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**A COMPARISON FLUORIMETER OF
HIGH SENSITIVITY**

by

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I. INTRODUCTION

Fluorometric measuring instruments have been valuable tools, for research workers and in routine analysis, for a number of years. Kauffman describes fluorescent techniques which are essentially those still followed (1). In 1933 Woodrow and Schmidt reported quantitative identification of fluorescence bands in fats (2). Visual observations of fluorescence have been used to identify many substances over a long period of time. Commercial photofluorometers are available and are regularly used. These instruments often use a photoemissive cell and a simple circuit to determine the intensity of fluorescence. Filters are commonly utilized to limit transmission to desired wave lengths, and a mercury vapor lamp is often used as the source of exciting radiation. In rather similar instruments, designed for color comparison measurements, a photovoltaic cell is frequently used.

According to Lothian, it is often necessary and sometimes desirable, even when unnecessary, to use dilute solutions of the fluorescing material (3). In many cases the intensity of the fluorescence produced may be exceedingly low. It seems very likely that a fluorimeter of greater sensitivity than those using photoemissive cells will be a valuable addition to existing types of instruments.

Since secondary emission multiplier phototubes have a luminous sensitivity which may be more than 100,000 times greater than the sensitivity of vacuum photoemissive cells, it seems probable that the use of a multiplier phototube in an appropriate circuit would improve the sensitivity of a fluorimeter.

To design a fluorimeter using a multiplier phototube, the characteristics of the tube should be considered, and best possible use of the tube should modify or eliminate as many undesirable features as possible. Dieke states that two individual tubes even of the same type may have very different properties (4). He reports differences in sensitivity by more than a factor of ten, and comparable differences in dark current. Saunderson suggests that dark currents may be cancelled out by a bucking potential (5). The response of the tubes is linear with respect to the light intensity incident on their photosurfaces, according to Engstrom (6). Current limitations are placed on the tubes to avoid serious fatigue effects. Another characteristic which must be considered is that the current from the multiplier tube will change greatly as the dynode supply potential varies.

A pair of multiplier phototubes operated as two sides of a bridge circuit, will have dark currents that tend to cancel, and the bridge may be balanced at a constant applied potential. Experience gained, while assisting Dr. P. H. Carr in the design of apparatus using this type of circuit, indicates that if the

potentials applied to the tubes vary, the bridge becomes unbalanced. Careful matching of tubes tends to minimize the unbalance, but it seems rather unlikely that two tubes which have the same characteristic curves can be readily selected. Any possible matching would, in time, probably prove unsuccessful, due to the large difference in fatigue rates which occurs between individual tubes. These difficulties lead to the consideration of circuits which involve only one multiplier phototube.

In some fluorometers using photoemissive cells a reading is obtained from a standard sample, then a comparison reading is made with the unknown solution. Obviously, this technique is only possible if the illumination and applied potential remain constant during the time required to take both readings. The required constancy is often attempted by the use of batteries. Unfortunately, the recently available, low current, high potential batteries which are being used with multiplier tubes are expensive and short lived. If an instrument using a single multiplier tube, can rapidly and alternately respond to the fluorescence from an unknown and a standard sample, some of these difficulties may be obviated.

If the accompanying circuit responds to the differences in currents caused by the standard and unknown solutions, dark current effects will be virtually eliminated, since dark current changes usually occur slowly. Kessler and Wolfe state that one component of dark current fluctuation has a period in the order of a second; other changes are slow drift (7).

The sensitivity of the multiplier tube will probably affect the magnitude of comparisons made on an unbalanced bridge circuit. If, however, a comparison between a standard and an unknown solution is made by balancing a bridge circuit, the change in sensitivity of a single multiplier tube, which is used in both sides of the bridge, should not unbalance the circuit.

After a consideration of some of these design essentials, research was begun to develop a sensitive comparison fluorimeter. It was deemed important to use a single multiplier tube, if at all possible, and to use that tube in a bridge circuit that would be balanced when taking readings of fluorescence intensity.

II. EXPERIMENTAL

A. The Apparatus

1. The electrical system

To make the determination of readings an easy task, it seemed desirable to indicate the relative intensity of fluorescence on a meter. This instrument could be either a galvanometer or a microammeter. Most sensitive galvanometers have a resistance which is less than 10,000 ohms. A secondary emission multiplier is a high impedance source, since, under constant low intensity illumination, a relatively large change in anode to final dynode potential will produce only a small change in anode current (8). Consequently, a direct connection between multiplier tube and galvanometer will not give maximum energy transfer.

To obtain better impedance matching, a cathode follower circuit was tested. This seemed a logical choice since the input resistance of a cathode follower is high and the output resistance may, by proper choice of vacuum tubes, be made as low as or lower than that of many sensitive galvanometers. In addition, the cathode follower is readily operated as a linear amplifier having negligible distortion and will not affect the preceding circuit components if the grid of the cathode follower

is not driven positive with respect to the cathode. The degenerative feedback inherent in the ordinary cathode follower results in circuit stability and minimizes variations in tubes and in their voltage supply.

The first cathode followers tested had a conventional capacitor input to the grid, and would respond only to the changes in the multiplier tube output which occurred as the tube was switched from one sample to another. Improvement in response was noted when a direct connection from multiplier anode to grid of the cathode follower was made. Figure 1 shows the basic circuit used.

A 6SN7-GT was the first tube tested in the cathode follower circuit. At the time, the multiplier tube was being operated on alternating current and the cathode follower's plate supply potential was taken from this source, as shown in figure 2. During the half cycle it was conducting, the current through the series voltage dividers was larger than during the triode's nonconducting half cycle. This caused a cyclic variation of potential between multiplier and cathode follower and an objectionable grid bias change during conduction. A brief trial of a 6SL7-GT, which caused the same difficulty in lesser degree, was made.

Simple mechanical switching at speeds up to thirty cycles per second is feasible according to Hoyt, who reports on the operation of a set of switches at fifteen cycles per second (9).

When non-precision workmanship and extreme simplicity were incorporated in a switch, difficulties were encountered at thirty cycles per second. Ordinary carbon brushes have a tendency to bounce if the commutator becomes rough or is slightly eccentric. If this does not lead to irregular breaking of contact, it will at least cause a resistance change in the brush contact. This was a serious objection to the first carbon brushes used in the circuit shown in figure 1, since erratic meter fluctuations occurred as the brush contact resistance changed. The brush commutator and the optical shutter were mounted on an extension shaft which had no auxiliary bearings.

A type of brush, which operates quite successfully at low currents and under adverse conditions, is made of a bundle of spring wires which make tangential contact with a commutator. Operation with only very infrequent small meter deflections was achieved with this type of brush operating on the same commutator and with the same circuit shown in figure 1.

Electronic switching and a combination of electronic with mechanical switching were tried and successfully operated with circuits indicated in figures 3 and 4. The addition of the vacuum tubes to the mechanical switching prevents brush contact fluctuations from affecting the meter. This occurs since the brush contact resistance change is usually one of only a few ohms, and this change is a negligible fraction of the total resistance included in the grid circuit. Switching in a high resistance circuit does not eliminate fluctuations due to contact

potential changes.

An Eccles-Jordan circuit was found superior to a "one-shot" or a "free-running" multivibrator for the electronic switching since the former operates in either direction only when a trigger pulse initiates the switching. Naturally, both multivibrators were synchronized with the sixty cycle supply, and operated at thirty cycles per second. The trigger for the Eccles-Jordan circuit was obtained from the sixty cycle supply by squaring, differentiating, and removing the negative pulse with a half-wave rectifier. The remaining positive pulse was applied to the grids of the Eccles-Jordan circuit. Synchronization of the optical and mechanical switching was excellent, and a variable resistor in a phase shifting circuit made this adjustment quite easy.

The vacuum tubes added between the cathode resistors and the cathode follower are not common to both circuits, and differences in tubes will cause changes in the meter reading. Plate potential supply changes may, for instance, cause meter fluctuations. The potential difference which exists across the tubes is larger than that across a mechanical switch, and this potential does not contribute to meter deflection. This useless potential drop was approximately one half of the potential difference developed across the cathode resistors, and represented a loss of energy which was negligible with a mechanical switch.

