A COMPUTER INTERFACED PHOTON COUNTING SPECTROPHOTOMETER

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This is to certify that the

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ABSTRACT

A COMPUTER INTERFACED PHOTON COUNTING SPECTROPHOTOMETER

By

David C. Wenke

A computer-interfaced, scanning spectrophotometer utilizing photon counting as a detection technique has been constructed. As a result of being on-line with a small laboratory computer all the advantages offered by this detection technique are achieved. These include: direct digital processing of the light intensity data which avoids error causing data domain conversions; increasing the S/N (the ratio of the mean value of a signal to its standard deviation) of light intensity measurements made with photomultiplier tubes through dark current rejection and equal weighting of photocurrent charge packets; and the stability which allows precise long time integration of the signal as a means of reducing the measurement bandwidth and increasing the S/N. As a result of these features, the system is capable of performing light intensity measurements on very weak sources with the S/N of these measurements being limited only by the available measurement time.

The automation exhibited by the system is considerable. From commands input at the teletype the computer controls three parameters during a scan: (1) monochromator wavelength setting; (2) a shutter which determines if the detector-photon counter observes signal or background count rates and (3) the integration time over which the

photon counter output is measured. Following initialization of the system prior to a scan, no input is required of the experimenter during scans which may take days. The data points are displayed on the teletype as they are obtained and stored on magnetic tape for further processing and display.

Computer control of the photomultiplier tube shutter makes possible the use of synchronous detection techniques which are necessary due to the presence of low frequency noise in the photon counter output. Modulation of the signal is accomplished under computer control by subtracting the results of alternate signal and background measurements made with the shutter opened and closed. By summing the results of repetitive measurements a very effective form of signal filtering is achieved with the result that low frequency drift noise ceases to be a problem.

With the problem of drift noise removed, the computer interfaced photon counting spectrophotometer is able to detect and measure very weak emission spectra and to optimize the measurement process. Prior to a scan the computer is instructed as to what S/N is desired for the spectral data points and also the maximum amount of time that should be spent on any one point. Using these data the computer decides on the basis of preliminary signal and background measurements if it is possible to obtain the spectral point at the desired S/N within the allowed time. If not the preliminary data is printed and stored and the next point in the scan is examined in the same way.

In addition to making this decision, the computer also determines at which spectral data points the signal count rate is great enough to make the background count rate unimportant in determining the S/N of the measurement. For these spectral points, synchronous detection is temporarily suspended and only the signal count rate is measured.

Due to the real time decision making abilities described above the computer interfaced photon counting spectrophotometer is able to optimally scan a source which may exhibit very strong and weak bands. To demonstrate these capabilities two emission spectra obtained with the system are presented. The first of these, the emission spectrum of fluorescein, demonstrates the system's ability to record spectra containing both weak and strong signals. To demonstrate the system's ability to recover signals buried in noise, an emission spectrum of anthracene requiring thirty hours to obtain is shown. Because of the low intensity of the exciting source, the maximum observed anthracene emission was equal to only 300 photons/sec.

Complimenting the data acquisition capabilities of the system are several forms of data processing and display. Spectra can be displayed on: (1) CRT, (2) an analog plotter and (3) the teletype. Any portion or all of a spectrum can be displayed in three modes on any desired scale. The modes are relative luminescence intensity, absorbance and transmittance. Programs are also included for digitally smoothing spectra and for adding, subtracting and multiplying two spectra.

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I. INTRODUCTION

Molecular luminescence spectroscopy is becoming increasingly important as an analytical and research tool in widely diverse areas. Its importance as an analytical tool is verified by its extensive use in the life science fields of clinical chemistry and biochemistry and the related areas of pharmacology and toxicology.

In the basic research area of chemical physics, the study of molecular luminescence is an important source of information concerning the quantum chemistry of excited molecular states. From luminescence spectra a great deal of knowledge can be obtained about the relative energies and configurations of excited states and intramolecular processes which occur after excitation.

Unfortunately, luminescence spectroscopy is all too often characterized by very low intensity light levels. In all but the most fortuitous instances, molecular emission is usually very weak and therefore exceedingly difficult to detect. Often, as might be expected, the weakest emissions are of particular interest since they usually signify the occurance of an unexpected event of low probability.

The desire to measure the weakest possible emission signals caused the advent of photon counting techniques to be met with a good deal of enthusiasm by emission spectroscopists. By being able to detect individual photons, photon counting systems exhibit an extreme sensitivity which makes them ideal for the detection of low intensity light levels.

However, the operational aspects of photon counting techniques are not without difficulty. To make one photon counting light intensity measurement requires a relatively complex and lengthy process of counting, timing, data storage and the calculation of an arithmetic difference. Because of the complexity of the operations which make up a photon counting measurement, the measurements are tedious and time consumming when performed manually. In such instances, when the time of the experimenter is being spent inefficiently in the repetition of a sequence of simple tasks, it is becoming increasingly popular to think in terms of interfacing a small laboratory computer to the experiment in the hope that the experimenter can be freed for more productive labors and that the experiment will be performed more efficiently and effectively.

The decision to construct a computer interfaced photon counting spectrophotometer was based on the arguments appearing in the preceeding paragraphs. The desire to measure low intensity molecular luminescence spectra led to the selection of photon counting as a sensitive detection technique which in turn made computer interfacing of the instrument almost mandatory.

Some of the basic considerations which govern the practical applications of photon counting techniques are discussed in Chapter II of this thesis. Especially emphasized in this chapter are the advantages and disadvantages of photon counting and the relative light levels for which they are most effective.

In Chapter III a description is given of the computer interfaced photon counting spectrophotometer after an initial discussion of some of the general methods and technology of interfacing small laboratory computers. An effort is made in this chapter to describe in some detail the information channels which exist between the computer and the spectrophotometer.

Some signal detection problems which occur during the use of photon counting techniques for the measurement of extremely low light levels are discussed in Chapter IV. Methods of filtering out low frequency noise components in the photon counter output are explained and an evaluation of the effectiveness of this filtering is given.

In Chapter V an evaluation of the performance of the computer interfaced photon counting spectrophotometer is given. The versatility, sensitivity and reliability of the spectrophotometer are discussed within the context of two spectra acquired with the instrument.

II. BASIC CONSIDERATIONS IN PHOTON COUNTING

A. Introduction

Photon counting is the term applied to a light detection scheme in which the arrival rate of individual photons at a detector is measured. Figure (1) illustrates the instrumental components needed to carry out a photon counting measurement. The detector is usually a high gain photomultiplier tube (1) in which each photon striking the photocathode results in a relatively large packet of charge arriving at the anode. These charge packets are collected at the photomultiplier anode, converted to voltage pulses, amplified and fed into a discriminator which passes only those pulses having a height greater than some preset level. The frequency of the pulses emerging from the discriminator provides a measure of the light intensity striking the photocathode.

