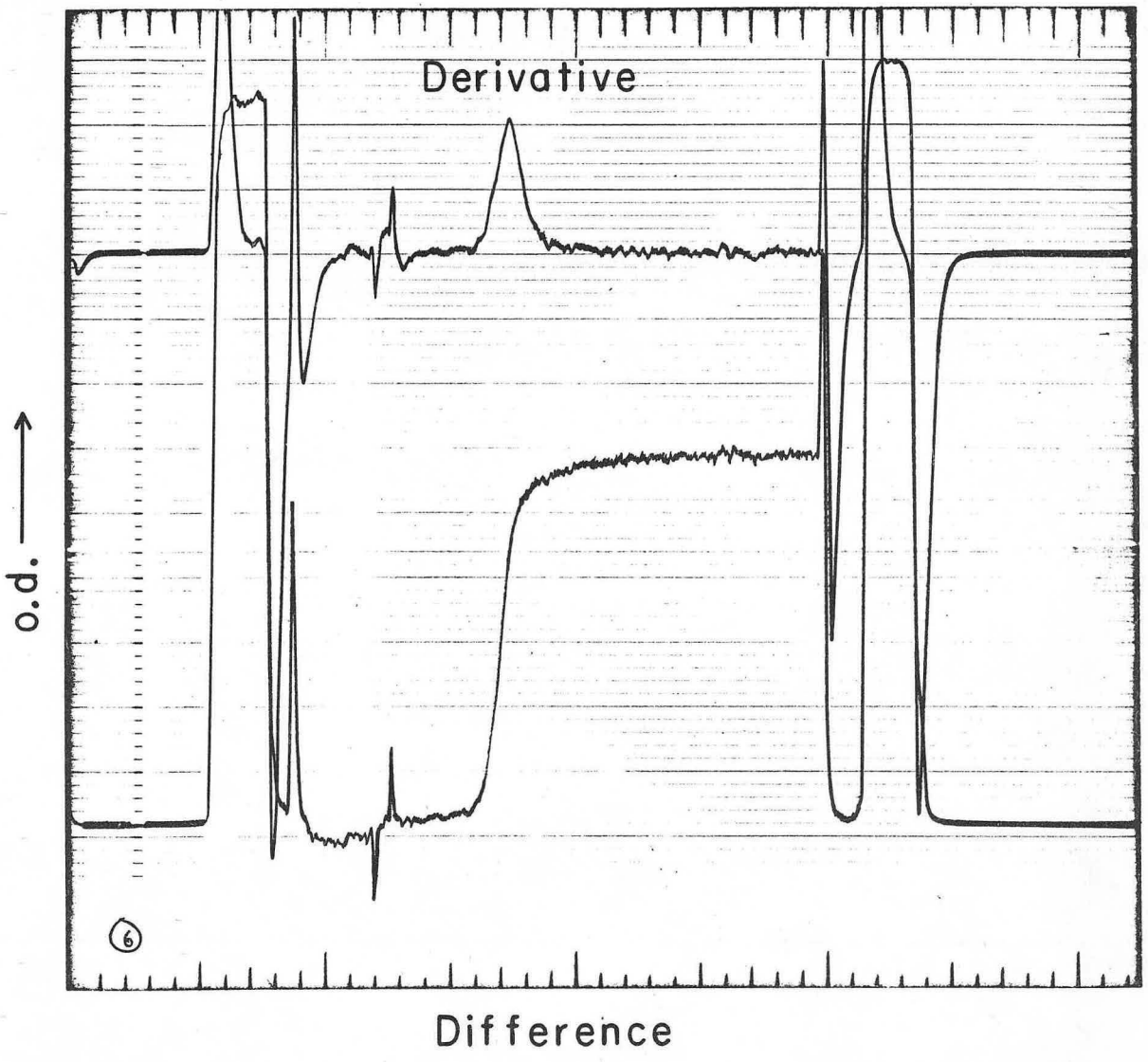
SPLIT BEAM SCANNING SYSTEM

$\lambda = 3400$ A°



ZN-4850

Fig. 13



MUB-7320

Fig. 14

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Kenneth W. Lamers

August 2, 1965

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ABSTRACT

This paper describes an electromechanical scanner used for displaying optical density changes that take place when light-absorbing material such as virus, protein, or nucleic acid is sedimenting in the centrifugal field of an ultracentrifuge--that is, a high-speed centrifuge employing an optical system. These materials absorb some of the light transmitted by the optical system, thereby projecting a pulsating image that varies in intensity with distance from the center of rotation. This image is scanned by a photomultiplier and compared with the pulsating image of a reference cell in order to compensate for nonuniform illumination and other optical imperfections.

This scanner eliminates the photographic process usually associated with this type of display. With photographic methods, density profiles are obtained by projecting the image upon film, then scanning that film with a photodensitometer. Photographic methods are frustrating because of the time lapse between experimentation and observation. They are also less accurate because film is linearly responsive to light intensity over a limited range.

The scanner described offers the following advantages: (a) Essentially direct viewing of the ultracentrifuge patterns is provided. A complete scan can be recorded in 6 seconds. Concentration is displayed as a function of radius. (b) Differential readout compensates for such optical imperfections as

nonuniform illumination or dirty lenses. (c) The system is linearly responsive to optical densities between zero and 1.8, with an accuracy ±1% of full-scale deflection. (d) An internal calibrator makes possible measurements of absolute optical density. (e) The ultracentrifuge incorporates a monochromator for spectral analysis, a powerful tool for disclosing the radial distribution of components responsive to different wavelengths. (f) Derivative of the function is displayed in time coincidence. (g) The system is designed for multiple-cell operation; thus two different experiments can be conducted simultaneously.

I. INTRODUCTION

An ultracentrifuge (Fig. 1) is a high-speed centrifuge employing an optical system.[1] This paper describes an electromechanical scanner (Figs. 2, 3, 4) used for displaying optical density changes that take place when light-absorbing material such as virus, protein, or nucleic acid is sedimenting in the centrifugal field produced by an ultracentrifuge. These materials absorb some of the light transmitted by the optical system, thereby projecting a pulsating image that varies in intensity with distance from the center of rotation. This image is scanned by a photomultiplier and compared with the pulsating image of a reference cell in order to compensate for nonuniform illumination and other optical imperfections.

This scanner eliminates the photographic process usually associated with this type of display. With photographic methods, density profiles are obtained by projecting the sample image upon film, than scanning that film with a photodensitometer. Photographic methods are frustrating because of the time lapse between experimentation and observation; they are also less accurate than the scanning method because film is linearly responsive to light intensity over a limited range.

The scanner described offers the following advantages: (a) Essentially direct viewing of the ultracentrifuge patterns is provided; a complete scan can be recorded in 6 seconds. Concentration (log of the transmittance) is displayed as a function of radius. (b) Differential readout compensates for such optical imperfections as nonuniform illumination or dirty lenses. (c) The system is linearly responsive to optical densities between zero and 1.8, with an accuracy ±1% of full-scale deflection. (d) The ultracentrifuge incorporates a monochromator for spectral analysis, a powerful tool for disclosing the radial distribution

of components responsive to different wavelengths. (f) Derivative of the function is displayed in time coincidence. (g) The system is designed for multiple-cell operation; thus two experiments can be conducted simultaneously.

The system described is designed for two double-sector cells separated 180 deg, as shown in Fig. 5. Each sector is 2 deg, and is separated approximately 2.5 deg from the other sector in the cell. It is possible to operate with only one double-sector cell or with two single-sector cells, also separated 180 deg. Single-sector cells are 4 deg; greater sector angles permit a longer slit[2] and illumination is increased proportionately. Discussion in this report, however, is restricted to operation with two double-sector cells.

Referring to Fig. 5, we see that each cell contains a reference and a sample sector, separated 2.5 deg and enclosed by a common cell window. The reference sectors compensate for such optical imperfections as nonuniform illumination or dirty lenses. This compensation provides continuous correction, an important feature because the lenses become coated with oil deposits from the centrifuge drive. Cell windows are less susceptible than the lenses to deposits and are cleaned prior to each run, whereas the lenses are not.

II. SYSTEM DESCRIPTION

A. General

A block diagram of the system is shown in Fig. 6. A photomultiplier with defining slit[2] scans the pulsating images at a constant speed.[3] It accepts light samples as the centrifuge makes them available, generating pulse amplitudes proportional to light intensity and slit area. Pulse duration is related to centrifuge speed, sector angle, and slit length.

Pulses generated at the photomultiplier are shown in Fig. 7. These actual photographs of oscilloscope traces show duration, amplitude, and

separation with the scanner stopped at several positions (see Fig. 5). In brief, the photomultiplier generates a pulse when the rotor makes light available. When scanning the cell images, the photomultiplier "sees" two quick light bursts, the first due to the reference sector of a given cell, the second due to the sample sector.[4] The light bursts are followed by a quiescent period, much longer than the time interval between "mates," that corresponds to the time required for the rotor to turn 180 deg. At the end of that time two light bursts appear as before, but these are due to the second cell. The net result is alternating pulse pairs, first from one cell, then from the other. If the oscilloscope sweep speed is increased, the pulses due to one cell only are displayed as illustrated in Fig. 8.