The switching system finally adopted is shown in figure 5a.

It uses two cathode followers with their grids interconnected and with the plates of the tubes connected to the secondary of a transformer. A grounded variable center-tap is provided to adjust the potential applied to each plate. Changing the plate potentials with respect to ground makes it possible to balance the circuit, if the tubes have different characteristics, or if a difference in grid signal exists during the switching cycle. With this circuit, the switching occurs at sixty cycles per second, since one of the tubes conducts during each half cycle of the applied transformer potential.

The electronic switching method finally adopted for the cathode follower amplifiers seems superior to a mechanical system using brushes which would eventually wear and require replacement. The system using an Eccles-Jordan circuit is much more complex than the simple plate switching circuit. For simplicity and durability, the plate switching cathode follower circuit seems to be the most advantageous of the methods tested.

Final choice of a 6AK5 tube, operated as a triode, and at slightly higher than normal plate potential, was largely made because successful operation was achieved at zero grid bias in the dual cathode follower circuit. The large grid potential range and the miniature size also seem worthwhile features. Satisfactory performance was also obtained from a 6V6-GT, but its miniature counterpart, the 6AQ5, was not available. Either of these tubes should perform well, and give a greater current gain than the 6AK5 cathode follower. After selection of a tube, circuit details were considered.

Two circuits which respond to the difference between two input signals are described by Gray and MacRae (10). A third is suggested, but apparently was not thoroughly tested at the time of publication of their report. These circuits are reported linear to two tenths of one percent of their output voltage over a relatively large range. In each case, both the input and output of the circuit are at high impedance.

An analysis of the dual cathode follower circuit shows that the meter current is, within reasonable limits, directly proportional to the difference of the grid signals. Figure 5a shows the cathode follower circuit, while figure 5b is an equivalent circuit with the condenser neglected. It should be recognized that μ_1 , μ_2 , r_1 and r_2 are functions of time, since the plate potential and current are constantly changing.

Since e_1 and e_2 , the grid signal potentials, are square waves, which in general are of different amplitudes, each may be expressed as an infinite sum of sine and cosine terms, as is customarily done in Fourier analysis.

$$e_1 = a_0 + \sum_{n=1}^{\infty} (a_n \cos nx + b_n \sin nx)$$

$$e_2 = c_0 + \sum_{n=1}^{\infty} (c_n \cos nx + d_n \sin nx)$$

This makes possible a conventional alternating current analysis which could be performed for each term of e_1 and e_2 . For a single component, from a consideration of figure 5b, we may write the following equations:

$$\mu_1(e_1 - i_1 R + i_3 R) - i_1(r_1 + R) + i_3 R + E_1 \sin \omega t = 0$$

$$\mu_2(e_2 - i_2 R - i_3 R) - i_2(r_2 + R) - i_3 R + E_2 \sin \omega t = 0$$

$$i_3(2R + R_1) + i_2 R - i_1 R = 0$$

These equations may be solved for i_3 , the meter current. The result is:

$$i_3 = \frac{(R)(E_2 \sin \omega t + \mu_2 e_2)(\mu_1 R + r_1 + R) - R(E_1 \sin \omega t + \mu_1 e_1)(\mu_2 R + r_2 + R)}{-(\mu_1 R + r_1 + R)(\mu_2 R + r_2 + R)(2R + R_1) + R(\mu_2 R + R)(\mu_1 R + r_1 + R) + R(\mu_2 R + r_2 + R)(\mu_1 R + R)}$$

The total current would be the sum of an infinite number of similar expressions.

The cathode followers alternate in their operation, each conducting for approximately one half cycle, and the mathematical expressions hold only for the conducting part of each cycle. At the beginning and end of each half cycle, when the plate potential is very low, the amplification factors are very small. They change quickly as rapid plate potential changes occur, but are relatively constant during the times of higher plate potentials. The effect of the changing amplification factor is to modify the current component represented by μe . If μ were a constant during a half cycle, these components, summed over all frequencies, would be a square wave, but the changing amplification factor will effectively degenerate this component to a form closely resembling a sinusoidal wave. With the capacitance removed from the circuit, and with grid signals which do not cut off the tubes for an appreciable part of the cycle, observation on an oscilloscope shows a sinusoidal variation of the

potential applied to the meter. As either grid signal becomes increasingly negative, a larger portion of the corresponding half cycle becomes nonconducting, and the wave form resembles portions of sinusoidal half waves separated by straight lines.

Since both cathode followers are the same type of vacuum tube, and since a higher plate potential is applied to the tube with a normally lower amplification factor, this factor during corresponding parts of each half cycle must have approximately the same value. If this is the case, assuming also that the plate resistances are similarly related, and that an average value for each can be selected, it is possible that the average meter current may be expressed as:

$$i_3 = a(e_2 - e_1)$$

The accuracy of this relation was checked by maintaining one cathode follower grid at ground potential while a battery and a potentiometer applied an increasingly negative potential to the other grid. A voltmeter indicated the potential difference of the grids, and the microammeter, normally used in the circuit, indicated the current. The circuit was initially balanced at zero grid potential.

Table 1

RELATIONSHIP BETWEEN GRID POTENTIAL AND METER CURRENT

Applied grid potential	Meter reading	
	Tubes normal	Tubes reversed
0.0 volts	0.0 u amperes	0.0 u amperes
-0.1	2.2	2.6
-0.2	4.2	4.6
-0.3	6.0	6.6
-0.4	7.6	8.4
-0.5	9.4	10.5
-0.6	11.0	12.0
-0.7	12.6	13.9
-0.8	14.2	15.6
-0.9	15.8	17.3
-1.0	17.4	19.1
-1.1	18.9	

A curve plotted from these data is shown in figure 6. This clearly shows the nearly linear relationship between the difference of grid potentials and the meter current, and confirms the simplification of the meter current expression. A measurement of the nonlinearity of the circuit was made by applying the same negative potential to both grids. Again the grid potential with respect to ground was measured with a voltmeter, and the meter current was determined by using the twenty microampere meter normally in the circuit.

A plot of the data on cathode follower nonlinearity appears in figure 7. When the tubes were interchanged, the meter deflection was in the opposite direction, indicating that the

Table 2

MEASUREMENT OF CATHODE FOLLOWER NONLINEARITY

Grid Potential	Meter Deflection
----------------	------------------

0.0 volts	0.0 u amperes
-0.3	0.3
-0.6	0.7
-0.9	0.9
-1.2	1.2
-1.5	1.5
-1.8	1.7
-2.1	1.9
-2.4	2.1
-2.7	2.3
-3.0	2.4
-4.0	2.8
-5.0	3.1
-6.0	3.4
-7.0	3.8
-8.0	3.9
-9.0	4.1
-10.0	4.2
-12.0	4.4
-15.0	4.5
-18.0	4.5
-21.0	4.5

nonlinearity is largely a function of tube characteristics. In an ideal circuit the meter deflection would remain zero as the grid potentials of the tubes were simultaneously changed.

By using data from both tables 1 and 2, an idea of the percentage deviation from linearity may be obtained. At -0.3 volts grid potential, the meter current is 6.0 microamperes, while the deviation from linearity is 0.3 microamperes. The current ratios indicate that the nonlinearity is about five per cent of the normal current. Similarly, at -0.9 volts grid potential, the deviation is 0.9 microamperes, and the meter current is 15.8 microamperes. Their ratio indicates that the nonlinearity is about 5.7 per cent. At increasingly negative grid potentials this error becomes smaller.

The current gain of the cathode follower circuit may also be obtained from table 1. The current in a one megohm grid resistor required to obtain a potential difference of 0.1 volts, is 0.1 microamperes. This value of grid potential change produces a 2.2 microampere current in the meter. A current gain of 22 is indicated. The least value of current gain indicated in this table is more than 17. The sensitivity of the cathode follower stage can also be evaluated from the data in table 1. The smallest scale division on the galvanometer used with the equipment was one-quarter microampere. A deflection of one half-scale division was readily discernible, and if a grid potential

change of 0.1 volt produced a current of 2.2 microamperes, a grid potential change of only $1/176$ volts would be required to produce a current of $1/8$ microampere. This grid potential change would be caused by a current of $1/176$ microamperes in the one megohm grid resistor. In round numbers, the cathode follower circuit enables a current of 10^{-8} amperes to be readily detected on a comparatively insensitive meter.