The simplicity of the block diagram is retained in the actual design and construction of photon counting systems. Recent advances in solid state amplifiers, high speed comparators and logic circuits have made the construction of high frequency photon counting systems straightforward and economical.

The methodology of photon counting suggests that this technique of light intensity measurement will exhibit characteristics superior to the more conventional dc techniques - especially in the measurement of low level light intensities. In practice however, these advantages

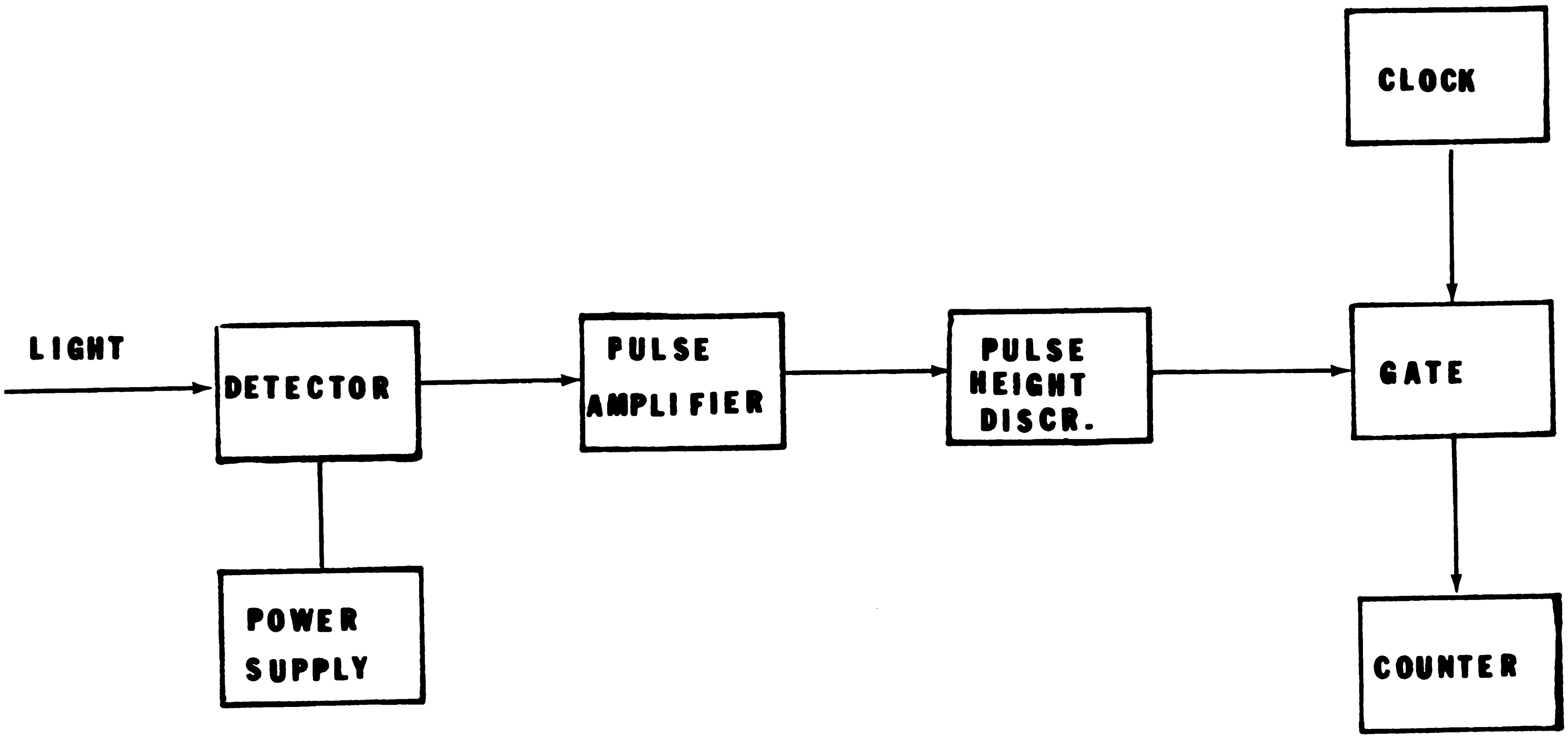


Figure 1. Block Diagram of Photon Counting System.

have not always been realized and, as will be shown, a number of authors have experimentally and theoretically determined that photon counting offers little or no advantage over conventional detection techniques (2,3). Even at relatively low light levels, lock-in techniques have been found to be competitive with photon counting measurements (3). There is, however, one region of light intensity in which all the advantages of photon counting are exerted. This is the region of extremely low light levels. It is in this region that photon counting can be said to be truly superior to any other existing technique for measuring uv and visible light intensities. The basis for this superiority is found in a consideration of the relative signal-to-noise ratio (S/N) characteristics for photon counting measurements and dc measurements.

B. Photon Counting S/N Expressions

The fundamental noise in a photon counting measurement is the fluctuation in count rate caused by the random arrival rate of photons at the photocathode. This photon or quantum noise is due to the quantized nature of light and the fact that photoemission is a statistical process. For ultraviolet and visible photons, these instantaneous random fluctuations about an average arrival rate can be described by Poisson statistics. Thus the probability of counting n pulses during a time interval, t, is given by the Poisson equation

$$P_n = \frac{(Rt)^n e^{-Rt}}{n!} \qquad (1)$$

where R is the true average count rate of an infinite sample.

Phenomena governed by Poisson statistics have the fundamental property that their mean and variance are equal. Therefore, if fluctuations in the signal count rate, R_S, are governed by Poisson statistics, the standard deviation, σ_n, of the number of photons, N_S, counted during a time interval, t, is given by

$$\sigma_n = (N_S)^{1/2} = (R_S t)^{1/2} \tag{2}$$

Defining the S/N as the ratio of the magnitude of the measurement to its standard deviation we can write the S/N for a photon counting measurement in terms of the count rate frequency as

$$S/N = \frac{R_S t}{(R_S t)^{1/2}} = (R_S t)^{1/2} \tag{3}$$

In terms of the number of counts obtained over a time interval t, the S/N expression has the form

$$S/N = \frac{N_S}{(N_S)^{1/2}} = (N_S)^{1/2} \tag{4}$$

It is important to state that these S/N expressions hold for measurements made with a perfect photon counting system in which the only source of noise is the inherent quantum noise of photons striking the photocathode.

