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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 regu-

lating 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 appli-

cations 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.[3] 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. As-

sume 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.[4] 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)[5] 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 pre-

ceding 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,[6] that presents a different set of con-

ditions 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,[7] 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 illus-

trated 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 errone-

ous routing.   In order to obviate that possibility, synchronizing signals

from a secondary optical system,[8] set pulses, are applied to the flip-flop

to ensure that it is in the proper state relative to the sector under obser-

vation (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 sepa-ration 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.
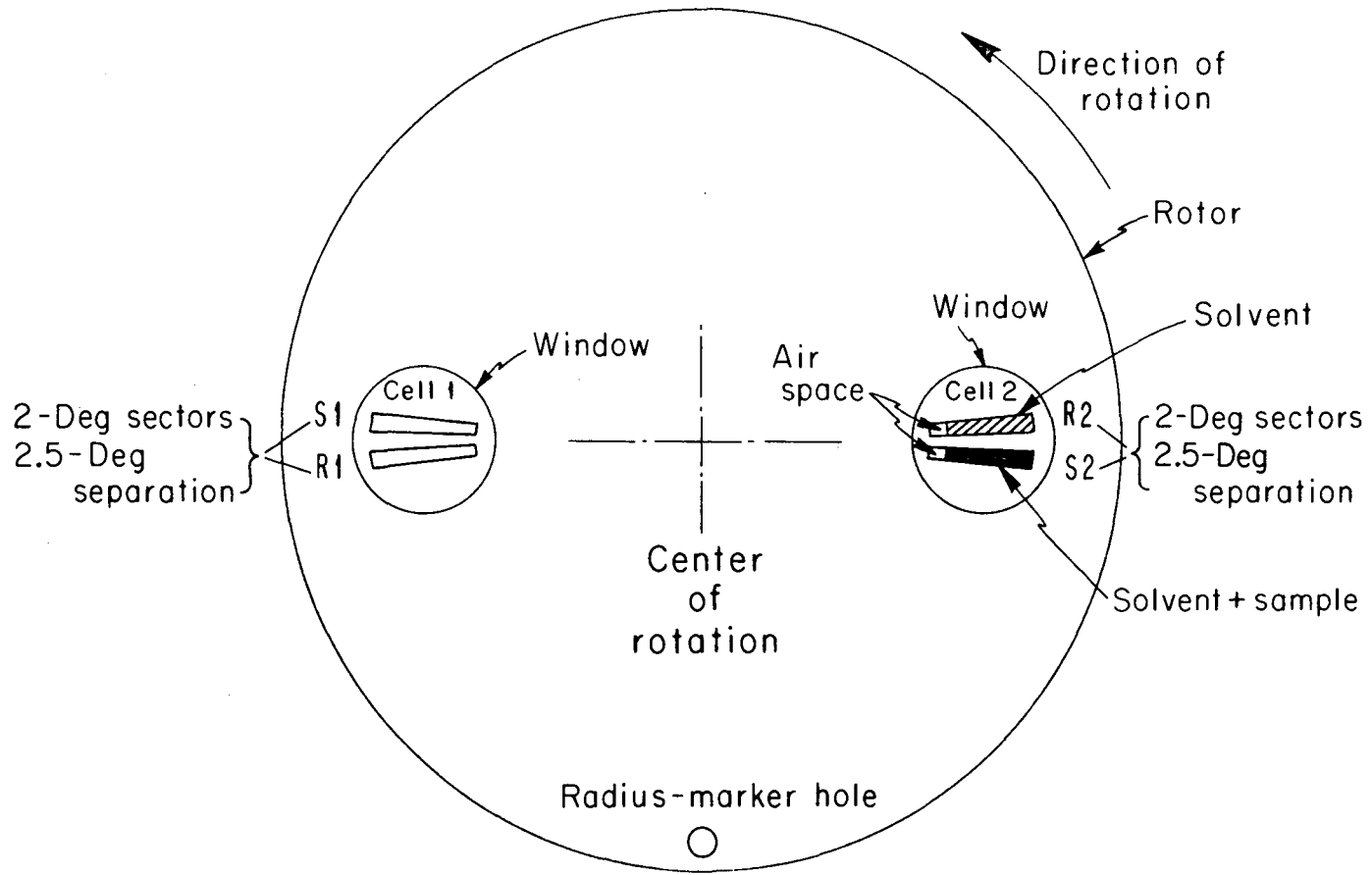
FOOTNOTES AND REFERENCES

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

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

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).