Pulses do not always appear in pairs, however; the rotor includes a radius-marker hole, as shown in Fig. 5. This hole is displaced 90 deg from the center line between cells and is used as a reference for detecting motion within the cells. When the scanner is "observing" the radius-marker image, the rotor allows but one burst per revolution, as shown in Figs. 7(c) and 8(d).

B. Scan Control

Scanning is automatic in the sense that the instrument need not be attended once operation has been initiated. The scanning mechanism remains at its start position until it receives an impulse from the ultracentrifuge, after which it moves the photomultiplier across the pulsating images, movement of the carriage being controlled by a synchronous motor driving a lead screw. Mounted on the end of the lead screw is a slotted disk which, in conjunction with a small light and a photosensitive element, generates marker pulses. These marker pulses determine positions of fiducial marks on the recorder chart in terms of distance traversed by the photomultiplier.

The recorder chart drive, an independent mechanism, is activated during the forward scan only. When the scanner reaches the end of its travel it actuates a limit switch and returns to its start position where it remains until the centrifuge provides another impulse. Manual scan control is also available.

The scanning mechanism includes a 4-speed transmission operated by solenoid-type clutches. The solenoids permit remote control of scanning speed and facilitate high-speed carriage return; i.e., the scanner automatically returns to the start position at its highest speed (6 seconds) unless the fast return is disabled.

C. Recorder

The recorder selected[5] (Fig. 4) employs an ultraviolet beam and self-developing paper. This recorder was selected mainly because its fast writing speed does not limit resolution, but also because (a) it permits the function and its derivative to be displayed across the full width of the paper while retaining a common time base, and (b) recording is rectilinear. The multiple-channel arrangement (12 channels plus two timing galvanometers) permits the same recorder to be used simultaneously by two (or more) centrifuges. The additional channels facilitate the recording of more information, if desired.

Also important, the ruling of grid lines on the paper as it issues from the recorder compensates for any lateral paper slippage that might occur. Marker pulses from the timing galvanometer provide a check for reproducibility of the mechanically independent scanner and chart drives.

D. Image Separation

1. Sector Images

The scanner output is a train of pulses; the system must detect which sector is responsible for a given pulse. The first requirement of image-separation circuitry is that it route the pulses from each of the four sectors to a

different holding circuit.[6] The second requirement is that it route the pulses from a given sector to the same holding circuit every time that the scanner moves across the images.

Holding circuits are necessary because reference and sample pulses do not occur simultaneously; the reference pulse must therefore be stored for comparison with its "mate." Because the holding circuits are designed to respond to the peak level of each pulse, output levels are high and the effects of drift are minimized.

If the pulses due to each sector are to be routed to the appropriate hold, we need some method for sensing which sector (or the radius-marker hole) is responsible for a given pulse. There are several methods by which this may be done: (a) One is to use a separate sensing element for detecting rotor position. (b) Another is to use circuitry that performs logic upon (deduces information from) pulses from the scanning photomultiplier. We decided to combine both techniques; in short, image separation is controlled partly by the scanner, partly by a secondary optical system (Fig. 6).

Assume for the moment that only the first requirement is to be satisfied, i.e., pulses from each sector are to be routed to a different hold. Assume further that photomultiplier pulses appear in the sequence indicated by Fig. 9.

It is practical to separate "mates" by use of a one-shot.[7] We designed the system (Figs. 6 and 9) so that the reference pair gate is open to the first pulse (which is from a reference sector). The trailing edge of each reference pulse activates the one-shot, closing the reference pair gate for a specified length of time. The transition opens the sample pair gate (previously closed) for the same period, permitting the following pulse (from the sample sector) to pass through. At normal operating speeds this time interval is comparatively short, permitting only one pulse through the sample pair gate. Furthermore,

the time duration of the one-shot pulse is short enough to ensure that a subsequent pulse passes through the sample pair gate if, and only if, it is mated to the preceding pulse.

The first pulse, however, is sometimes associated with a sample sector. Since the reference pair gate is open, the first pulse inadvertently passes through it. This confusing condition is rectified when the one-shot returns to its original state and allows the following pulse to pass through the reference pair gate. Since that pulse is produced by a reference sector, gating is restored to normal.

The one-shot has particular utility for this application because it has a built-in recovery time (determined by the length of its quasi-stable state) that prevents it from responding to consecutive pulses of a given pulse pair. This recovery time is effective in discriminating between "mates," but it does not discriminate between cells. Additional circuitry is required to ensure that every other pulse pair is routed identically. This indicates the need for a binary device, the flip-flop,[8] that presents a different set of conditions to alternate pulse pairs.

We next consider the gates operated by a one-shot and flip-flop. The system herein is designed so that each pulse must pass through two gates,[9] the first operated by a one-shot (pair gate), the second by a flip-flop (cell gate) as shown in Fig. 6. As either gate can be open or closed, there are four possible states for a given pair of gates (a pair gate plus a cell gate). The trick is to drive each gate with a properly timed signal so that a given pair of gates is open only to the pulse that it is supposed to pass.

The one-shot is triggered by the trailing edge (delayed) of each reference pulse as shown in Fig. 9.[10] The flip-flop, in turn, is triggered by the trailing edge of each one-shot pulse. This triggering mode is important

because no reliance is placed upon sample pulses, some of which are highly attenuated at large optical densities.

The pair gates, operated by the one-shot, are driven out of phase so that each input pulse passes through only one pair gate. The cell gates are operated by the flip-flop, which opens them in synchronism so that one cell gate passes the reference pulse and its counterpart passes the "mate."

To record the image from the other cell, one must change the cell-selector switch accordingly. This inverts flip-flop phasing to both cell gates so that they transmit the alternate pulse pairs.

Referring again to Fig. 9, we see that the pulse relationship illustrated is a special case; i.e., we assume that the first pulse finds the flip-flop in its low-level state. We further assume that the first pulse pair is due to cell 1, although this is not necessarily true in practice. The first pulse can come from any of four sectors--which sector is dependent upon the position of the rotor when the scanner intercepts the first pulse. Because these assumptions are not necessarily true, we may get an erroneous routing. In order to obviate that possibility, synchronizing signals from a secondary optical system[11] (Fig. 2) (set pulses) are applied to the flip-flop to ensure that it is in the proper state relative to the sector under observation.

Set pulses do not usually change flip-flop status. In most cases they merely confirm it, applying corrective action if necessary. Figure 10 illustrates corrective action taken when pulses arrive with the flip-flop in an improper state. The first pulse pair, R2a and S2a, is not permitted through the cell gates even though the cell-selector switch has been set for cell 2. The following pulses, R1a and S1a, pass erroneously through the cell gates (reference and sample). In the absence of set pulses, all subsequent pulses admitted to the holding circuits would be due to cell 1. This erroneous routing would occur even though one had selected cell 2 for observation. This, perhaps, would be

tolerable if one could turn the cell-selector switch to cell 1 and record the alternate cell. That, however, is not practical. Subsequent scans sometimes "find" a different pulse relationship, and routing becomes erroneous again. Without set pulses the recorded traces vacillate between cells, recording the desired cell only by chance. For a single scan only, a loss of triggering (due to factors such as meniscus and light fluctuations) could cause the recorded output to switch, some parts of the trace representing one cell, other parts another. Set pulses preclude both possibilities.

Refer again to Fig. 10, for which corrective action is as follows: The first set pulse finds the flip-flop in its low-level state. This is improper, and the set pulse promptly shifts the flip-flop to its higher level, at which the cell gates pass cell-2 pulses only (cell selector at 2). Once the system is "back in step," set pulses play no further role because they have no influence when the flip-flop is in its high-level state, one in which all subsequent set pulses find it.

2. Radius-Marker Image

Although it is necessary that both difference traces include the radius-marker image [Fig. 11(c) and (f)], they do not do so unless a scanner-activated switch (Fig. 2) is added to disconnect set pulses when the scanner is at the radius-marker image. Refer to Fig. 12, which shows the switching signal to cell gates when the cell-selector switch is set at 1; here we find that the cell gates are open to every radius-marker pulse. If we changed the selector switch to cell 2 and did nothing to alter the presence of set pulses, the switching signal to the cell gates would be identical (but inverted) with the signal obtained with the cell-selector switch set at 1. This signal would cause the cell gates to prevent passage of the radius-marker pulses in cell-2 position. In order

to circumvent this, set pulses are disconnected when the radius marker image is being scanned (Fig. 2) and the cell-selector switch is set at 2. The switching signal to cell gates then corresponds to that shown for cell 2, a relationship that admits every other radius marker to the reference hold. The one-shot prevents radius-marker pulses from entering the sample hold, so that the radius marker appears in both difference traces.

In summary, the reference gates (pair gate and cell gate) transmit every radius-marker pulse with the cell selector at 1, but every other radius-marker pulse with the cell selector at 2.