Unfortunately, these very simple expressions do not describe the S/N of a photon counting measurement made with a real system. It is well known that all photomultiplier tubes exhibit a background or dark current which results in charge packets arriving at the anode in addition to those due to photons striking the photocathode. The presence of these background counts means that photon counting measurements made over a time t will include counts due to the signal,

N_S, and counts due to the background, N_B. Because dark current pulses can also be assumed to follow Poisson statistics (4), the noise they add to a photon counting measurement is also quantum noise and as such can be accommodated in the S/N expression in much the same way as the signal quantum noise.

The specific form of a S/N expression allowing for a background count rate depends on the manner in which the background is measured. In the first case the average number of background counts, $\overline{N}_B$, per counting time, t, is determined in a separate experiment with the detector shielded. To carry out a light intensity measurement, this average number of background counts is subtracted from the total number of counts obtained during the measurement time t, $N_T = (N_S + N_B)$, to give the number of signal counts N_S:

$$S = N_S = N_T - \overline{N}_B = (N_S + N_B) - \overline{N}_B \tag{5}$$

Because both the signal and background count rates are assummed to follow Poisson statistics, the standard deviation and thus the noise associated with this measurement is given by

$$N = (\sigma^2 N_T)^{1/2} = (N_T)^{1/2} = (N_S + N_B)^{1/2} \tag{6}$$

The desired S/N expression is given by

$$S/N = \frac{[(N_S + N_B) - \overline{N}_B]}{(N_S + N_B)^{1/2}} \tag{7}$$

An alternative way to carry out this measurement is to record the number of counts obtained for a time t with the detector exposed to the light flux and then again, for the same time with it covered. A measure of the light intensity is again obtained by finding the difference between the total number of exposed counts N_T, and the dark counts, N_B.

$$S = N_T - N_B = (N_S + N_B) - N_B \tag{8}$$

The noise for such a measurement is greater than for the first case because the precision with which N_B is known is not as great. The noise for this measurement is equal to the standard deviation of the signal and background measurement as is shown:

$$N = (\sigma^2 N_T + \sigma^2 N_B)^{1/2} = (\sigma^2 N_S + \sigma^2 N_B)^{1/2} = (N_S + 2N_B)^{1/2} \tag{9}$$

Forming the usual ratio, the S/N expression is given by eq (10).

$$S/N = \frac{[(N_S + N_B) - N_B]}{(N_S + 2N_B)^{1/2}} \tag{10}$$

Writing equation (10) in terms of signal and background count rates, R_S and R_B, the following form of the S/N is obtained.

$$S/N = \frac{R_S t^{1/2}}{[(R_S + 2R_B)t]^{1/2}} \tag{11}$$

A manipulation of this expression yields the following convient form

$$S/N = \frac{R_S^{1/2} t^{1/2}}{(1 + 2R_B/R_S)^{1/2}} \tag{12}$$

from which the effects of signal count rate, integration time and background count rate can be seen. Of special interest to this discussion is the form of the S/N expression in the limit of very low light levels, i.e. $R_S << R_B$. Equation (12) becomes

$$S/N \approx \frac{R_S^{1/2} t^{1/2}}{(2R_B/R_S)^{1/2}} \tag{13}$$

It can be seen that the S/N increases as $t^{1/2}$ and is also increased for an individual measurement when the ratio $2R_B/R_S$ is decreased.

C. Advantages of Photon Counting

As has been mentioned, a number of advantages have been claimed for photon counting relative to conventional dc detection techniques; especially in the measurement of very low light levels. (By conventional dc detection techniques is meant the conversion of the photoanodic current to a voltage by a high quality operational amplifier current-to-voltage converter followed by some type of voltage measurement and readout.) A compilation of these advantages by a photon counting proponent (5) has resulted in the following list:

(1) excellent long term stability

(2) linearity of response over a wide dynamic range

(3) discrimination against both dc surface leakage and low amplitude events not originating from the cathode

(4) high speed digital processing capability without the use of analog to digital conversion

(5) optimum S/N ratio for quantum limited signals.

A recent study by Crouch and Ingle (6) has examined each of these claimed advantages in some detail. They find that except in the case of very low light intensities, dc and photon counting techniques give comparable results. At relatively high light levels dc techniques are superior because photon counting suffers from a loss of linearity due to peak overlap. However, at very low light levels under which quantum noise dominates, photon counting systems compared to dc measurement systems can provide advantages of 5-22% higher S/N, greater stability, and better linearity.

The 5-22% increase in S/N offered by photon counting stems from the fact that the dynode amplification of photoelectrons in the photomultiplier tube is a statistical process which results in an amplitude distribution in the charge packets arriving at the anode. When the photoanodic current is measured by dc techniques, the amplitude distribution introduces an additional uncertainty in the light intensity measurement because all photons are not weighted equally. Photon counting measurements remove this uncertainty by weighting equally all pulses having heights greater than some preset discriminator level.

One of the principal advantages often claimed for photon counting and one that should lead to an additional increase in the S/N ratio is the ability to discriminate against background counts because of their generally lower heights. In this manner the ratio R_S/R_B of equation (12) could be minimized and an improved S/N ratio attained relative to a dc measurement. Unfortunately, background discrimination has not proved to be very fruitful in raising the S/N and in some cases has been responsible for decreasing it. To understand some of the difficulties encountered in background discrimination it is helpful to consider a typical differential and integral peak height distribution curve as shown in Fig. (2).