When less sensitivity is desired, the grid resistor may be changed to a smaller value by a switch. Changing to a smaller grid bias resistor also extends the range of the instrument, since a greater current must exist in the resistor to reach the grid cutoff potential. The cathode followers are cut off when the grids are thirty volts negative with respect to the ground of the circuit. This means that on the most sensitive range, a thirty microampere change in multiplier current is possible; while on the others, the current changes are sixty and one hundred twenty microamperes.

A simple half wave power supply is used for the secondary emission multiplier. The filter is of two condensers, separated by a resistor which also functions as the regulating resistor in the voltage stabilizing system. Six OD3 tubes and one OC3 tube are in series across the one thousand volt supply for potential stability. The voltage divider for the dynode potentials is at the multiplier socket to minimize the number of leads. The positive side of the power supply is grounded to keep the cathode follower circuit and the meter near ground

potential, while the negative lead to the multiplier tube is broken by a microswitch, when the cover housing this tube is opened. The switch was modified to open two or three times farther than normal. This change has eliminated any arcing when the one thousand volt potential is across the switch.

2. The optical system

The exciting source of illumination for the fluorimeter is a General Electric H-4 mercury vapor lamp. According to Radley and Grant, mercury vapor lamps are commonly used to excite fluorescence (11). A fifteen centimeter focal length lens is that distance away from the ultraviolet source and immediately in front of two apertures through which the light passes toward the sample holder. The lamp and the motor which drives the rotating shutter are in the central compartment. The shutter rotates between a double wall, each part of which has two apertures. The shutter permits light to pass through one pair of holes and illuminate a single cuvette for a quarter cycle of motor operation. During the next quarter cycle, the other cuvette is illuminated. A mask immediately in front of the cuvettes prevents illumination of the sides, top and bottom of these cells. Since the front and back of the cuvettes do fluoresce and scatter light, a hinged cover may be dropped over their top. This cover has two holes which expose the solution, but not the edges of the cuvettes. When in use, the secondary emission multiplier is above the cuvettes, and some fluorescence

of the solution in the cells will strike the multiplier cathode.

A filter holder is attached to the right side of the wall between compartments, and permits ultra violet transmitting filters to be readily changed whenever the cover of the right compartment is open. The filters which will usually be ultra violet absorbing are mounted in a similar filter holder on the multiplier housing. Both filter holders are spring loaded to accommodate various thicknesses of standard two inch by two inch filters. The beam nearer the operator, may be controlled by a movable slit mounted on the lamp side of the compartment wall. This slit is immediately behind the lens, and is adjusted by rotating a wheel engraved with one hundred equal divisions along its circumference, and a vernier permits direct reading of tenths of divisions. Twenty turns of the wheel move the slit completely across the one half inch aperture. A diagram of the optical system is shown in figure 8.

An auxiliary system which improves the method of taking null readings is included in the instrument. It consists of a standard photovoltaic cell and a microammeter. They are connected through a press-to-contact switch. This type of switch is used since the current produced by the photocell when it is exposed to room light is greater than the range of the meter. The photocell is installed directly behind the cuvette which will usually contain the solution of unknown concentration. In this position the beam passing through the cuvette strikes

the photovoltaic cell. Any change in the beam due to slit width adjustment will cause a change in the current existing in the cell when the switch is closed.

Since the photocell responds to the total illumination passing through the slit, it eliminates the effect of slit errors. If the slit jaws do not remain parallel as their position is changed, or if the spring return does not maintain a constant tension, the micrometer reading on the slit adjustment may not be linearly related to the light striking the cuvette. The photovoltaic system, on the other hand, is independent of slit errors, if its surface has a uniform response. A simple check was made by projecting a small light beam on the cell while it was connected to a meter. When the cell was moved, the light spot shifted over the photosurface. No appreciable change in meter reading was discernible.

A photovoltaic cell must be attached only to a low resistance meter, if the current is to be proportional to the incident light. The curves shown in many good electronics texts, such as Ryder's, show that absolute linearity of current with respect to incident light flux is achieved only with a short circuit (12). However, these graphs also show that the deviation from linearity is very slight if a low resistance meter is used. Since the low intensity portion of the graphs with any resistance is comparatively linear, and the illumination intensity used is very low in this apparatus, the response of the photocell may be

regarded as directly proportional to the light striking its surface.

3. The mechanical arrangement

The fluorimeter is housed in a black wrinkle-finished box, twenty-five inches long, eleven and a half inches wide, and eleven inches high. A one quarter inch sheet of aluminum forms a rigid mounting base for the motor and transformers, and prevents distortion of the box. The housing is divided into three compartments. The central space houses the motor and mercury vapor lamp, and has a number of three-quarter inch ventilating holes. The motor drives a circulating fan as well as the optical shutter, and is carried on rubber shock mountings to minimize vibration.

The smallest enclosure is on the right and has a hinged cover containing the secondary emission multiplier tube. The lower portion of this space contains the cuvette holders, the filter holder, and the photovoltaic cell. The left compartment has a hinged cover, which may be entirely removed by pulling the hinge pin. The cover is provided to protect the instruments and switches which are mounted on a bakelite panel just below the cover. One microammeter indicates the degree of cathode follower circuit unbalance; the other, operating only when a switch is depressed, shows the current produced by the

photovoltaic cell. The power switch, the grid resistor switch, and two balancing potentiometers are also on this panel. Electronic circuits, including the lamp transformer, the high voltage supply, and the cathode follower circuit are in the space below the instrument panel.

B. Operation of the Instrument

In using the fluorimeter, the left cover is opened or removed and the switch turned on. The source of power should be the usual 115 volt, sixty cycle supply. Since the mercury vapor lamp reaches full intensity rather slowly, at least five minutes should elapse before any attempt to take readings is made. If the waiting period is ten or fifteen minutes, temperature equilibrium of all parts will be approached, and readings will probably be slightly more reliable. A single switch starts the motor, turns on the lamp, and applies potential to the cathode follower and multiplier tubes.

After the warmup period has elapsed, cuvettes containing a standard solution are inserted, the right cover closed and the twenty microampere meter brought to zero, by the two balancing potentiometers immediately below it. One of these potentiometers is a vernier control, the ratio between the two being approximately one hundred to one. Experience will quickly indicate, to the operator, settings of the potentiometers which will prevent excessive current from existing in the meter, or an auxiliary meter of less sensitivity may be connected to the binding posts on the instrument panel. A toggle switch thrown toward the mounted meter connects it to the cathodes of the 6AK5's; in the opposite direction, the cathodes are connected to the binding posts. When using an insensitive meter, it should show a near zero potential difference of the cathodes

before the operator transfers to the panel meter.

Next, a drop of the solution under test is added to the cuvette nearer the operator, or this cuvette is removed, filled with the solution under test, and replaced in its holder. The compartment cover is closed, and the meter should indicate an unbalance of the circuit. Balance is restored by regulating the width of the variable slit. A reading which is proportional to the slit width may be taken from either the meter in the photovoltaic cell circuit or directly from the vernier of the slit control. Since the cathode follower circuit is restored to its original balanced condition, any non-linearity in its response is eliminated, and only the linearity of the multiplier tube and the slit mechanism or the photovoltaic circuit can affect the final reading.

In an alternate method, which is less recommended, the degree of meter unbalance is assumed proportional to the fluorescence of the solution being tested. Obviously, the non-linearity of the cathode follower circuit, and lamp intensity fluctuations will affect the result to a greater degree than in the previously described method. When using this system, irregularities of the slit system or photovoltaic circuit have no effect. Unless an auxiliary meter of greater range is used, this system is limited to a small variation of fluorescence in the samples. In the method which restores the cathode follower to a balanced condition after each sample is added, a meter of great sensitivity may be used. This does not limit the range of fluorescent samples,

since the circuit continues to respond until it is cut off by relatively large currents in the multiplier tube. These currents approach the values which should not be exceeded unless serious multiplier fatigue is tolerated.