3. Eliahu I. Jury, Sampled Data Control Systems (John Wiley and Sons, Inc., New York, 1958), p. 48.

4. J. Millman and H. Taub, Pulse and Digital Circuits (McGraw-Hill Book Company, Inc., New York, 1956), p. 174.

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 synchro- nizing pulses to a stationary photomultiplier (Fig. 2). The photomulti- plier 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.
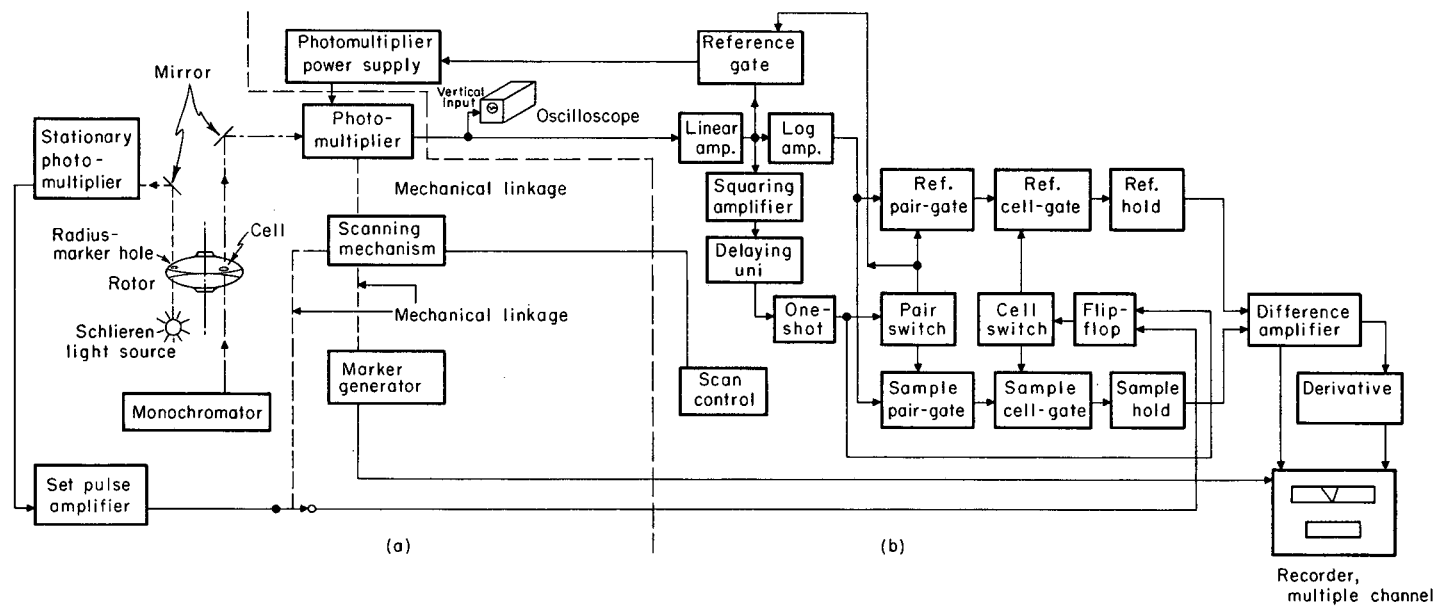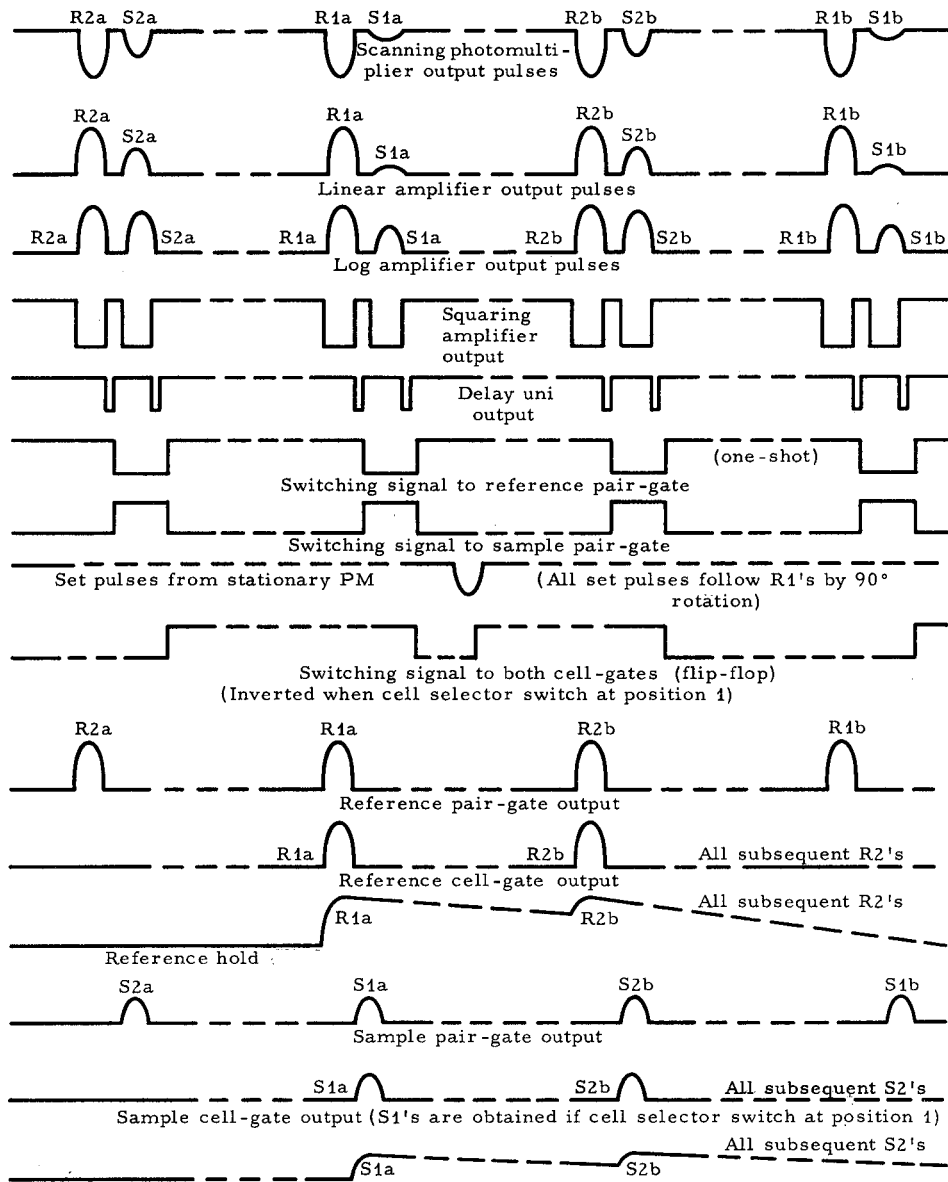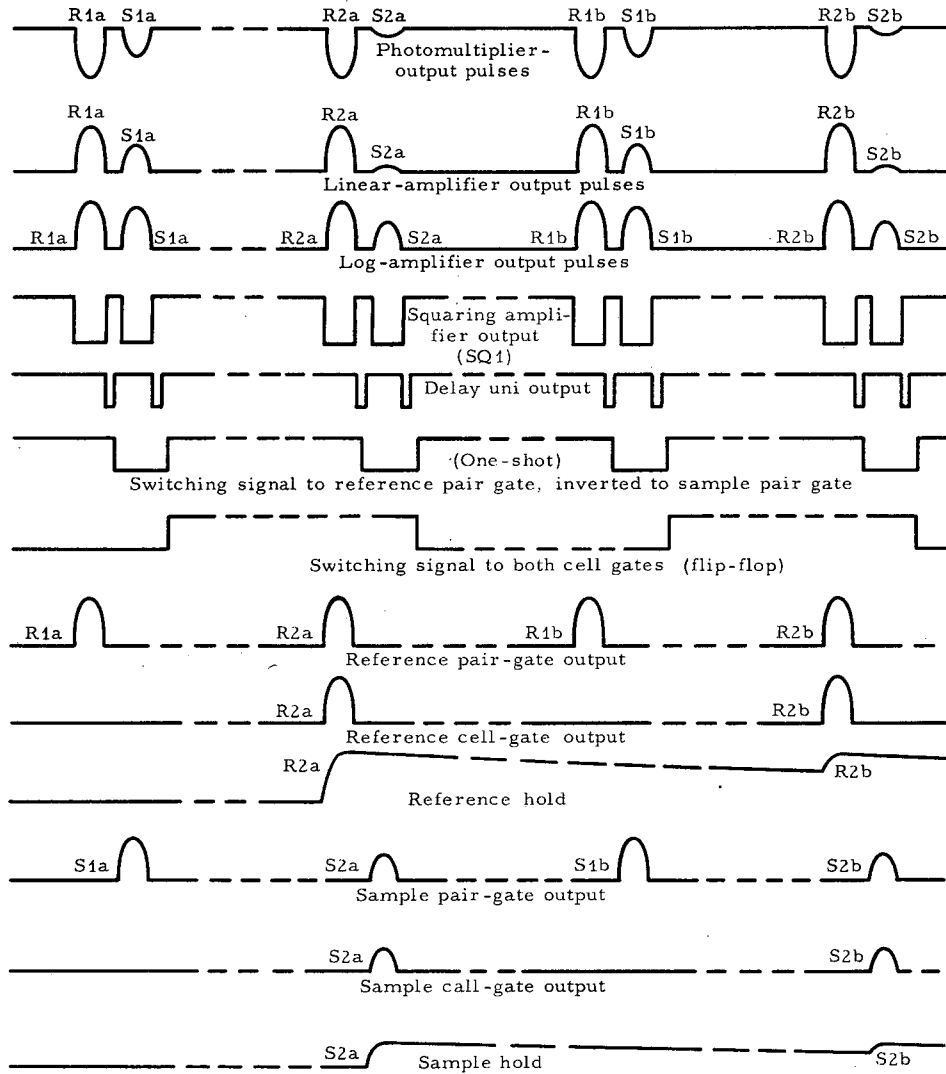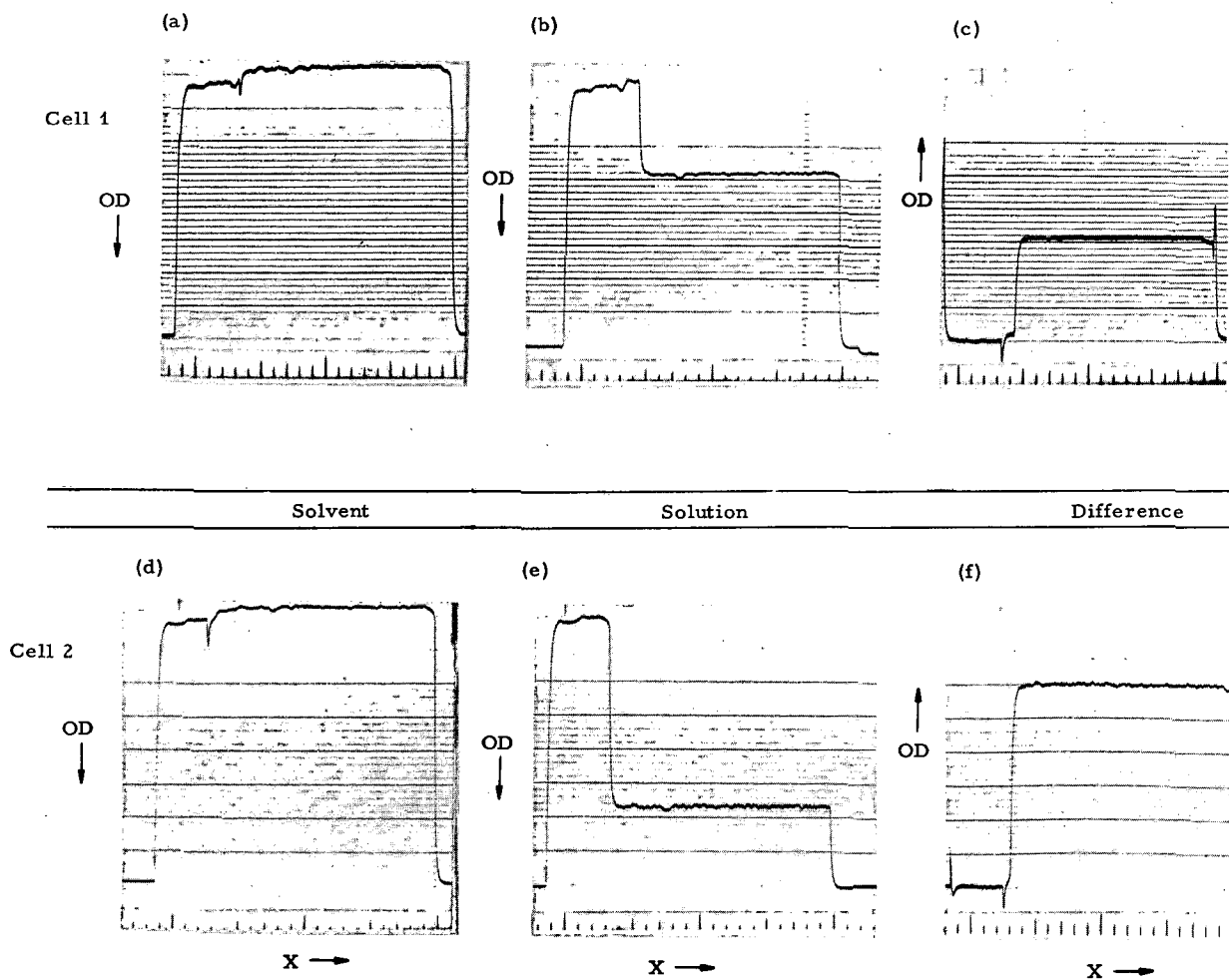
Fig. 1