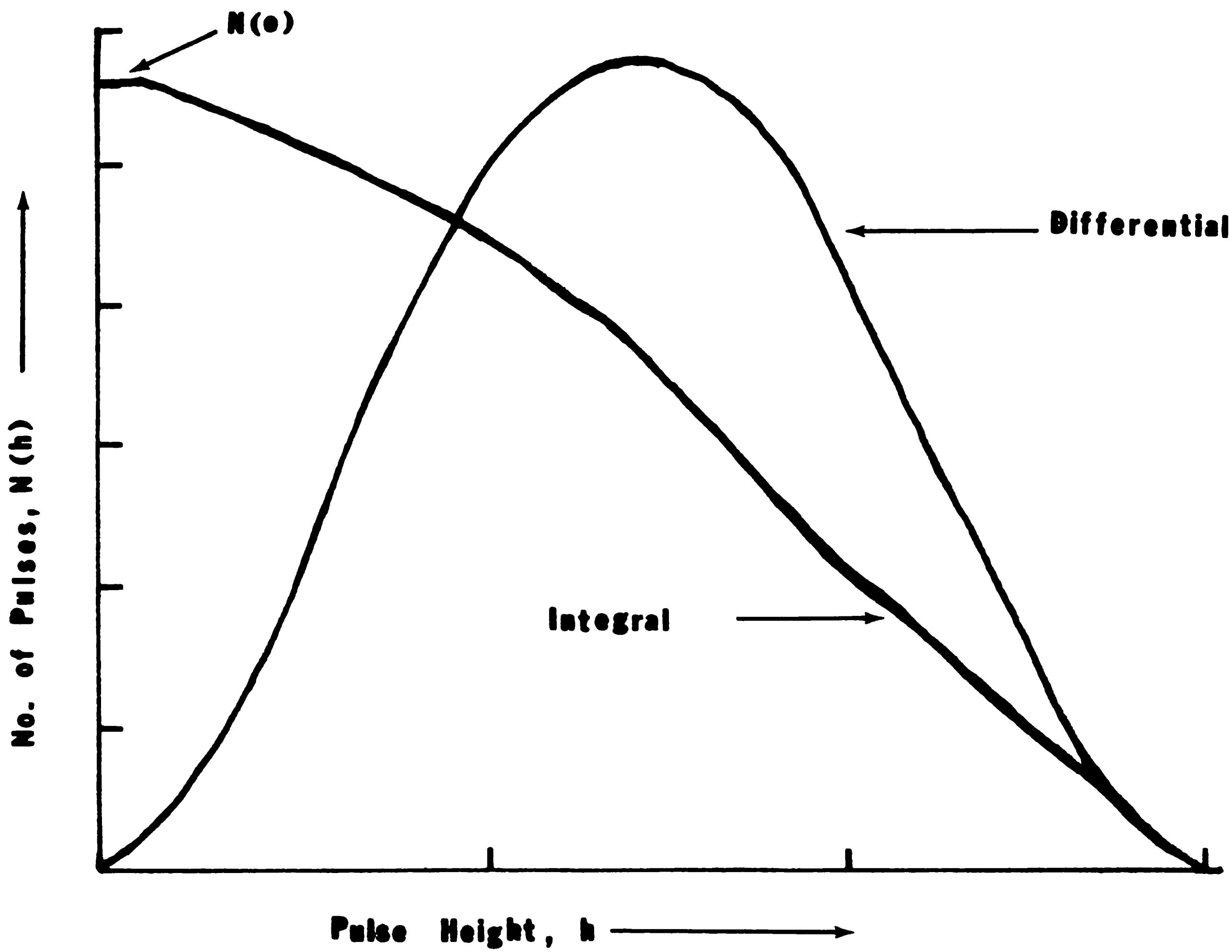


Figure 2. Typical Integral and Differential Pulse Height Distribution Curves.

The differential peak height distribution curve, obtained with a multichannel peak height analyzer, shows the number of pulses N(h) (ordinate) in a given time interval that have heights (charge) between h and h + Δh as a function of h (abscissa). The single-electron integral peak height curve shown in the same figure is a closely related description of the same distribution. This curve shows the number of pulses in the given time interval that have a height equal to or greater than h. The value of N(o) at which this curve intersects the zero pulse height axis is the number of electrons entering the photomultiplier's dynode structure. The shape of the differential pulse height distribution indicates that secondary emission follows Poisson statistics to a large extent but not exactly. It has been shown (7) that the distribution becomes more exponential if the activation of each dynode is non-uniform.

As might be expected, the dark current pulse height distribution is somewhat related to the photon pulse height distribution. Fig. (3) shows a rather idealized comparison of integral pulse height distribution curves for photon and dark current pulses. Although similar, differences in the two distributions are evident; one of the main differences being the very large number of dark current pulses having low to very low pulse heights. These low height dark current pulses are thought to be primarily due to thermal and cold field emission and ion pulses (8). Because these pulses often originate down the dynode chain and thus do not undergo full amplification, their charge content is less than that of photon pulses which enjoy full amplification.