C. Results

A fluorimeter was designed and constructed, using a secondary emission multiplier tube as the fluorescent sensitive element, and with a cathode follower circuit for impedance matching and to switch the multiplier tube output from one terminal of a meter to the other in synchronism with the optical switching. Tests of the fluorimeter were made to determine its response to a standard fluorescent material. In testing the response of the machine a solution of quinine sulphate in distilled water was prepared by Professor Gladys Everson of the Foods and Nutrition Department. One drop of this solution in thirty cubic centimeters of distilled water produced a dial deflection of forty divisions on a commercial photofluorometer in her laboratory.


The quinine sulphate solution was diluted to one part in one hundred parts of distilled water, and the resulting dilute solution added drop by drop to six milliliters of distilled water in a cuvette. A Corning number 587, 5.05 mm filter was used to absorb visible light, and a Corning number 306, 1.25 mm filter prevented scattered ultraviolet light from reaching the

multiplier tube. Readings were taken on the meter indicating the photovoltaic cell current as the width of the light beam was varied to rebalance the cathode follower circuit. Three trials were made in a check on reproducibility. A tabulation of the results follows. The current values shown are the difference between the original current of the photovoltaic cell with distilled water in both cuvettes, and the current produced as the circuit was rebalanced.

Table 3
FLUORIMETER RESPONSE

Drops of quinine sulphate solution	Difference in photovoltaic current		
	Trial 1	Trial 2	Trial 3
0	0.0 uamp.	0.0 uamp.	0.0 uamp.
2	0.0	0.0	0.0
4	0.0	0.0	0.3
6	0.8	1.0	0.5
8	1.3	2.4	1.6
10	3.3	3.7	2.5
12	4.8	4.7	4.3
14	6.0	6.0	4.9
16	7.0	7.0	6.6
18	8.0	8.1	7.9
20	8.9	8.9	9.0
22	9.7	9.7	10.1
24	10.1	10.6	10.8
26	10.7	11.0	11.5
28	11.3	11.6	12.0
30	11.8	--	12.4

A curve showing the relationship between the fluorimeter response and the quinine sulphate concentration is shown in figure 9. Only data taken in trial 2 of the above table are

plotted on the graph. The graph illustrates the nearly linear relationship of the fluorimeter reading with respect to concentration. The deviation from linearity may be largely accounted for by the technique used in adding the quinine sulphate solution. Since thirty drops from the pipette used to add solution are equal to one milliliter, the first drops were added to approximately 5.5 milliliters of solution; the last to 6.5 milliliters. If the lower portion of the graph is extrapolated as a straight line, it would indicate that a reading of 13 microamperes should have been recorded instead of the 11 microamperes actually observed. These have the same ratio as the volume of solution at the beginning and end of the trial, and show that a graph corrected for the volume change would closely approach a straight line. For the higher concentrations of quinine sulphate, the response does not increase uniformly, as the cathode follower circuit approaches cutoff. 

An indication of sensitivity may be gained by a comparison with the commercial photofluorometer. One drop of quinine sulphate solution in thirty cubic centimeters of distilled water produced a deflection of forty divisions on a meter with a total of one hundred divisions. In the fluorimeter built during the course of this investigation, one drop of the same quinine sulphate solution in six hundred milliliters of distilled water produced a six microampere current in a twenty microampere meter, and produced a deflection larger than full scale with a portable Leeds and Northrop galvanometer. The galvanometer used was a

small 250 ohm, 0.25 microamperes per scale division instrument and not a delicate, high sensitivity meter.

The zero response of the instrument to extremely low concentrations is apparently the result of change in the solution. A transient response is produced, but this rapidly and steadily drops to zero, indicating that a change is taking place in the fluorescence emission. The temperature rise of the solution, when checked, was 9 degrees Centigrade, but the transient response disappears too rapidly for any appreciable temperature change to occur. No explanation for this effect is offered.

Neither "drops" nor "dial divisions" are satisfactory scientific terms. The size of drops will vary with the circumference of the pipette tip, and to a lesser degree, with the amount of liquid in the pipette. The commercial machine was not readily available for a more exacting comparison than dial divisions.

To evaluate the range of the instrument, a solution of 1 ml of the previously mentioned quinine sulphate solution was added to 10 ml of distilled water, and this solution was dropped into one of the cuvettes. Table 4 gives the result of this test.

Table 4
FLUORIMETER RANGE

Drops of quinine sulphate solution	Difference in photovoltaic cell current
0	0.0 microamperes
1	3.6
2	7.0
3	10.0
4	11.4
5	12.0
6	12.7
7	12.9

The range of the instrument is graphically indicated in figure 10. The initial instrument response is nearly linear, and the range is excellent. The concentration when 6 drops of the solution was added to the cuvette is three times as great as the concentration tested on the commercial photofluorometer.

The completed fluorimeter is easy to operate. All switches, potentiometer knobs, and slit adjustment are accessible, and, it is hoped, conveniently located. The cuvettes are exposed by opening the right cover and may be readily changed. Rotation of potentiometer controls in a clockwise direction produces a greater meter deflection; when a toggle switch is thrown toward a meter that meter is in the circuit. Each electrical unit such as the high voltage power supply, or the cathode follower circuit

is assembled on a separate chassis, and that chassis is fastened to the base plate with machine screws. Interconnecting leads are cabled, and are long enough so the instrument panel may be removed while the fluorimeter continues to function. These features should make the instrument easy to service, when that is required.

III. DISCUSSION

The purpose of this investigation was not only to design and construct a fluorimeter, but to partially evaluate its performance and construction. It is recognized that the most adequate test of the instrument can probably be best performed during regular use in a laboratory, and it is hoped that such use can be arranged. In criticizing the construction, it should be recognized that to some extent, readily available components were used rather than ordering, and waiting for, parts which would be slightly better. For example, the synchronous motor used is certainly larger than is required. If a physically smaller motor had been available, the light shutter could have been smaller, and the entire housing might have been more compact.

Electrometer tubes or small current tubes such as those used by Nielsen (13) could have been employed to achieve a higher amplification than is the case with the cathode followers used. While the voltage amplification of a cathode follower stage is less than unity, and the usual analysis is made entirely in terms of voltage gain, the current amplification of a cathode follower may easily be one hundred or more. If the power amplification is defined as the product of the voltage gain and the current amplification, it will approach the current amplification in magnitude since the voltage gain is often nearly unity. Power is required to produce a meter deflection during any finite time

interval, and the cathode follower is an excellent device for producing the desirable power gain, and having the correct impedance matching characteristics.

In many types of equipment which use a light source to excite some form of photocell, and auxiliary, identical photocell which responds directly to light variations is often used in a bridge circuit. Claims are sometimes made that this type of circuit eliminates the effect of light intensity fluctuations. This is not always the case, however, unless a zero input signal to the measuring circuit is maintained. If a difference in the currents of the photocells exists, a change in lamp intensity may modify the difference, since each current will probably be increased or decreased by the same percentage.

This effect is probably one of the causes of slight fluctuations in the fluorimeter's balance indicating meter, because the lamp autotransformer was operated directly from a line with severe potential fluctuations. When the lamp was removed, and the fluorimeter turned on, these fluctuations were negligible. Actually one cuvette is always illuminated while the sixty cycle current in the lamp is in one direction, and the fluorescence of the other sample is excited by the lamp while current is in the opposite direction. Statistical variations in lamp intensity may occur as one lamp terminal is regularly negative, for example, and the variations during the

other half cycle may be different. This too, would contribute to fluctuations of the meter.

The phase relationship between the optical shutter and the electronic switching was adjusted by using an electronic switch and an oscilloscope. No hunting of the motor was observed while making this adjustment. However, the method was not accurate enough to detect small variations in the optical-electronic switching, and a very slight hunting of the motor would also cause meter fluctuations.

The meter fluctuations are semi-regular, and it is not difficult to adjust the median position of the needle to zero. The regularity of the graph in figure 10 is evidence that this may be successfully accomplished. When the largest grid resistor is in the circuit, these fluctuations are approximately plus and minus one half scale division, while a deflection of six scale divisions is produced by a dilution of 1 drop of the quinine sulphate solution used, in 600 milliliters of distilled water. This indicates that the possible error due to meter fluctuations is six or seven percent of this dilution. When using the instrument with either of the smaller grid resistors, the fluctuations are less noticeable, and can only contribute slightly to possible errors in the results.