E. Reference-Pulse Regulator

The system includes provisions for regulating the reference pulse to an amplitude that remains constant though illumination changes. When so operated, the scanning-photomultiplier supply voltage varies to compensate for changes in illumination, light intensity, linear-amplifier gain, and photomultiplier sensitivity. Such a scheme does not influence the relative amplitude of reference and sample pulses. Regulation (a) ensures that the log compressor operates over a prescribed region, and that none of the compressor's linear region is wasted in compensating for nonuniform illumination; (b) increases accuracy of the calibrating system (the calibration is based upon 25-V reference pulses to the log compressor input—a significant departure from that level results in error); (c) is helpful in sustaining switching when illumination is extremely nonuniform or when a solvent of appreciable optical density is used.

If regulation were perfect, one could dispense with the difference system. The sample traces, in that event, would represent the corrected density profile. Regulator performance is very good for illumination profiles of moderate nonuniformity, but subtraction enhances performance even more. Subtraction is

also helpful because: (a) It facilitates trace expansion at low optical densities; i.e., recorder deflection per optical density can be increased. The recorded trace need not be displaced when the regions of interest are expanded, and the profile can be recorded in its entirety. (b) It discriminates against fluctuations in light intensity, especially those of higher frequency.

F. Calibrator

The system includes an electronic calibrating circuit that makes possible the measurement of absolute optical density. The measurement is made by comparison of the solution's attenuation (optical effect) with a known electrical attenuation. The electrical attenuator is continuously variable and empirically calibrated in terms of optical density.

G. Derivative

The function recorded is differentiated electronically, and the derivative applied to another recorder channel. The function and its derivative are recorded in time coincidence.

III. PERFORMANCE

A number of performance tests were conducted. Many involved oscilloscope presentations and some were photographed to convey performance characteristics more accurately. The oscilloscope traces are not presented here, but they are included in another report.[12] Performance is best deduced from Visicorder traces, however, and these appear as Figs. 11, 13, and 14. System response to solutions of measured optical density is given in Fig. 15. Articles relevant to scanner applications, indicated in references 13 through 16, chronicle scanner evolution and indicate some of the studies that have been made. Future articles will emphasize derivative, spectral analysis, and calibration applications.

IV. FUTURE CONSIDERATIONS

The opportunities for extending scanner performance appear to be unlimited. Among them are:

1. Operation with more cells.

2. A servo arrangement for synchronizing the scanning mechanism with the recorder chart drive. Recorder traces, in that event, would have a magnification factor independent of scanning speed.

3. A printer for recording such information as run number, time, and date on each recorded trace. This could prevent confusion should traces be filed erroneously.

4. Computer techniques for extending accuracy. Recording techniques could permit data processing subsequent to scanning.

ACKNOWLEDGMENTS

The photoelectric scanner was designed for the Molecular Biology and Virus Laboratory, University of California, Berkeley. The work was under the direction of Professor Howard K. Schachman, who conceived the application of double-sector cells to centrifuge work, and is responsible for most of the optics relevant to the transition from photographic recording. The scanning mechanism was designed by Messrs. Frederick H. Bierlein, Robert K. Johnson, George Lauterbach, and Franz X. Plunder. Messrs. Charles G. Dols and Paul Salz contributed useful suggestions. Miss Boihon Chin conducted many of the experiments.

FOOTNOTES AND REFERENCES

* Work performed under the auspices of the U. S. Atomic Energy Commission.

1. Howard K. Schachman, Ultracentrifugation in Biochemistry (Academic Press, New York, 1959).
2. The slit moves across the images in a radial direction with its length perpendicular to the direction of travel. A rotary head permits selection of one of six slits. The slit dimensions presently used are (1) 0.00099 by 0.4 inch, (2) 0.003 by 0.093 inch, (3) 0.00133 by 0.081 inch, (4) 0.00617 by 0.107 inch, (5) 0.00150 by 0.4 inch, and (6) 0.00354 by 0.4 inch. The slit most commonly used is number (2).
3. Four scanning rates are available. The images are scanned in 6, 30, 60, or 360 seconds. The scanner returns to the start position in 6 seconds unless the fast return is disabled.
4. The cells are loaded with regard to rotation.
5. Visicorder Model 906B. Visicorder is the commercial name for a recording oscillograph (Honeywell) employing an ultraviolet beam and self-developing paper. The maximum writing speed is greater than 10 000 inches per second. The galvanometers used are M100-120A with a sensitivity of 10 μA per inch and a frequency response (flat ∓5%) of 0 to 60 cps.
6. Eliahu I. Jury, Sampled Data Control Systems (John Wiley and Sons, Inc., New York, 1958), p. 48.
7. J. Millman and H. Taub, Pulse and Digital Circuits (McGraw-Hill Book Company, Inc., New York, 1956), p. 174.
8. Ibid, p. 140.
9. One gate would be adequate if the gating signal were conditioned differently. The method used was chosen for expediency.
10. A slight delay, added in order to improve pulse separation, is subject to speed.

11. The secondary optical system, Schlieren type, is standard equipment for this centrifuge; it is used for diffraction-type measurements. Schlieren optics are displaced 180 deg from the absorption optical system. The stationary photomultiplier (Fig. 6) could have been positioned to intercept light from part of the radius-marker image projected by the absorption system. The absorption images projected change position with wavelength (lens must be moved), however, so we chose to use the Schlieren optical system, which operates at a fixed wavelength.

12. Kenneth W. Lamers, Ultracentrifuge Photoelectric Scanner, Multiple Cell, Lawrence Radiation Laboratory Report UCRL-11623, 1965 [a comprehensive report (121 pages) including schematics, operating instructions, and maintenance adjustments].

13. S. Hanlon, K. Lamers, G. Lauterbach, R. Johnson, and H. K. Schachman, Ultracentrifuge Studies with Absorption Optics, I. An Automatic Photoelectric Scanning Absorption System, Arch. Biochem. Biophys. 99, 157 (1962).

14. H. K. Schachman, L. Gropper, S. Hanlon, and F. Putney, Ultracentrifuge Studies with Absorption Optics, II. Incorporation of a Monochromator and its Application to the Study of Proteins and Interacting Systems, Arch. Biochem. Biophys. 99, 175 (1962).

15. K. Lamers, F. Putney, I. Z. Steinberg, and H. K. Schachman, Ultracentrifuge Studies with Absorption Optics, III. A Split-Beam Photoelectric, Scanning Absorption System, Arch. Biochem. Biophys. 103, 379 (1963).

16. Kenneth W. Lamers, Ultracentrifuge Photoelectric Scanner, Split-Beam, Lawrence Radiation Laboratory Report UCRL-10499, 1962.

FIGURE CAPTIONS

Fig. 1. An overall view of the ultracentrifuge, which was manufactured by Spinco Division, Beckman Instruments, Inc. (Model E). The speed range is 600 to 67 000 rpm, which corresponds to 260 000 g's at 60 000 rpm.

Fig. 2. Scanning mechanism mounted in the ultracentrifuge. This view shows the upper right-hand end of the centrifuge. The transmission cover plate has been removed to show the clutches (foreground). The micrometer adjustment (lower left) determines scanner position at which set pulses are disconnected. Stationary photomultiplier (upper right) which straddles the large pipe, receives light pulses from a secondary optical system (set pulses). Lid of the ultracentrifuge, opened for this picture, clears the scanning mechanism and is normally closed to prevent room light from interfering with the operation.

Fig. 3. A view of an earlier version of the scanning mechanism removed from the ultracentrifuge (prior to addition of the slit selector, the set-pulse disable switch, and the variable-speed transmission). Light rays passing through the optical system (perpendicular to the plane of the photograph) enter the photomultiplier through a narrow vertical slit covered by a small snout that is no longer required.

Fig. 4. Front view of the control console, now adapted to include operating controls for two ultracentrifuges. The top two panels are operational units, one for each machine. Directly below them is the Visicorder. Below the Visicorder, a scan control panel includes separate controls for each scanning mechanism. The bottom panel includes the stationary-photomultiplier power supplies, convenience outlets, and a master power switch for the entire console. Directly above the bottom panel is the scanning-photomultiplier power supply, which includes separate controls for each scanning photomultiplier.

Fig. 5. Top view of rotor with two double-sector cells. As the cells are not filled completely, air spaces form at the inner radii. Cell 1 is filled in the same manner as cell 2, usually with a sample of different density. The radius-marker hole is located as shown.

Fig. 6. Block diagram showing the important electrical and mechanical components of the photoelectric scanner: (a) ultracentrifuge, and (b) control console.

Fig. 7. Oscilloscope traces showing photomultiplier pulses with the scanner stopped at several positions (refer to Fig. 5): (a) Scanner positioned to intercept the images projected by the air spaces of all four sectors. The lower pulses, which are set pulses from the stationary photomultiplier (Fig. 6), are due to the radius-marker hole. (b) Scanner at the images projected by both solutions. One sample sector is filled with a solution of higher density than its counterpart. The lower pulses are set pulses. (c) Scanner at the radius-marker hole. The lower pulses are set pulses.