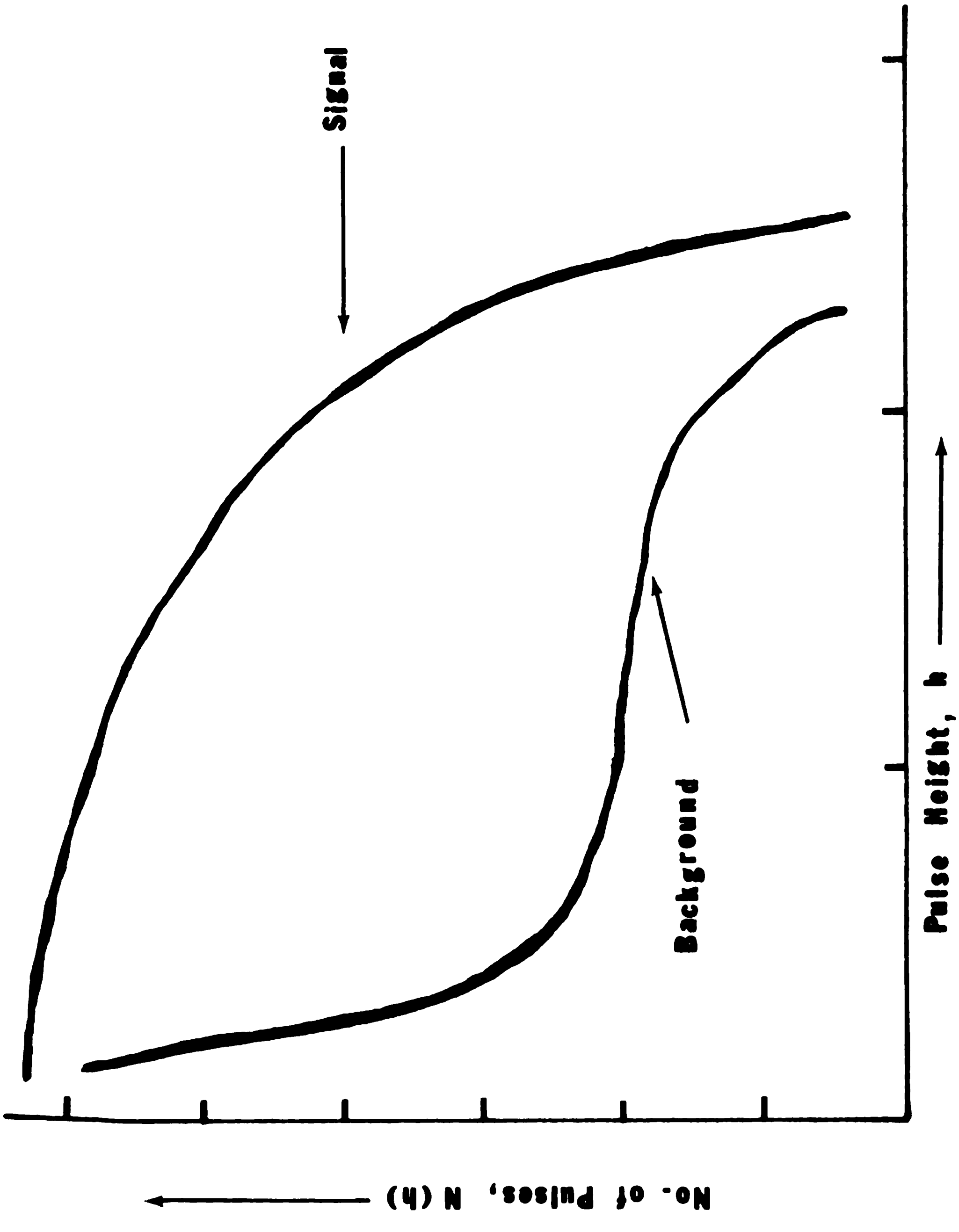


Figure 3. Typical Signal and Background Integral Pulse Height Distribution Curves.

In addition to the dark current sources mentioned, several others exist which, because they result primarily in the much smaller number of large dark current pulses, are not of importance to this discussion. The important point is that due to the difference in amplitude distributions of the photon and dark current pulses, pulse height discrimination can be used to reject the large number of low amplitude dark current pulses while passing most of the photon pulses.

Theoretically the optimum discriminator level, h_o, can be found for a low intensity measurement by the equation (9)

$$\frac{\partial}{\partial h_o} [\int_{h_o}^{\infty} N_S(h)dh]/[\int_{h_o}^{\infty} N_B(h)]^{1/2} = 0 \qquad (14)$$

in which $N_S(h)$ and $N_B(h)$ are the signal and background differential pulse height distribution functions respectively. The actual determination of h_o as defined by eq. (14) is performed by plotting the photon and background integral pulse height distribution curves on log paper and finding the point at which $(\frac{d}{dh})\ell n\ N_S(h) = 1/2 \frac{d}{dh}\ \ell n\ N_B(h)$ (4). (The derivation involves the assumption that $S/N = \frac{N_S}{(N_B)^{1/2}}$.)

In practice it is quite difficult to determine just where the discriminator should be set since pulse height distributions are very sensitive to operating conditions and are rarely as well behaved as theory predicts. Many workers find no optimum discriminator level (3) and in fact some workers find that the S/N is independent of the discriminator level except at high levels at which point the S/N is actually decreased (2). A recent theoretical treatment by Ingle and Crouch (6) confirms the decrease in S/N with increasing (high) discriminator levels.

In the absence of any well defined methodology for optimum discriminator level selection, the most desirable approach seems to be the direct one of actually measuring the S/N as a function of discriminator level. To optimize the S/N in this manner for a scanning experiment in which the incident intensity is always changing would seem to be impossible. Fortunately, the optimum discriminator setting does not appear to vary significantly with incident intensity in the low intensity range in which photon counting is most applicable (3).

D. Disadvantages of Photon Counting

The numerous advantages of photon counting techniques - real and imagined - are an inverse function of the light intensity being measured. As the light intensity being measured increases, a very definite point is reached beyond which photon counting techniques are not applicable. At this point, the charge packet arrival rate at the anode is too rapid to allow individual packets to be distinguished by the counter circuits and pulse overlap occurs. The net result is that the arrival rate of photons at the photocathode as observed by the photon counter becomes a smaller and smaller fraction of the true arrival rate. This phenomenon called dead time or resolving time loss is well known to workers in the area of scintillation counting (10).

In a recent study, Ingle and Crouch (11) have considered this problem in some detail. They have divided photon counting circuits into two types depending on the source of the dead time loss. In the first type of circuit, described as paralyzable, each pulse that occurs extends the dead time, whether or not the pulse is counted.

Thus a pulse which follows a previous pulse in a time less than the system dead time will not be counted but will extend the system dead time. In the second type of circuit, called nonparalyzable, pulses occurring within the dead time of another pulse will not be counted but will not extend the system dead time. For type I circuits, it is possible to distinguish between those in which the RC load and amplifier limit the frequency response and a second type in which the dead time is determined by the discriminator or counter.

The loss of linearity in count rate as a function of intensity severely limits the usefulness of photon counting techniques. Using state-of-the-art electronics, counting circuits can be built which are linear to within 0.1% at count rates up to 10^4 counts/sec. This range can be extended for one type of paralyzable counting circuits by a technique known as dead time compensation which maintains linearity by the judicious selection of discriminator levels. Using this technique Ash and Piepmeir (12) were able to obtain good linearity up to about 10^5 counts/sec.

A perhaps more fruitful technique for compensating for dead time loss involves the mathematical correction of the observed count rate to the true count rate. Ingle and Crouch (11) have derived expressions showing the true count rate as a function of the observed count rate and the circuit dead time parameter. With these equations, the useful light intensity range can be extended by roughly 10%. Of course this would be very tedious to do manually.

E. Summary of Photon Counting Applicability

As the discussion thus far has shown, several of the often quoted advantages of photon counting are somewhat suspect. Dark current discrimination, which appears to have a sound theoretical basis, has proved difficult to implement experimentally in a beneficial manner. Pulse overlap problems, as has also been discussed, severely limit the upper level of light intensity that can be measured. The elimination of readout error as a photon counting advantage loses some of its importance when one considers the high quality analog readout systems presently available.

Of more importance as a real advantage is the direct digital processing of photon counting data which results in the elimination of data domain conversions (15). In the limit of low intensity measurements in which maximum resolution and stability are needed from each member of the data acquisition and domain conversion sequence, photon counting offers distinct advantages due to its drift free nature. Bandwidths in photon counting measurements can be made vanishingly small by simply increasing the counting time.

This is not possible to the same degree in conventional analog systems since drift and 1/f noise ultimately limit the minimum useful bandwidth. Robben (13) found that his photon counting system was five times more stable than a dc system for long integration times. Other workers have also confirmed the advantages of photon counting techniques due to long term stability. Young (8) concludes that "At low levels, where dark noise dominates over photon noise ($R_B >> R_S$), pulse counting is very nearly the optimum method and is certainly better than other methods of comparable complexity". Tull (14) finds that when $R_S \stackrel{\sim}{\sim} R_B$ photon counting measurements give a S/N which is better by a factor of 3.5 than charge integration measurements with comparable bandwidths. Nakamura and Schwartz (3) find that photon counting gives a S/N over three times better than even analog synchronous detection when measuring light levels on the order of 10^{-17} watts, and this advantage increases with decreasing light intensity.