It should be realized that the recommended method of taking readings makes it possible to achieve great range and yet retain considerable sensitivity. A sample may cause the meter needle

to go off scale, but a reading may still be taken by using the adjustable slit to balance the circuit.

IV. SUMMARY

A comparison fluorimeter, utilizing a secondary emission multiplier as the sensitive element, has been built. It consists of an optical system, an electronic switching system and power supply, and a housing for the entire assembly.

A single secondary emission multiplier was used to alternately determine the intensity of the samples to be compared.

A simple dual cathode follower system was designed to switch the multiplier tube, and to simultaneously provide impedance matching between the multiplier tube and the meter being used in the circuit.

Tests on the cathode follower circuit show that it is a highly stable, comparatively linear system for detecting potential differences up to thirty volts.

The entire unit was housed in an aluminum box designed to have all necessary controls readily accessible.

The fluorimeter was found to be considerably more sensitive than existing devices using older types of phototubes.

The fluorimeter has considerable range, and when used as recommended, was found to be nearly linear in its response to variations in concentration.

Many of the design features are readily adaptable to similar devices, such as turbidity meters.

V. CONCLUSIONS

As a result of this investigation, the following conclusions have been reached:

1. It is possible to build a comparison fluorimeter using a secondary emission multiplier tube as the fluorescence sensitive element.
2. A single secondary emission multiplier tube may be readily switched by a simple electronic system from one side of a bridge circuit to another.
3. Synchronization of the electronic switching with optical switching of the exciting lamp illumination may be readily achieved and maintained.
4. The entire instrument may be portable and may be operated from a standard sixty cycle alternating current supply.
5. Comparatively large range may be combined with a high degree of sensitivity in the fluorimeter.
6. Linearity of response to solution concentration may be closely approached.
7. Voltage stabilization of the secondary emission multiplier's power supply improves performance.
8. Voltage stabilization of the potential supplying current to the lamp exciting the fluorescence, is highly desirable. The lack of voltage stabilization for this lamp

is probably the major limitation on accuracy, at very low concentrations, and on greater sensitivity.

9. A cathode follower circuit is an efficient system for transferring energy from a secondary emission multiplier to a much lower impedance device, such as a meter.

VI. ACKNOWLEDGEMENTS

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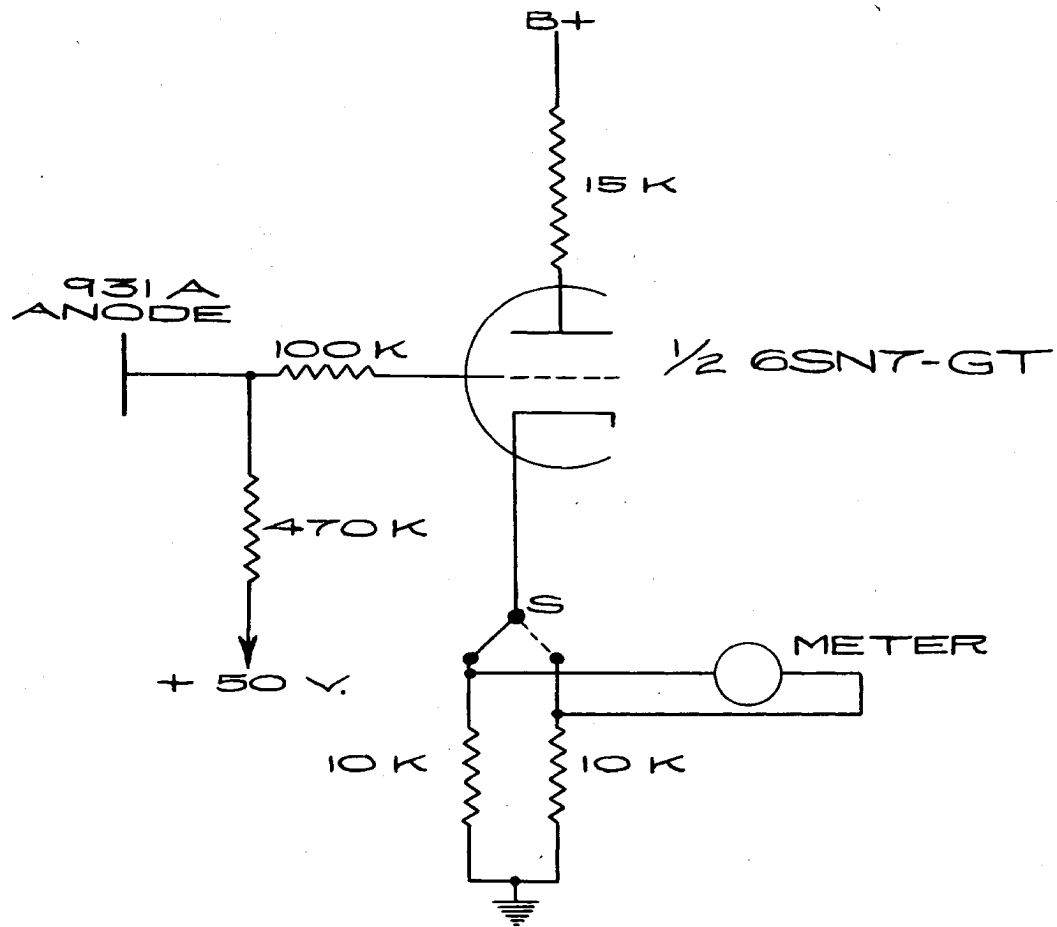