Fig. 8. Oscilloscope traces showing photomultiplier pulses (inverted) with the scanner stopped at various positions (Fig. 5). The oscilloscope sweep speed is faster than that of Fig. 7, therefore the pulses due to one cell only are displayed. (a) Scanner positioned to intercept the image projected by one solution. (b) Scanner positioned at the air-solvent boundary within one reference sector. (c) Scanner at the air space of one cell (both sectors). (d) Scanner positioned at the radius-marker hole. Pulse width is slightly longer and is related to the diameter of the hole. The photomultiplier "sees" only one light burst per revolution.

Fig. 9. Pulse relationships with the scanner positioned to intercept the images projected by both solutions (Fig. 5). Each gate is open when its switching signal is at the higher level. This is an idealized case for which set pulses would not be required.

Fig. 10. Pulse relationships illustrating set-pulse corrective action with cell selector at 2. The scanner is positioned to intercept the images projected by both solutions (Fig. 5). Each gate is open when its switching signal is at the higher level. The set pulse brings the flip-flop "in step," after which the trailing edge of each one-shot pulse dictates switching (unless the flip-flop falls "out of step"). R1a and S1a pass into the holding circuits erroneously, but all subsequent pulses are routed correctly.

Fig. 11. Visicorder traces illustrating performance with two double-sector cells. The reference-pulse regulator is disabled in order to indicate the improvement resulting from the difference system. (a) A scan recorded with mode selector at reference, cell selector at 1. (b) A repeat scan with mode selector at sample. (c) A third scan with mode selector at difference. (d) Cell selector at 2, mode selector at reference. (e) Cell selector at 2, mode selector at sample. (f) Cell selector at 2, mode selector at difference.

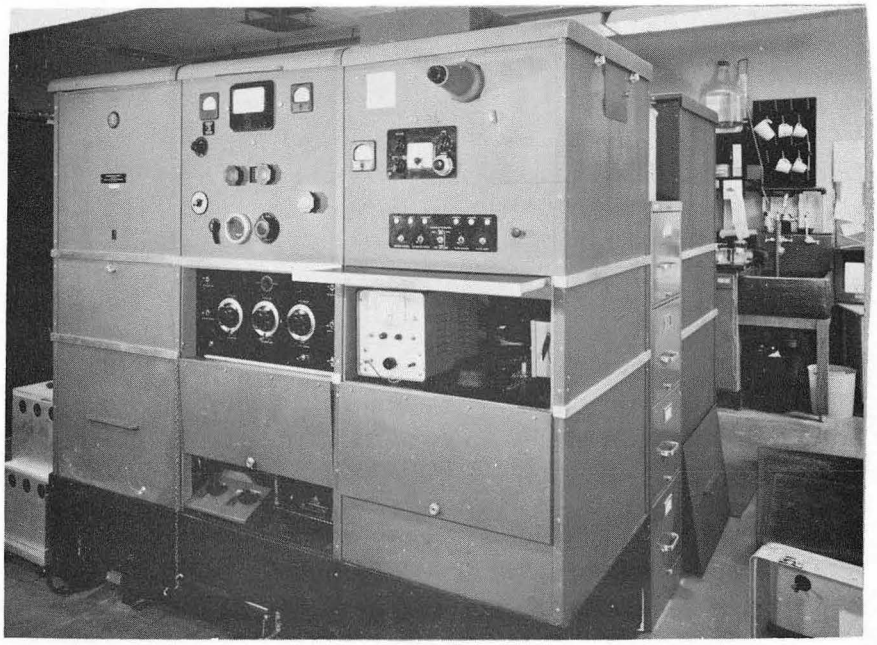
Fig. 12. Pulse relationships with the scanner positioned to intercept the image projected by the radius-marker hole (Fig. 5). Each gate is open when its switching signal is at the higher level. The waveforms below the heavy line indicate the significant changes resulting when the cell-selector switch is changed from cell 1 to cell 2 position. Cell 1 position admits every radius-marker pulse, cell 2 position every other radius-marker pulse. Set pulses are disconnected with the

the cell-selector switch at position 2; the flip-flop spends equal time in each state. Set pulses have no influence when the flip-flop is in the high-level state.

Fig. 13. Visicorder traces illustrating operation in each of three modes. The response is logarithmic, and therefore indicates optical density as shown: (a) A scan recorded with the mode switch at reference position. The system responds to reference-sector content. The upper trace records the derivative. (b) A repeat scan with the mode switch at sample. The radius-marker pulses are rejected by the gating circuits. The derivative is inverted but important information is derived from the difference traces. (c) A third scan with the mode switch at difference. Radius-marker amplitudes have no significance in terms of optical density. The sharp spike following the left-hand radius marker results from a disparity between the radial positions of the reference and sample cells. The spike adjacent to the right-hand radius marker results from sedimentation in the sample cell.

Fig. 14. A Visicorder trace illustrating derivative performance, with 30-second scan rate. This trace was taken in the difference mode, internal regulation. The lower trace represents the difference profile with a solution density of 0.9 (1-cm path length); trace amplitude was set for 40% of maximum gain. The upper trace is its derivative; with derivative amplitude set for about 60% of maximum gain. Visicorder chart speed is 5 mm/sec; centrifuge speed is 50 740 rpm. Note fiducial marks top and bottom.

Fig. 15. System response to solutions of measured optical densities, mode at difference. The deflections are approximately 25% of those obtainable at maximum gain. The calibration indicates that the system is linearly responsive (accuracy ± 1% of full-scale deflection) to optical densities between zero and 1.8. The photomultiplier supply was adjusted for 2-V reference pulses from the scanning-photomultiplier. Optical density in the centrifuge is 1.2 times the value of a 1-cm path length.