The arguments and evidence discussed thus far indicate that photon counting is by far the most advantageous method of measuring very low intensity light levels. Furthermore, because of its practically infinite stability, the photon counting technique is able to obtain light measurements of a quality limited only by the available measurement time.

III. A COMPUTER INTERFACED PHOTON COUNTING SPECTROPHOTOMETER

A. Applicability of Photon Counting Measurements to Computer Control

Photon counting systems are ideally suited for interfacing to small digital laboratory computers for a number of reasons. The phenomena they measure are inherently digital and digital processing avoids error causing data domain conversions (15). A second reason is that photon counting data must often be arithmetically processed to be most useful, i.e. background counts must be subtracted from signal counts in order for meaningful measurements to be made. Another reason for computer control of photon counting measurements is the need for data storage when scanning experiments are being performed. The alternative to computer storage and display of the digital data is manually recording the count rates or the use of some type of frequency to voltage converter followed by an analog display. A third alternative is an automated data storage system such as a paper tape punch. The first method is tedious at best while the second reintroduces the problem of interdomain conversions. The third possibility is less attractive than immediate real time storage of data since it inevitably involves a time lag between data generation and processing.

In addition to the specific data processing advantages obtained by being on-line, all the operational characteristics of a scanning photon counting spectrophotometer lend themselves to computer control.

The entire process of obtaining a spectrum can be described by the words increment, count, and calculate. The recorded spectrum consists of photon count rates measured over precise time intervals at regular wavelength increments. The experimental sequence to obtain a data point involves five steps; (1) set monochromator at the desired wavelength; (2) measure signal and background count rate; (3) measure background count rate; (4) find the difference between the count rates and (5) display and/or record this difference. How this process could be carried out manually for more than a few points is difficult to imagine. This is especially true when one considers that to obtain a reasonable S/N for low intensity measurements, repeated measurement and summing of background and sample count rates is often necessary. The only viable alternative to computer control is automation of the entire process by a very complex hardwired controller which amounts to designing a small custom computer capable of performing only one task. That this is a very inefficient and far less flexible approach than interfacing the experiment to a small general laboratory computer will be demonstrated.

B. Overview of System

1. System Components

A block diagram of the computer interfaced system is shown in Fig. 4. The arrows indicate the flow of information and control. As can be seen the computer forms the heart of the system. Information and control words pass to and from it to all other system components.

The PDP8/I is a general purpose computer using TTL (transistor-transistor-logic) integrated circuit modules and parallel data

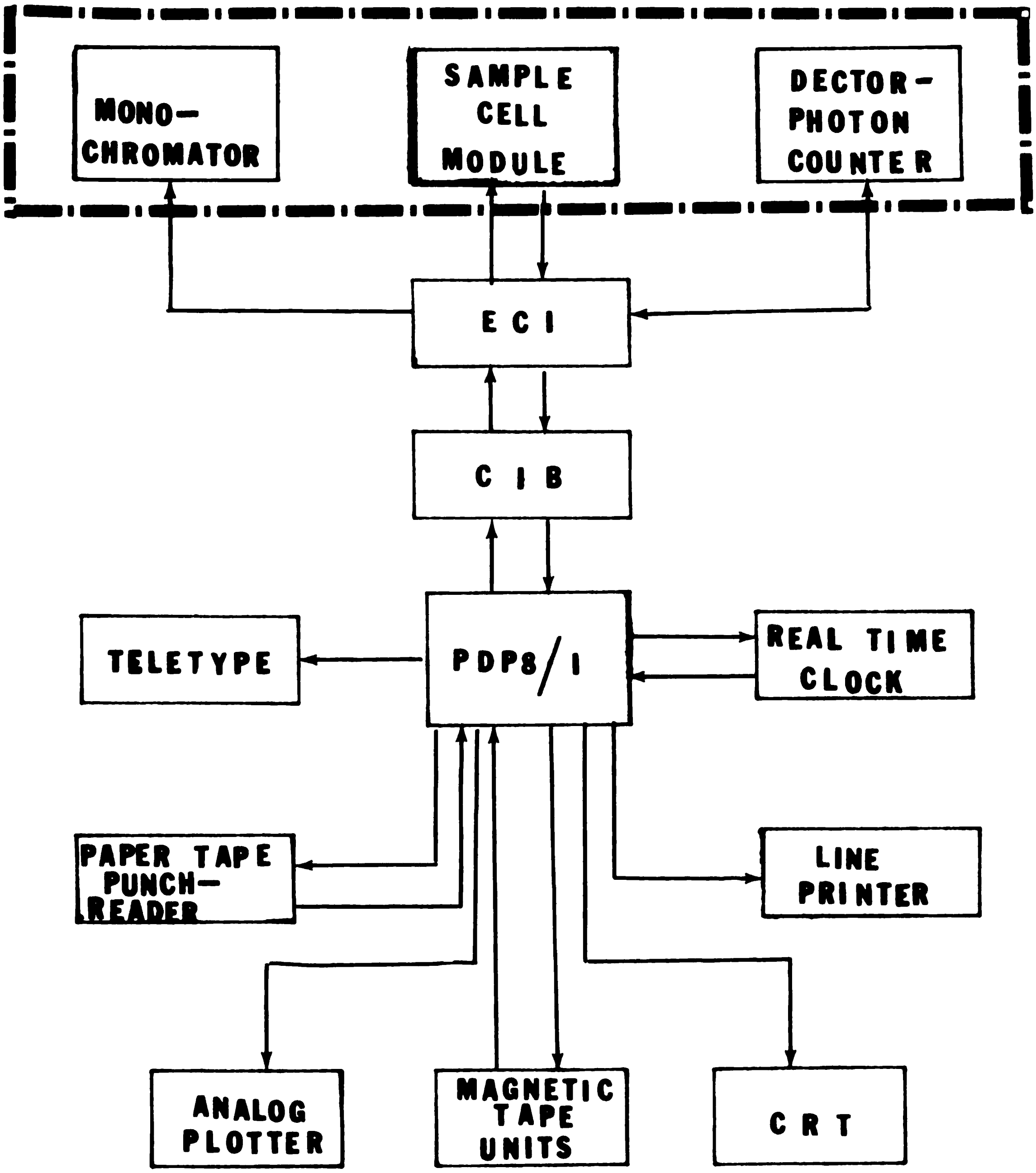


Figure 4. Block Diagram of Computer Interfaced Photon Counting Spectrophotometer.

transfer. The computer is organized into a central processor, a core memory and input/output (I/O) lines. Word size is fixed at 12 bits and core memory consists of two fields of 4096 words each. Two DEC TAPE transports are also available as memory extension devices.

