Lawrence Berkeley National Laboratory
Recent Work

Title
ULTRACENTRIFUGE PHOTOELECTRIC SCANNER, MULTICELL

Permalink
https://escholarship.org/uc/item/0460n12w

Author
Lamers, Kenneth W.

Publication Date
1966-04-07
University of California

Ernest O. Lawrence Radiation Laboratory

ULTRACENTRIFUGE PHOTOELECTRIC SCANNER, MULTICELL

TWO-WEEK LOAN COPY

This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545

Berkeley, California
DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.
UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California
AEC Contract No. W-7405-eng-48

ULTRACENTRIFUGE PHOTOELECTRIC SCANNER,
MULTI CELL

Kenneth W. Lamers
April 7, 1966
ULTRACENTRIFUGE PHOTOELECTRIC SCANNER,
MULTI CELL

Kenneth W. Lamers

Lawrence Radiation Laboratory
University of California
Berkeley, California

April 7, 1966

ABSTRACT

We describe modifications for converting an ultracentrifuge photo-electric scanner to multi-cell operation. The scanner records concentration of light-absorbing material (log of the transmittance) as a function of radius. Derivative of the concentration is recorded in time coincidence. The modified scanner is designed for two double-sector cells separated 180 deg or for two single-sector cells, also separated 180 deg. Attention is focused on the techniques used for recovering the time-interlaced images resulting from centrifuge motion.
I. INTRODUCTION

A single-cell scanner has been described previously.¹ The modified version described here is designed for two double-sector cells (Fig. 1), and is therefore concerned with the recovery of four images, each projected to the same location in time sequence. Each image (although present less than 1% of the time) is recreated electronically as though it were not pulsating, and as if no other images were present. Differential readout compensates for such optical imperfections as nonuniform illumination or dirty lenses.

The scanner described here differs from previous versions in that it is designed for operation with (a) two double-sector cells or (b) two single-sector cells, separated in both cases by 180 deg.

II. SYSTEM DESCRIPTION

A. General

The instrument described here has been reported in detail.² A block diagram is shown in Fig. 2. A photomultiplier with defining slit scans the pulsating images, generating pulses with amplitude proportional to light intensity. These pulses are processed as shown in Figs. 2 and 3.

The scanning mechanism activates the recorder chart drive during the forward scan only. It also operates a marker generator that produces fiducial marks on the recorded traces, providing a check for reproducibility of the mechanically independent scanner and chart drives.

Referring to Fig. 2, the instrument includes provisions for regulating the reference pulses to constant amplitude even though illumination and other factors change. Regulation, which does not influence the relative amplitude of reference and sample pulses, (a) is helpful in sustaining.
switching when illumination is extremely nonuniform or when a solvent of appreciable optical density is used and (b) increases accuracy.

As mentioned earlier, the scanner is also designed for operation with two single-sector cells separated 180 deg. Greater separation between sectors permits us to use cells with larger sector angles (usually 4 or 6.5 deg), and therefore to increase the length of the scanning slit used. A longer slit provides more illumination to the photomultiplier in those applications where light intensity is not enough. Discussion in this article, however, is restricted to operation with two double-sector cells.

B. Image Separation

Output from the scanning photomultiplier is a train of pulses (Fig. 3); 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. 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. 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 holding circuit. Assume further that photomultiplier pulses appear in the sequence indicated by Fig. 4.

Experience with the earlier scanner indicated that it is practical to separate reference and sample pulses due to a given cell, called mates, with a one-shot. We apply that same technique to the scanner described here. Referring to Figs. 2 and 4, the reference pair gate is open to the first pulse (which is from a reference sector). The trailing edge of each reference pulse (delayed) 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 (one-shot duration) 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 (sample) 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 separating mates 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, that presents a different set of conditions to alternate pulse pairs.

We next consider the gates operated by the one-shot and flip-flop. The system is designed so that each pulse must pass through two gates, the first operated by a one-shot (pair gate), the second by a flip-flop (cell gate), as shown in Fig. 2. 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. 4. 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. 4, 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; the specific 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, set pulses, are applied to the flip-flop to ensure that it is in the proper state relative to the sector under observation (see Figs. 2 and 3).
Set pulses do not usually change flip-flop status. In most cases they merely confirm it, applying corrective action if necessary. Figure 3 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, so that some parts of the trace represent one cell and other parts another. Set pulses preclude both possibilities.

Refer again to Fig. 3, 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, a state in which all subsequent set pulses find it.
III. PERFORMANCE

Performance is best deduced from the recorded traces; image separation is illustrated in Fig. 5. These traces represent six separate scans, each recording a different profile. Derivative performance, considerably better than previously reported, is illustrated in Fig. 6.

ACKNOWLEDGMENTS

The photoelectric scanner was designed for the Molecular Biology and Virus Laboratory, University of California at Berkeley. The work was under the direction of Professor Howard K. Schachman who is responsible for most of the optics. The scanning mechanism was designed by Messrs. F. H. Bierlein, R. K. Johnson, G. Lauterbach, and F. X. Plunder.
FOOTNOTES AND REFERENCES

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


2. Kenneth W. Lamers, Ultracentrifuge Photoelectric Scanner, Multiple Cell, Lawrence Radiation Laboratory Report UCRL-11623, April 1965 (a comprehensive report including derivations, schematics, operating instructions, and maintenance adjustments).


5. A slight delay is added in order to improve pulse separation.

6. Ref. 4, p. 140.

7. One gate would be adequate if the gating signal were conditioned differently. The method used was chosen for expediency.

8. The secondary optical system, standard equipment for this centrifuge, is displaced 180 deg from the absorption optical system.
FIGURE CAPTIONS

Fig. 1. Top view of rotor. Each cell contains two sectors, a reference and a sample. 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 provides synchronizing pulses to a stationary photomultiplier (Fig. 2). The photomultiplier defining slit scans the images in a radial direction with its length perpendicular to the direction of travel.

Fig. 2. Block diagram showing the important electrical and mechanical components: (a) ultracentrifuge and (b) control console.

Fig. 3. 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. 1). 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. R1a and S1a pass into the holding circuits erroneously, but all subsequent pulses are routed correctly.

Fig. 4. Pulse relationships, an idealized case for which set pulses would not be required.

Fig. 5. Recorder traces illustrating image separation (see Fig. 1). These traces represent six separate scans, each recording a different profile. The response is logarithmic as shown. [The fiducial marks are produced by the marker generator (Fig. 2).] (a) R1; (b) S1; (c) differential readout, R1 minus S1; (d) R2; (e) S2; (e) differential readout, R2 minus S2.

Fig. 6. A recorder trace illustrating derivative performance. The lower profile represents concentration (differential readout), the upper profile its derivative. The scanning time is 30 sec.
Fig. 1
Fig. 2
Scanning photomultiplier output pulses

Linear amplifier output pulses

Log amplifier output pulses

Squaring amplifier output

Delay unit output

Switching signal to reference pair-gate

Switching signal to sample pair-gate

Set pulses from stationary PM

Switching signal to both cell-gates (flip-flop)
(Inverted when cell selector switch at position 1)

Reference pair-gate output

Reference cell-gate output

Reference hold

Sample pair-gate output

Sample cell-gate output (St's are obtained if cell selector switch at position 1)
Fig. 4
Solvent | Solution | Difference
--- | --- | ---
(a) | (b) | (c)
(d) | (e) | (f)

Cell 1

Cell 2

Fig. 5
This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.