Fig. 2

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R2a S2a   R1a S1a   R2b S2b   R1b S1b

Scanning photomulti-
plier output pulses

R2a   R1a   R2b   R1b
S2a   S1a   S2b   S1b

Linear amplifier output pulses

R2a   S2a   R1a   S1a   R2b   S2b   R1b   S1b

Log amplifier output pulses

Squaring
amplifier
output

Delay uni
output

(one-shot)

Switching signal to reference pair-gate

Switching signal to sample pair-gate

Set pulses from stationary PM   (All set pulses follow R1's by 90°
rotation)

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

R2a   R1a   R2b   R1b

Reference pair-gate output

R1a   R2b   All subsequent R2's

Reference cell-gate output

R1a   R2b   All subsequent R2's

Reference hold

S2a   S1a   S2b   S1b

Sample pair-gate output

S1a   S2b   All subsequent S2's

Sample cell-gate output (S1's are obtained if cell selector switch at position 1)

All subsequent S2's

S1a   S2b

Fig. 3

Fig. 4

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(a)   (b)   (c)

Cell 1

OD ↓   OD ↓   OD ↑

Solvent   Solution   Difference

(d)   (e)   (f)

Cell 2

OD ↓   OD ↓   OD ↑

x →   x →   x →

ZN-5459

Fig. 5

Derivative

MUB-7320

Fig. 6