Inputs into the computer in addition to the I/O lines are a KSR-33 teletype and a high-speed paper tape reader. Outputs in addition to the I/O lines are a high-speed paper tape punch, teletype, analog plotter (Heath Model EU-205-11), CRT display (Tektronix Model 603) and a modified RCA Model 301 line printer. A real-time clock is also available to provide computer-controlled time measurement with microsecond accuracy.

For an experiment to be on-line with a small laboratory computer a flow of information between the experiment and the computer is required. This flow of data and control information is carried out over the computer input/output (I/O) lines. Binary control and data words in the form of buffered TTL level voltages can be exported and imported by the computer over its I/O lines to and from the experiment. As obtained from the manufacturer, the computer I/O lines are somewhat inaccessible and not totally immune to damage. This fact makes the physical joining of the computer I/O lines with the experiment, which may be geographically quite distant from the computer, a difficult and sometimes traumatic process. In the past it was necessary for each experimenter to design an interface to bridge the gap between the computer I/O lines and the experiment. This is no longer the case. The Heath EU801E Computer Interface Buffer (CIB) has completely eliminated the difficulty of I/O line connections and concern about mixing on the computer I/O lines. With this device and its attendant hardware it is possible to design and implement on-line experiments in terms of days rather than weeks or months.

Standing at the end of the I/O lines exported by the CIB and serving as a link between the computer and the experiment is the experiment computer interface (ECI). The function of this device is to encode and decode data and control information. Commands from the computer to the experiment are decoded by the ECI into the appropriate action, i.e. a computer command to start the experiment may be translated by the ECI into the closing of a relay or the switching of a transistor. In the same manner, data generated by the experiment are encoded by the ECI into a word which the computer can accept and process. Also, it is through the ECI that the experiment signals the computer when some action on the part of the computer is required. In summary, the ECI is the apparatus through which the computer perceives and controls events in the outside world.

Intimately joined to the ECI both physically and philosophially is the experiment hardware itself. The degree of interaction between these two entities is a measure of the sophistication and flexibility of the system. Inflexible unsophisticated on-line computer applications in which the computer is restricted to a data logging role are characterized by the small number of information lines linking the experiment hardware and the ECI. In these applications, the experiment hardware used in the on-line system need differ little from that used in the conventional off-line experiment. As more use is made of the computer's ability to take an active role in the control of the experiment increased demands are made on the experiment hardware. It must become more modular in design and construction. A useful rule-of-thumb in determining the ease and sophistication with which an experiment can be put on-line is to count the number of external programming jacks present in the experiment.

An example of this can be found in the field of spectroscopy when one compares the Cary 14 and the Heath EU 700 Spectrophotometer. The high quality of the Cary 14 can not be questioned. However, compared to the Heath equipment it is decidedly limited in ease of interfacing. The only straightforward manner in which the Cary 14 could be used in an on-line application would be for the computer to record and display the digitized output of the transducer. In contrast, the Heath equipment allows for external and independent control of (a) source intensity, (b) gain of the transducer, (c) monochromator setting and (d) position of the sample cell with respect to the optical path. Obviously, with these many parameters available for computer control, on-line applications involving very active participation by the computer are possible. How this participation is accomplished necessitates a brief discussion of the information flow between the experiment and the computer.

2. Information Flow Through the System

When used in an interfacing application, a laboratory computer must be able to communicate with the experiment over the computer/laboratory boundry. In general three forms of communication must exist between the computer and the devices which make up the ECI. The computer must be able to request a peripheral device to begin performing some action, a device in the ECI must be able to inform the computer that it needs servicing by the computer and the computer and devices in the ECI must be able to exchange information and control words. In the computer interfaced photon counting spectrophotometer the exchange of information and control words between the computer and devices in the ECI is made via a technique known as programmed data transfer.

Programmed data transfers take place through a special twelve bit computer register known as the accumulator. Under program control, words can be imported into the accumulator from the ECI over the I/O lines which link the twelve bits of the accumulator to the ECI via the CIB. Similarly, control words present in the accumulator can be exported from the computer to the ECI over a second set of I/O lines. The I/O lines over which information transfers are made into the accumulator ($\overline{AC}$) and out of the accumulator (BAC) are shown pictorially in Fig. (5).

As the terminology programmed data transfer indicates, the exporting and importing of information between the computer and the devices in the ECI is controlled by specific program statements. The instruction to export a word from the accumulator to the ECI causes the contents of the accumulator to be read from the BAC I/O lines by a device in the ECI. Effecting the importation of a word from the ECI into the accumulator is somewhat more involved because unlike the BAC I/O lines, the $\overline{AC}$ I/O lines are active only during the transfer of data into the accumulator. Thus the program statement causing a word to be imported into the accumulator does so by activating the $\overline{AC}$ I/O lines at the exact moment that the word to be transferred is available in the ECI and acceptable to the computer.