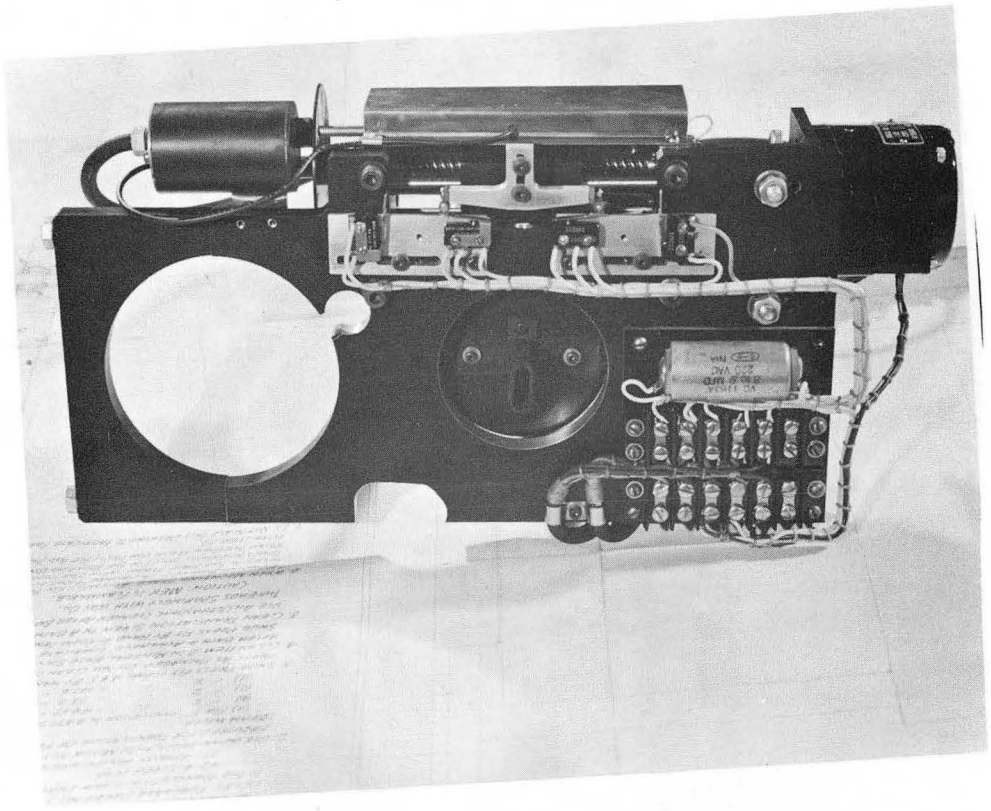
ZN-3502

Fig. 1


MODEL
Spinco ULTRACENTRIFUGE
SERIAL NO.
DATE
SPECIALIZED INSTRUMENTS CORP
BERKELEY, CALIFORNIA

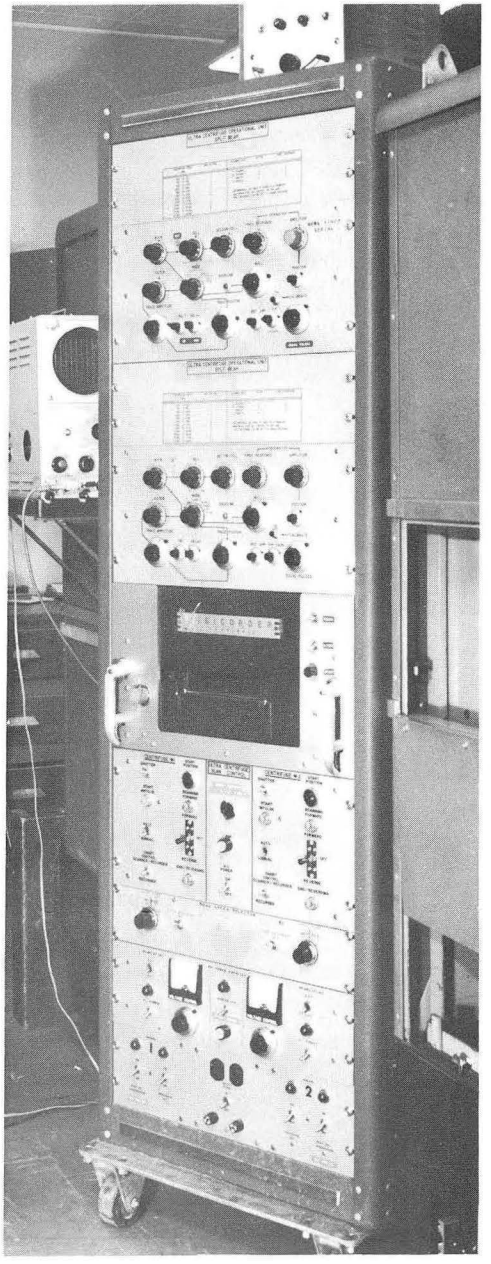
ZN-4851

Fig. 2



ZN-3570

Fig. 3



ZN-4852

Fig. 4

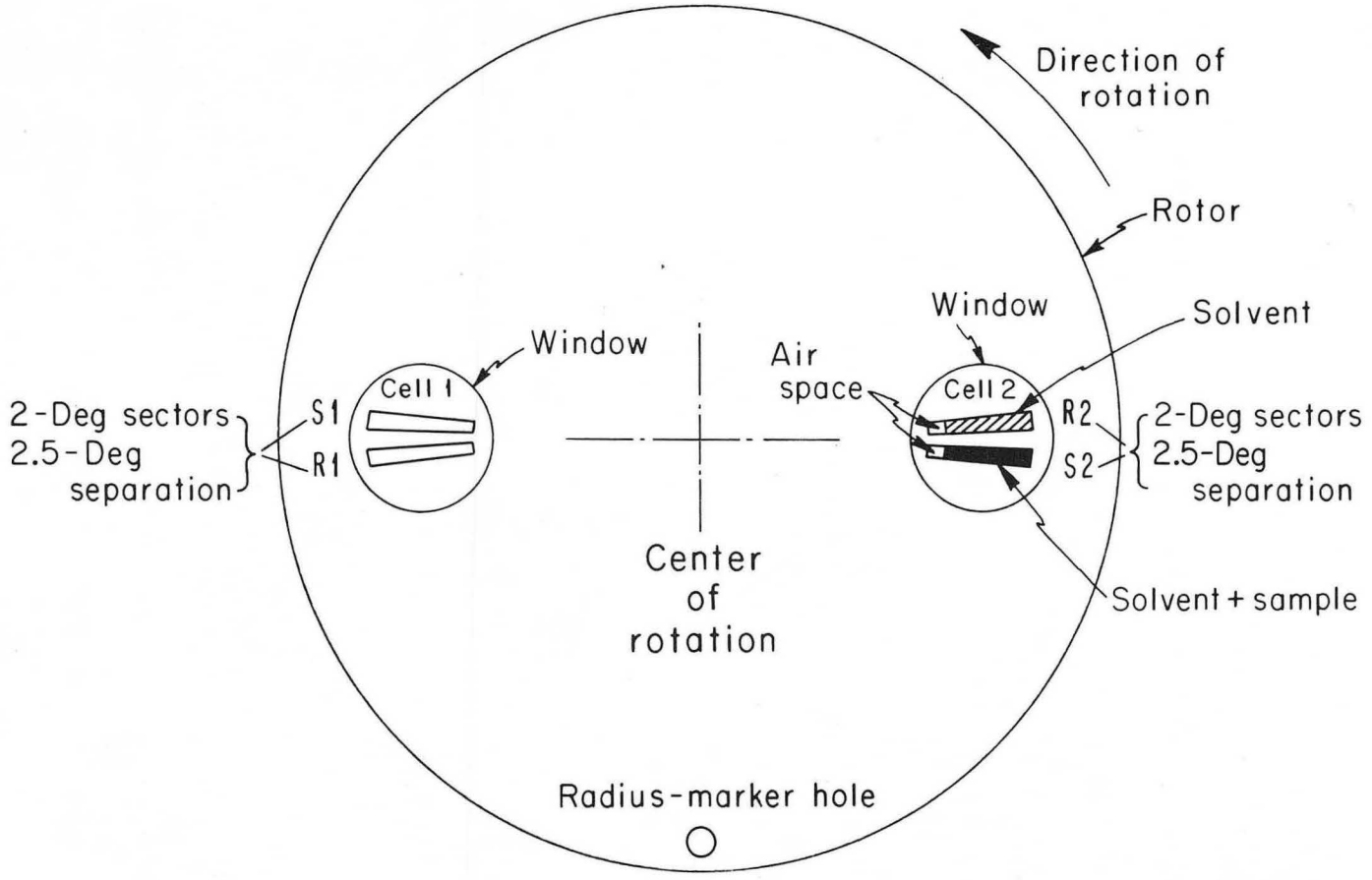

Direction of rotation
Rotor
Window
Solvent
Window
Air space
Cell 1
Cell 2
2-Deg sectors
2.5-Deg separation
S1
R1
R2
S2
2-Deg sectors
2.5-Deg separation
Center of rotation
Solvent + sample
Radius-marker hole
MUB-7324