FIG. 1 BASIC CATHODE FOLLOWER CIRCUIT

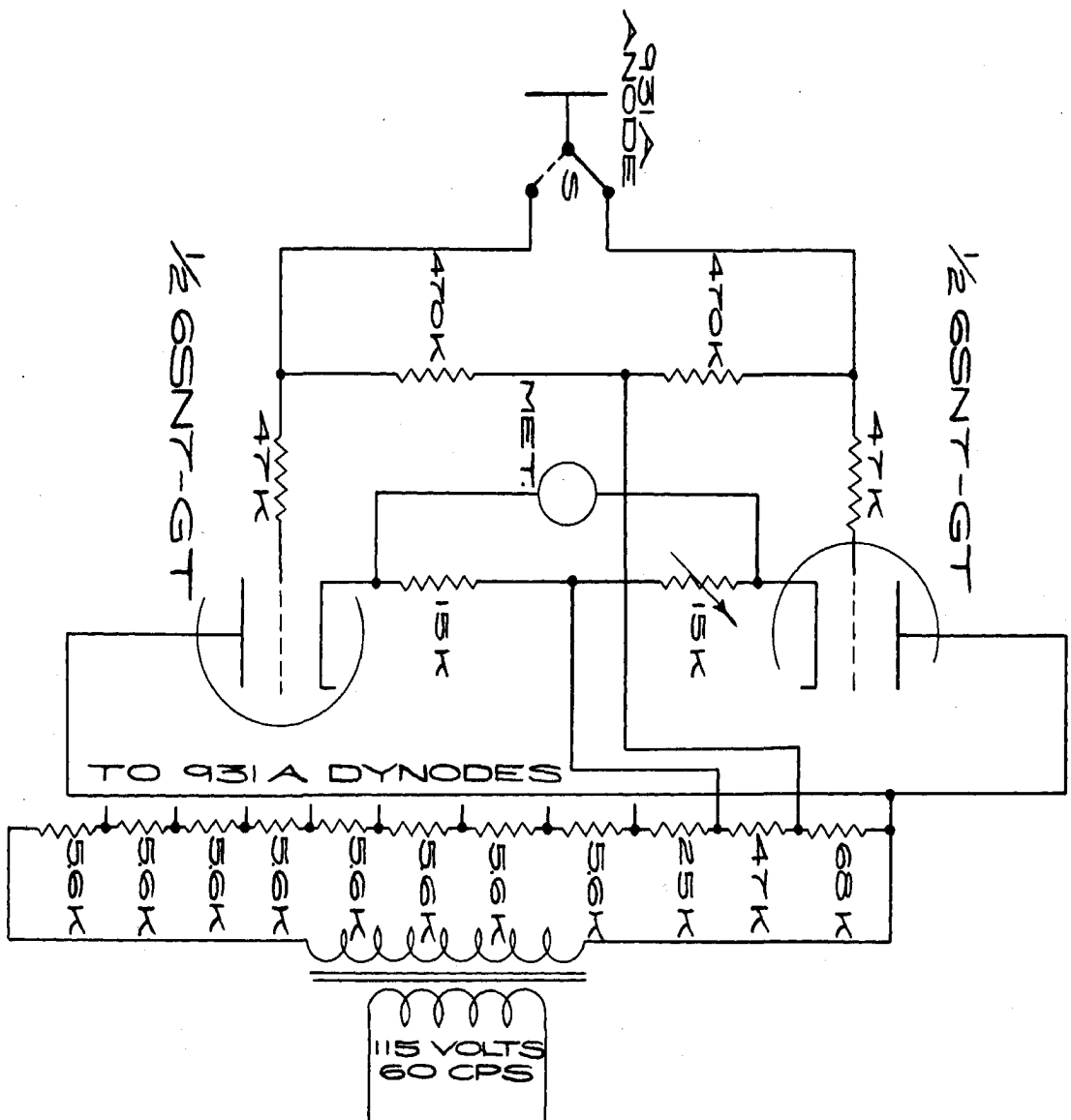


FIG. 2. DUAL CATHODE FOLLOWER BRIDGE

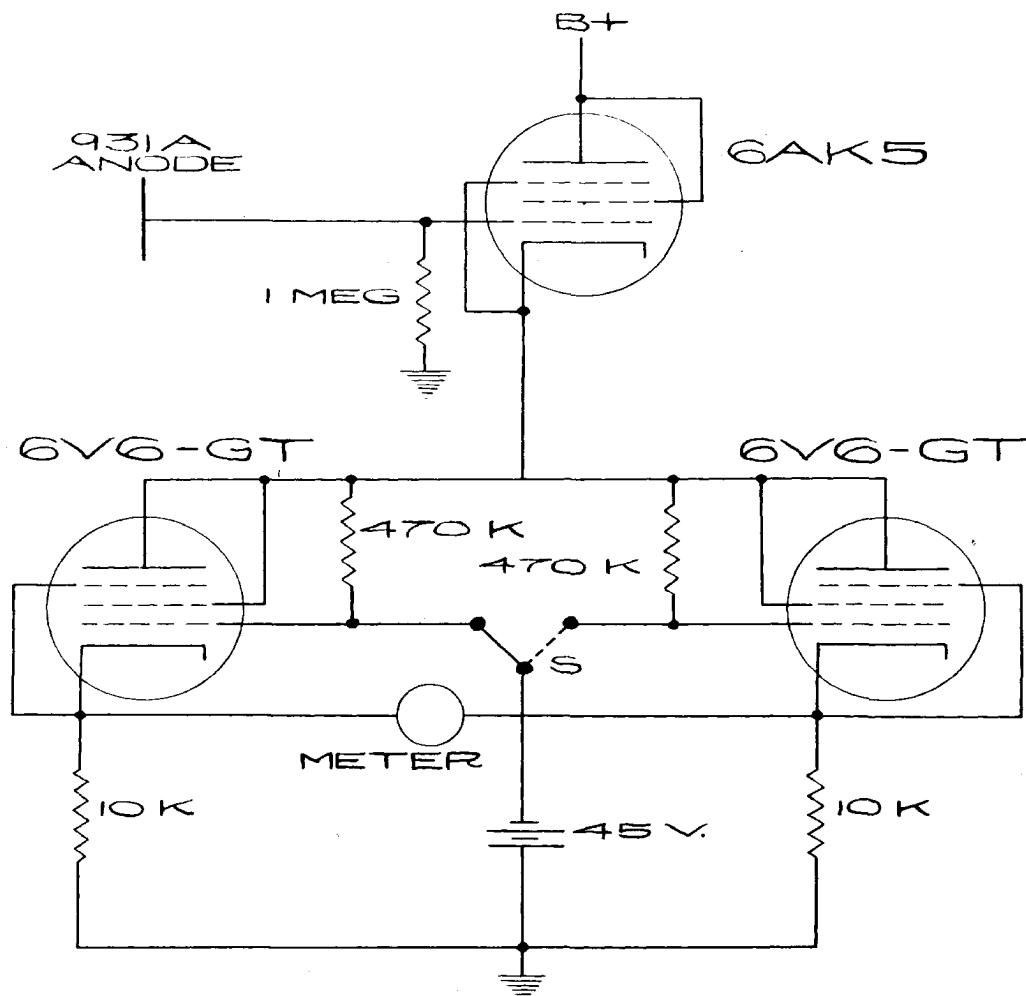


FIG. 4. MECHANICAL CONTROL OF SWITCHING TUBES

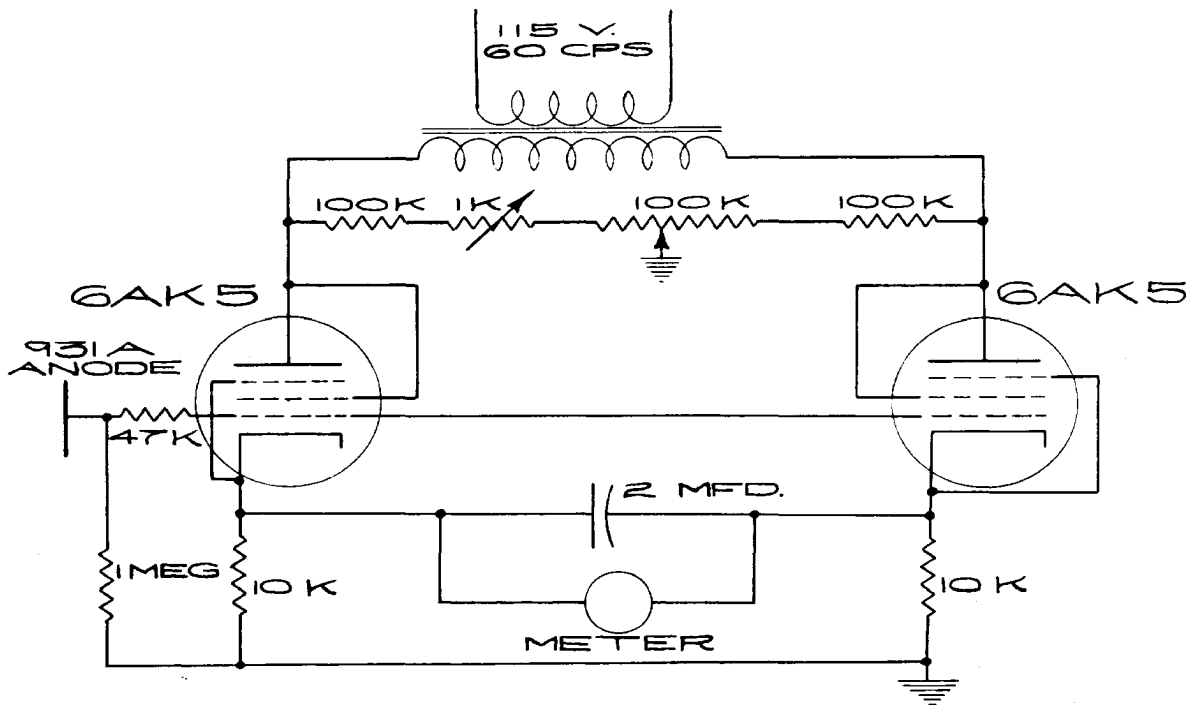