Fig. 5

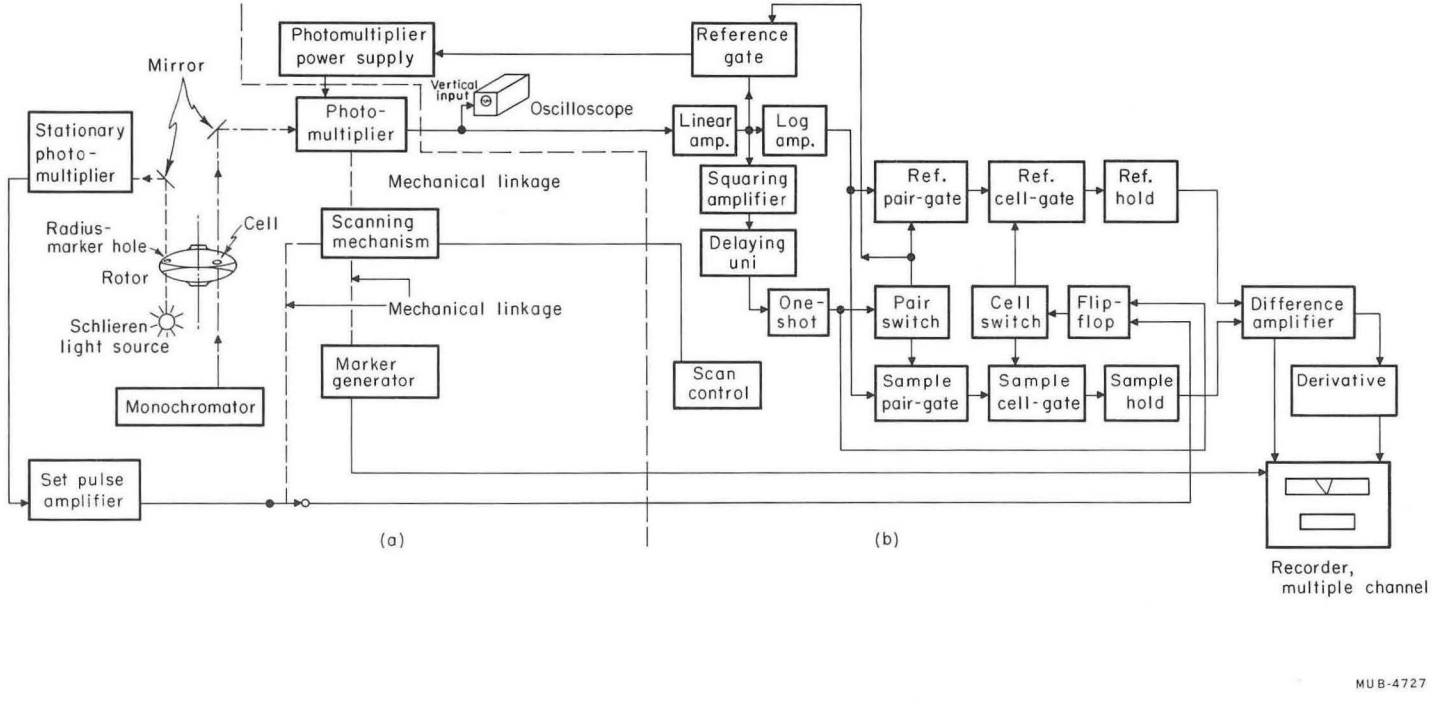
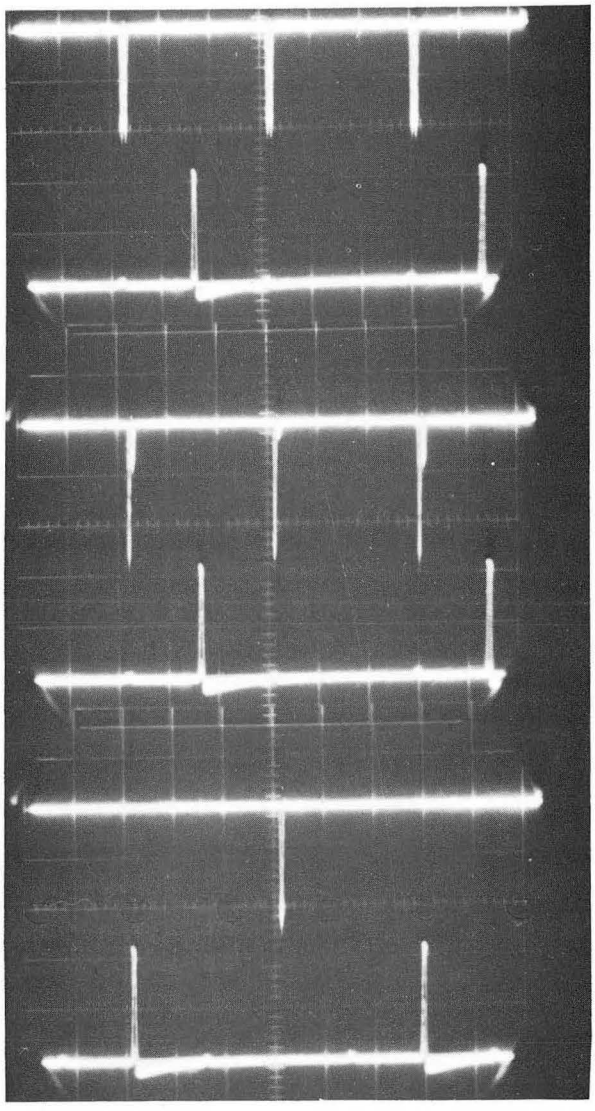
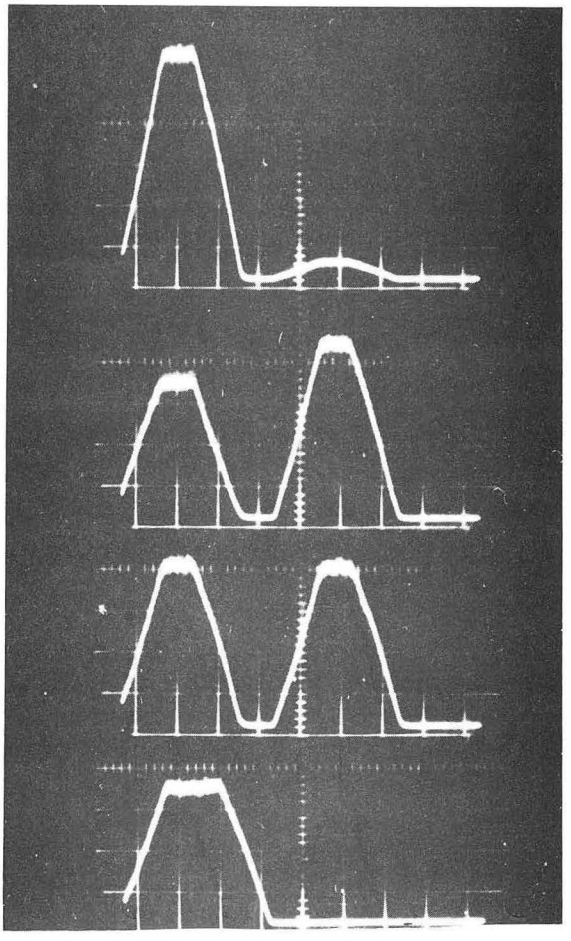

Photomultiplier power supply
Reference gate
Mirror
Vertical input
Oscilloscope
Stationary photo-multiplier
Photo-multiplier
Linear amp.
Log amp.
Squaring amplifier
Ref. pair-gate
Ref. cell-gate
Ref. hold
Mechanical linkage
Radius-marker hole
Cell
Scanning mechanism
Delaying uni
Rotor
Mechanical linkage
One-shot
Pair switch
Cell switch
Flip-flop
Difference amplifier
Schlieren light source
Marker generator
Scan control
Sample pair-gate
Sample cell-gate
Sample hold
Derivative
Monochromator
Set pulse amplifier
(a)
(b)
Recorder, multiple channel
MUB-4727

Fig. 6



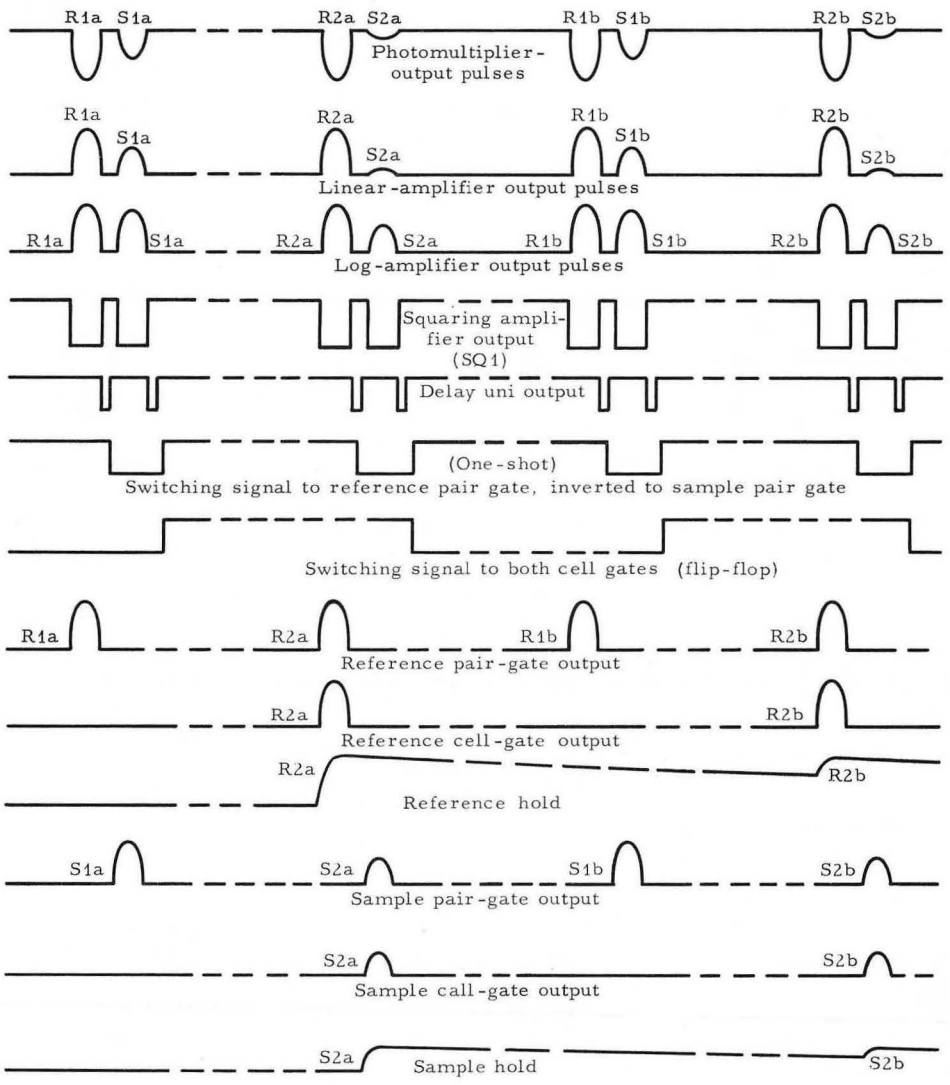
ZN-4848

Fig. 7



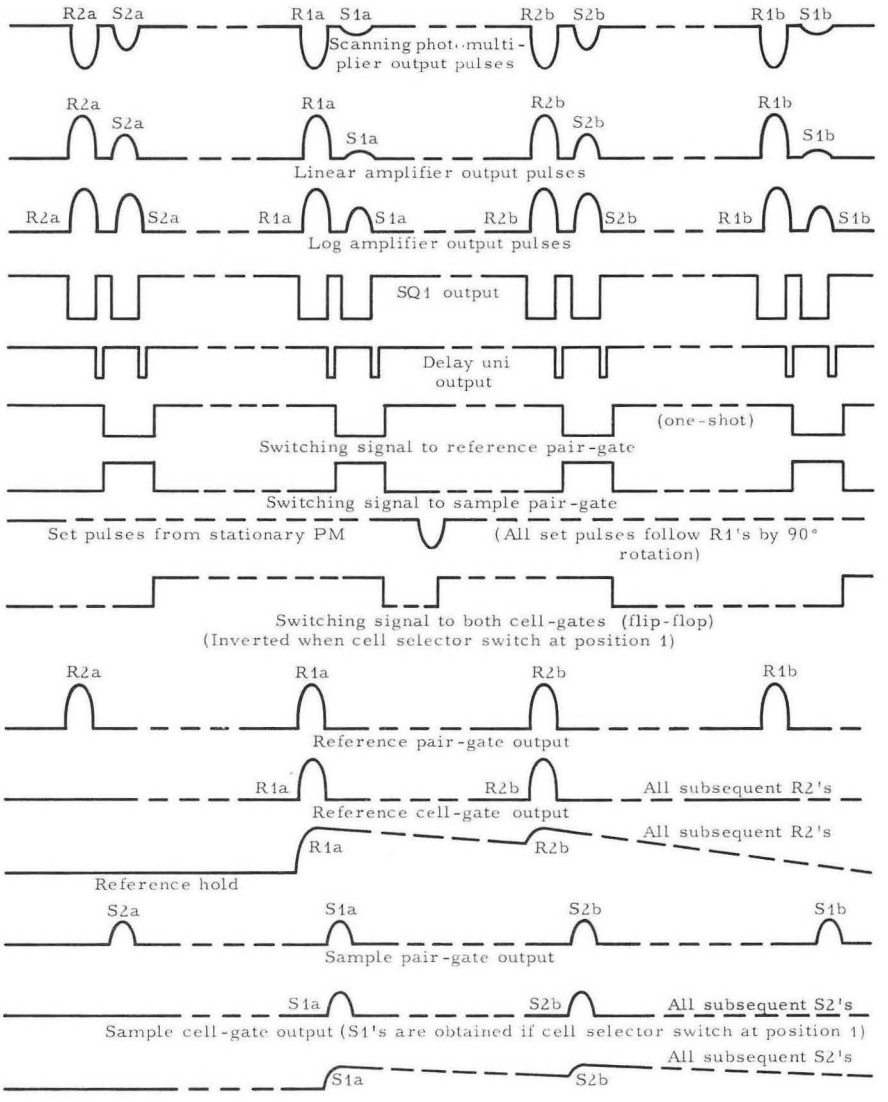
ZN-4849

Fig. 8



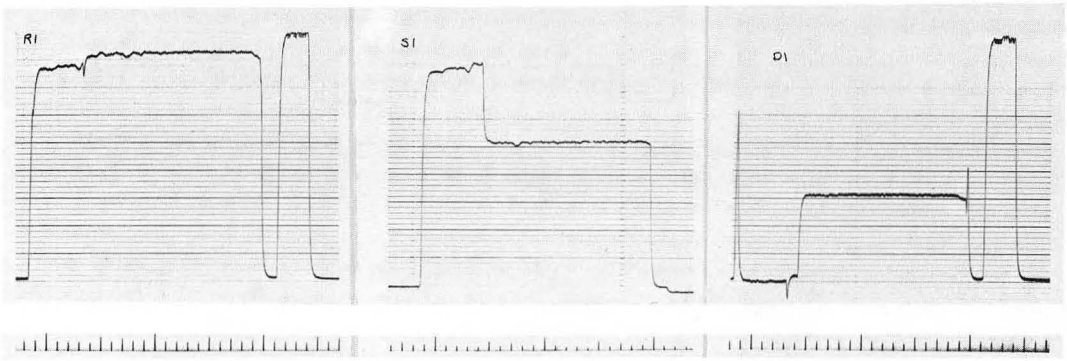
MUB-5846

Fig. 9



MUB-5841

Fig. 10



(a) (b) (c)

ZN-4904

Fig. 11

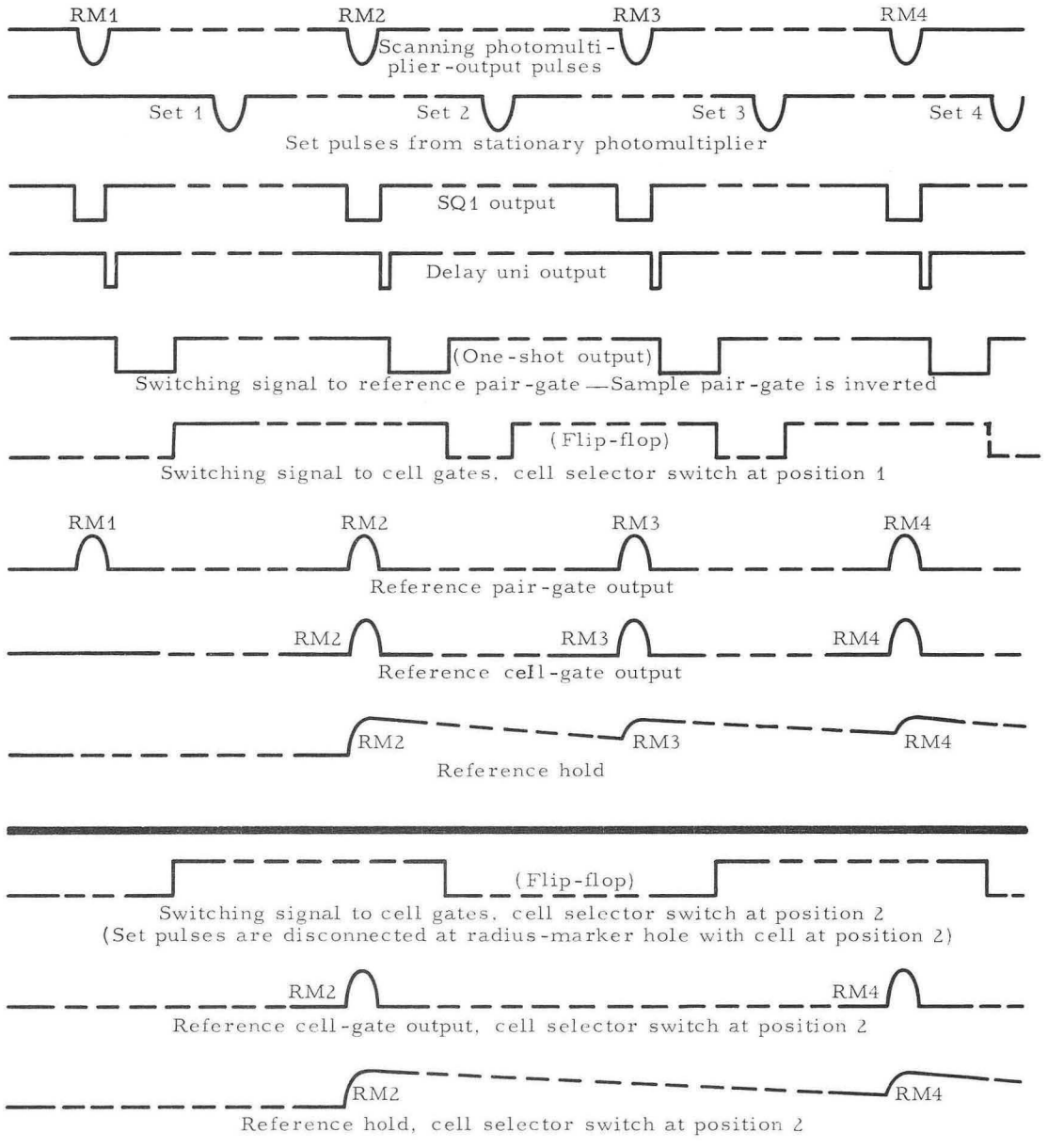
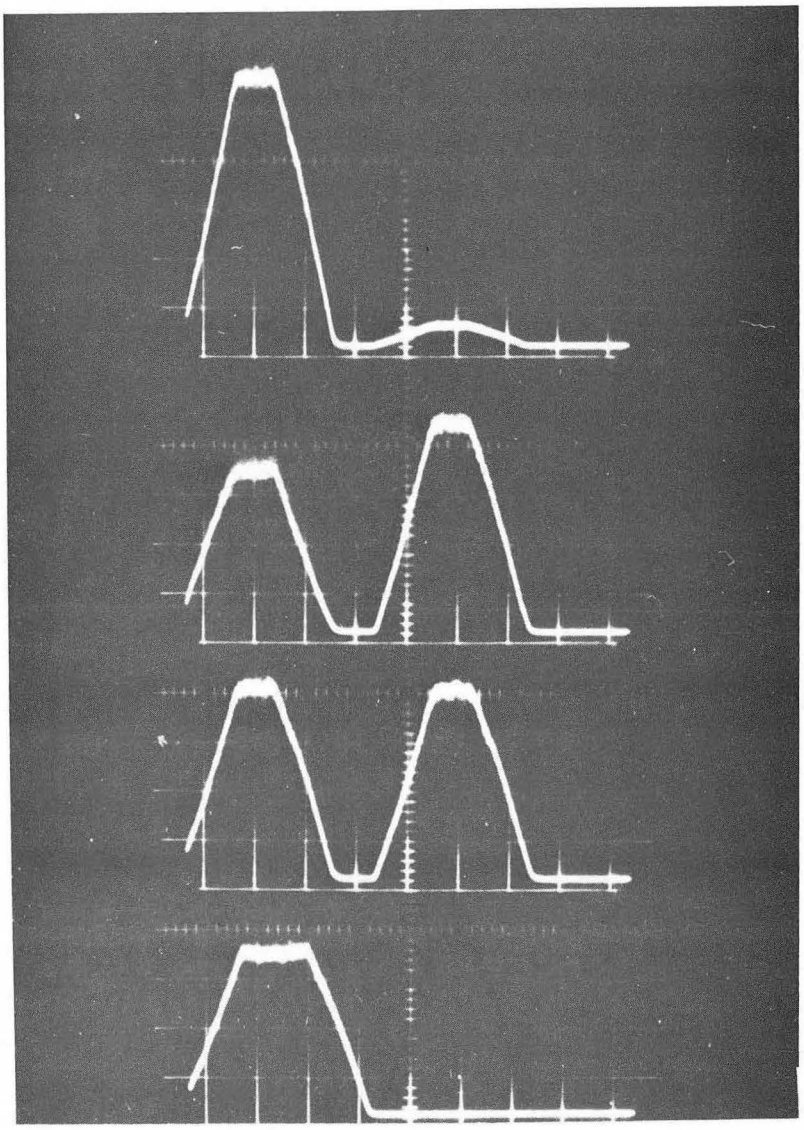
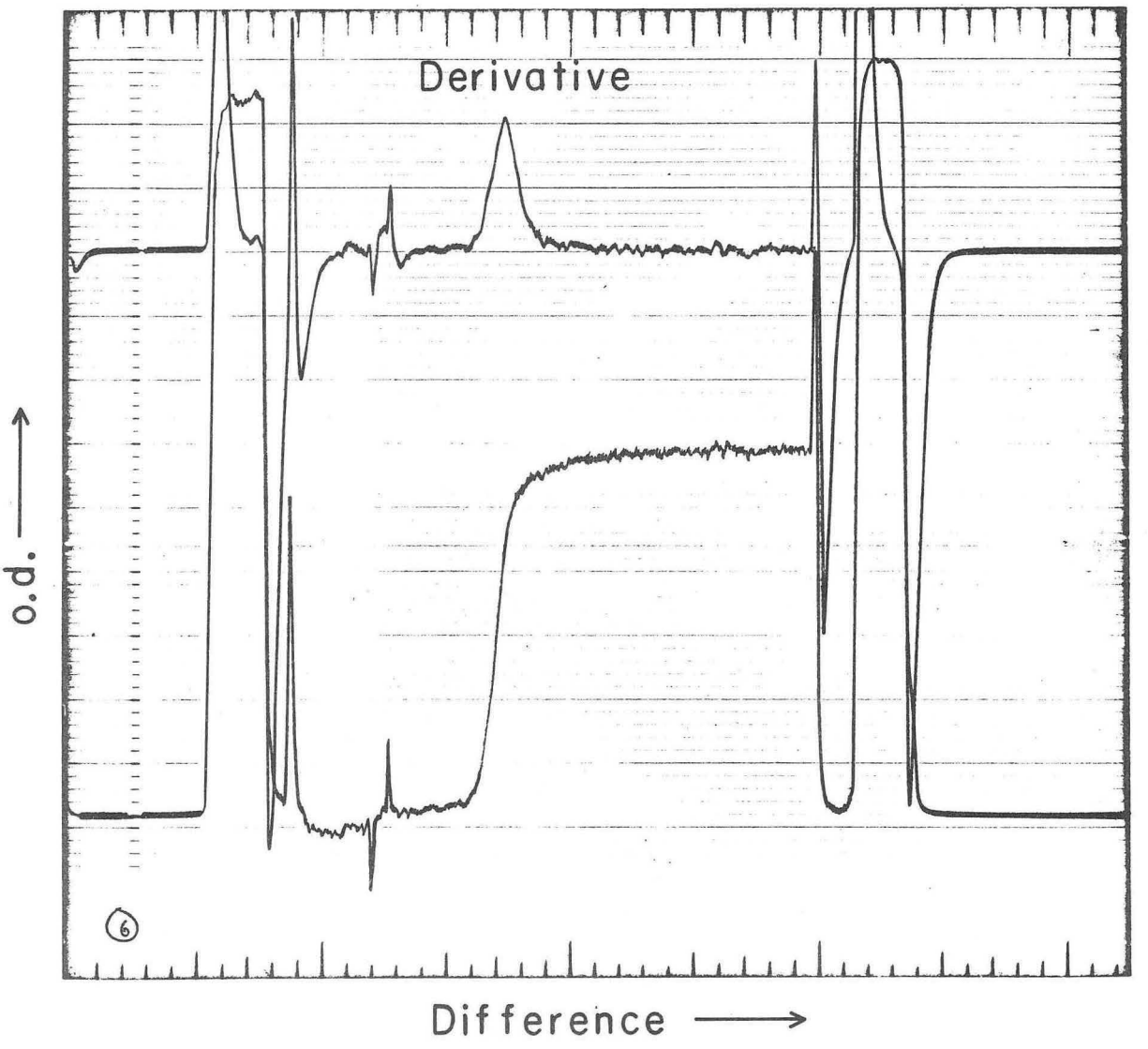