FIG. 5A CATHODE FOLLOWER SWITCHING CIRCUIT

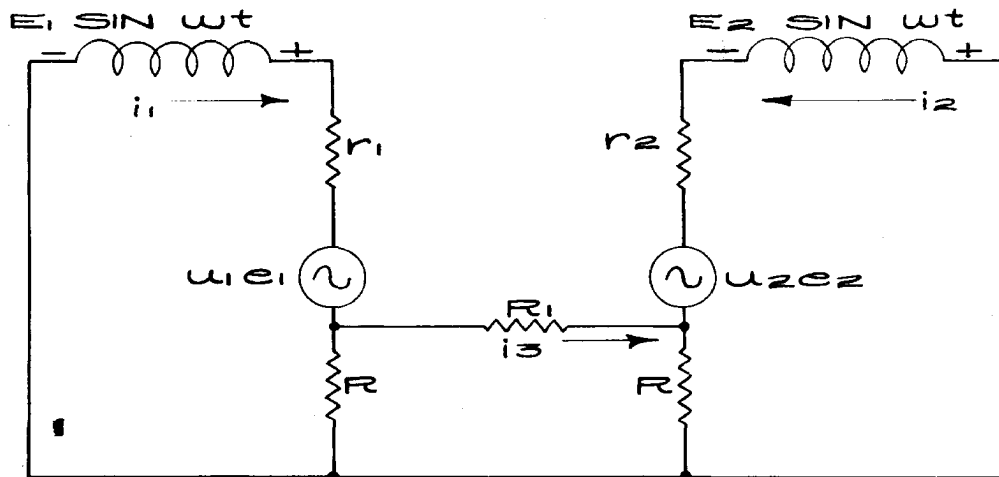


FIG. 5B EQUIVALENT CIRCUIT OF CATHODE FOLLOWER SWITCHING SYSTEM

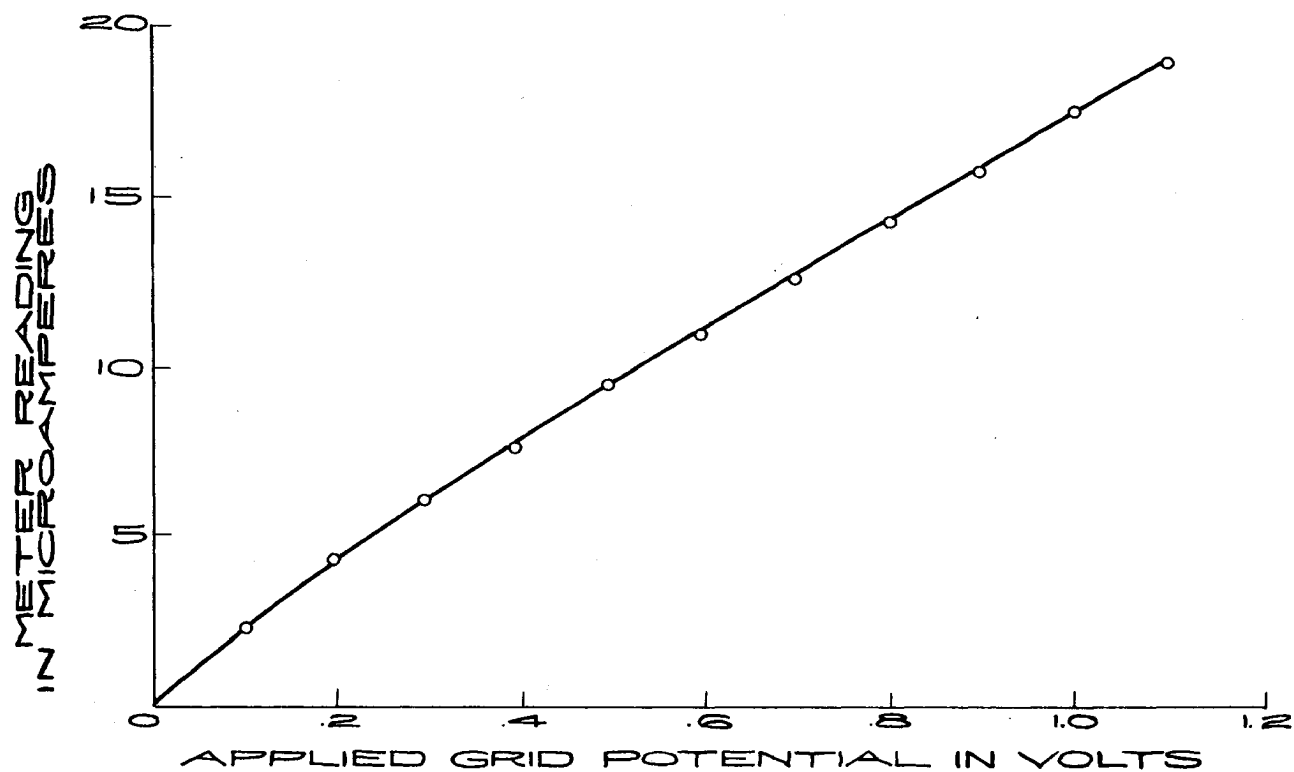
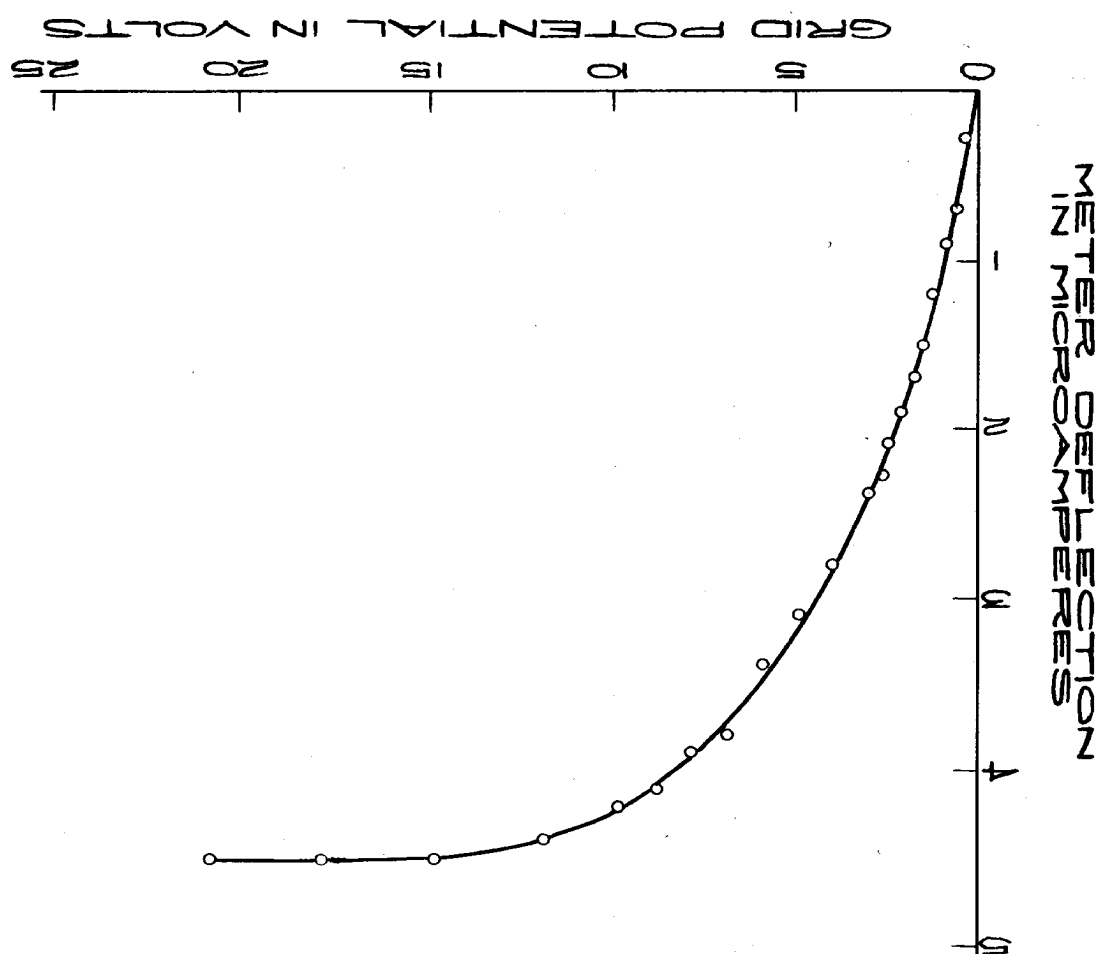


FIG. 6. RELATIONSHIP BETWEEN GRID POTENTIAL AND METER CURRENT

FIG. 7. MEASUREMENT OF CATHODE FOLLOWER NONLINEARITY



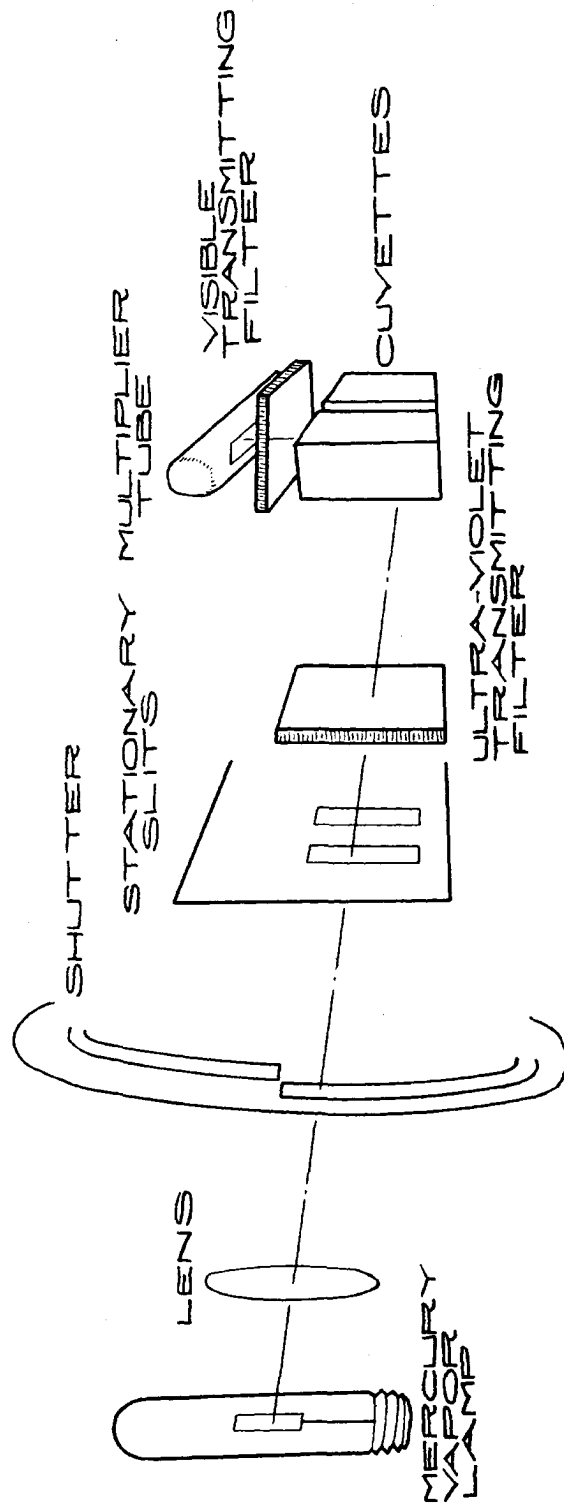


FIG. 8. OPTICAL SYSTEM OF FLUORIMETER

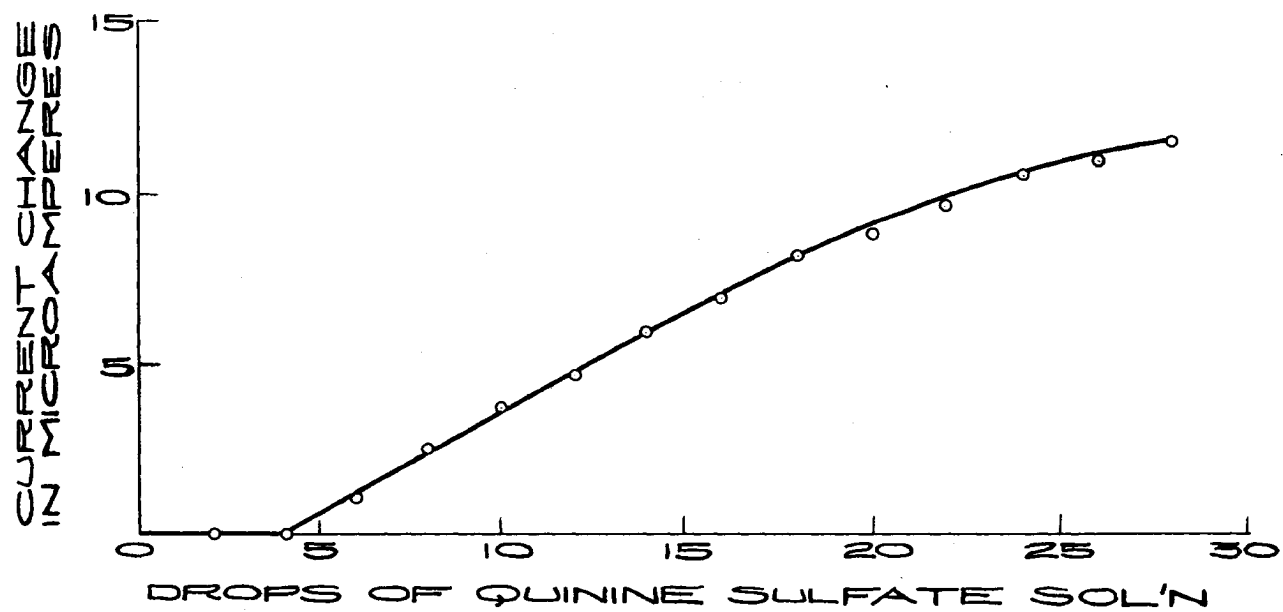


FIG. 9. FLUORIMETER RESPONSE

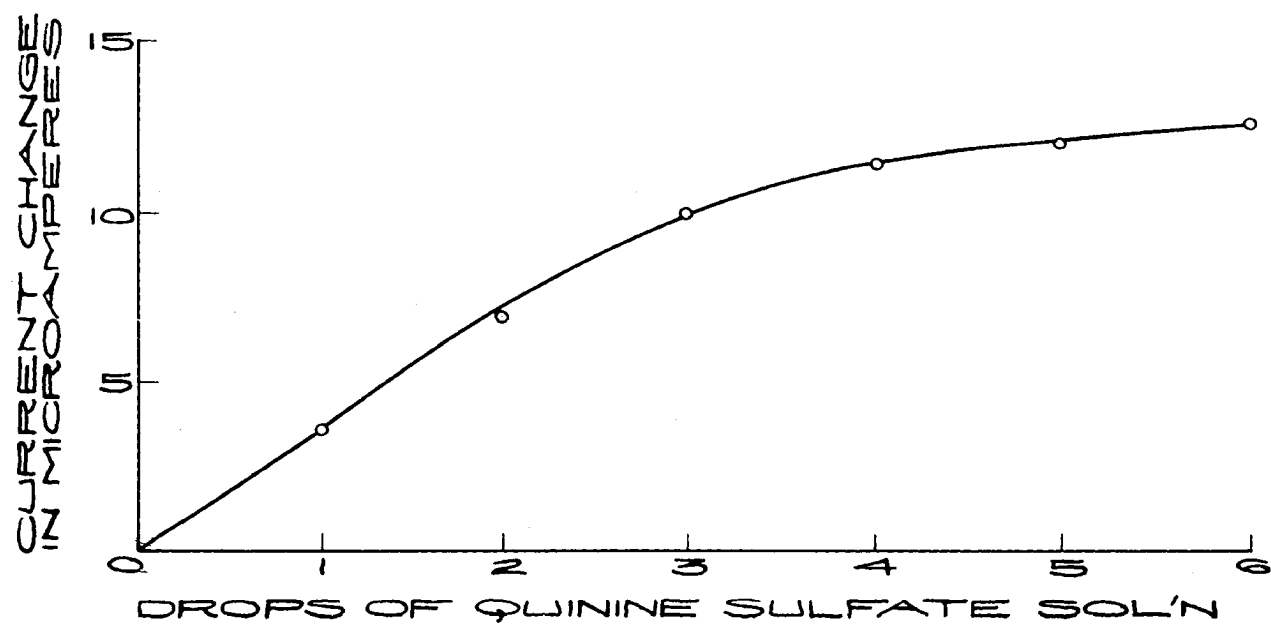


FIG. 10. FLUORIMETER RANGE