RM1
RM2
RM3
RM4
Scanning photomultiplier-output pulses
Set 1
Set 2
Set 3
Set 4
Set pulses from stationary photomultiplier
SQ1 output
Delay uni output
(One-shot output)
Switching signal to reference pair-gate — Sample pair-gate is inverted
(Flip-flop)
Switching signal to cell gates, cell selector switch at position 1
RM1
RM2
RM3
RM4
Reference pair-gate output
RM2
RM3
RM4
Reference cell-gate output
RM2
RM3
RM4
Reference hold
(Flip-flop)
Switching signal to cell gates, cell selector switch at position 2
(Set pulses are disconnected at radius-marker hole with cell at position 2)
RM2
RM4
Reference cell-gate output, cell selector switch at position 2
RM2
RM4
Reference hold, cell selector switch at position 2
MUB-5882

Fig. 12



ZN-5098

Fig. 13



MUB-7320

Fig. 14

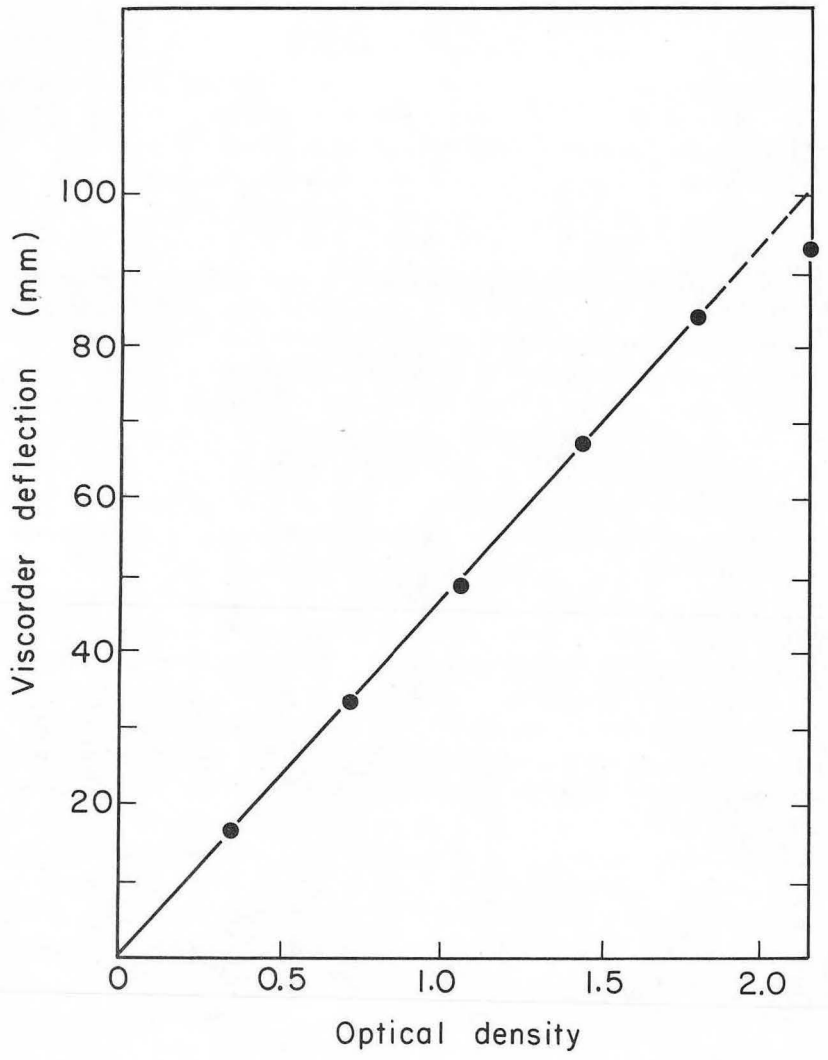

Viscorder deflection (mm)
100
80
60
40
20
0
0.5
1.0
1.5
2.0
Optical density
MU-29379

Fig. 15