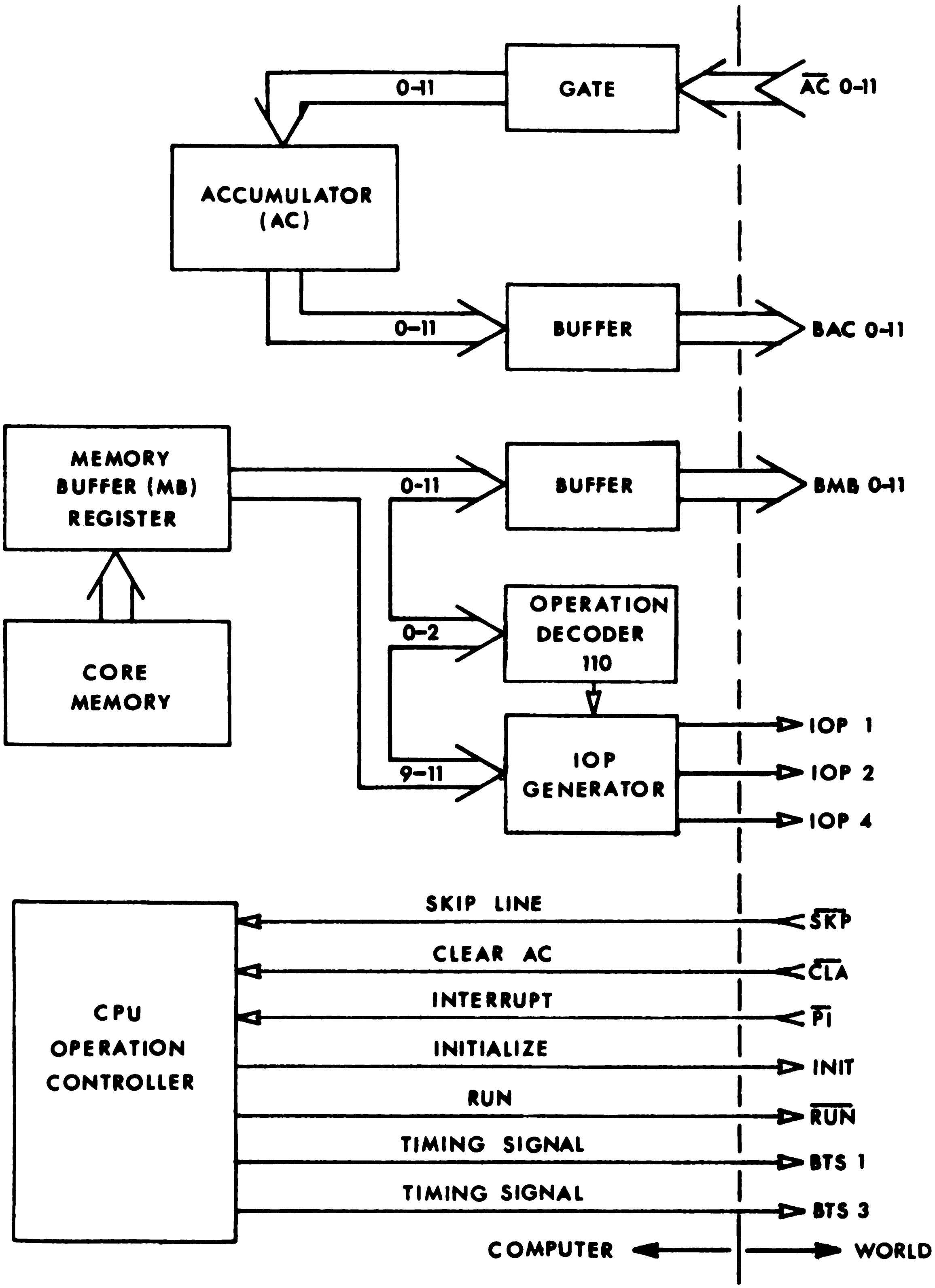


Figure 5. Computer Connections for Programmed Data Transfer.

Due to the speed with which the computer cycles through the instruction controlling programmed data transfers, each transfer must take place within a fraction of a microsecond. As a result there is a need for a high degree of synchronization between the program statements which instruct the computer to execute an information transfer and the existence and reception of the information being transferred. For example, to transfer a word from a device in the ECI to the accumulator, the $\overline{AC}$ I/O lines must be active during exactly the fraction of a microsecond in which the word to be transferred is both available and acceptable to the computer. Similarly, the transfer of data from the accumulator requires an exact synchronization between the existence in the accumulator of the word to be transferred and the acquisition of the word from the BAC I/O lines by the peripheral device.

To obtain the synchronization necessary for reliable information transfers between the computer and peripheral devices, the program statements which control the computer during the information transfers (I/O instruction) also generate the signals which make the synchronization possible. These signals are in the form of three data transfer timing pulses, IOP1, IOP2 and IOP4 which are generated by the computer during every I/O instruction. Depending on how the I/O instructions are written, they cause one, two or all three of the IOP pulses to appear at the IOP generator I/O lines shown in Fig. (5).

With the aid of the IOP pulses, synchronization between the individual events making up a data transfer is achieved. During transfer into the accumulator, an IOP pulse synchronizes the appearance on the $\overline{AC}$ I/O lines of the data to be transferred with the activation of the $\overline{AC}$ I/O lines. During transfers from the accumulator an IOP pulse is used to inform the appropriate device in the ECI of the exact moment at which data are to be acquired from the BAC I/O lines.

Because all information transfers are synchronized by the same identical IOP pulses, some means must be found to insure that each IOP pulse generated by an I/O instruction results in only a single information transfer being carried out. This is easily accomplished by assigning a code or device number to each device in the ECI with which the computer communicates. (This applies to other peripheral devices like the teletype whose device number is 03.) By also coding the IOP pulses, assurance can be had that each IOP pulse will initiate only the information transfer for which it is intended.

Decoding of the IOP pulses is accomplished by a circuit in the ECI called the device selector. This circuit examines all the IOP pulses arriving from the computer and allows each IOP pulse to activate only the device for which it was coded.

IOP pulses are coded by the manner in which the I/O instructions which generate them are written. Each I/O instruction which causes the computer to generate an IOP pulse also contains in its middle six bits the number of the device to which the IOP pulse is to be directed. As the computer executes the I/O instruction, these six bits along with the remaining bits of the I/O instruction, are routed along a third set of I/O lines to the device selector circuit where they arrive slightly before the IOP pulse. By arriving slightly before the IOP pulse, the middle six bits can be decoded before the IOP pulse arrives and then used to guide the IOP pulse to its proper destination. The I/O lines over which the I/O instruction word is brought to the device selector circuit are labelled BMB in Fig. (5).

All the information transfers that have been discussed require access to the computer $\overline{AC}$, BAC and BMB I/O lines. This access is provided directly in the ECI by devices known as I/O Patch Cards (Heath # EU-801-21). These devices consist of a printed circuit board placed in the ECI which is attached to a cable whose other end is plugged into the CIB. Depending on how the cable is plugged into the CIB, wire connectors on the printed circuit board in the ECI are linked directly to either the $\overline{AC}$, BAC or BMB I/O lines. In addition to providing these connections, the I/O Patch Cards also provide access in the ECI to all the computer operating signals such as the IOP pulses.

The devices which exist in the ECI to send and receive data and control words are known respectively as Gated Driver Cards (Heath # EU-800-JL) and Data Latch Cards (Heath # EU-800-FA). Each of these devices, which is assigned its own device code and can be activated by a properly coded IOP pulse, is connected to the appropriated set of I/O lines by an I/O Patch Card.

A Gated Driver Card is used to transmit information in the form of twelve bit words from some device in the ECI into the accumulator. During a data transfer, the Gated Driver Card is activated by an IOP pulse so that data present at the inputs of the card appears at its outputs during the time that the $\overline{AC}$ I/O lines are active and capable of transferring a word into the accumulator.

Information transfers from the computer to the ECI occur through Data Latch Cards. Upon reception of the properly coded IOP pulse, a Data Latch Card acquires from the BAC I/O lines the word currently contained in the accumulator and holds this word until instructed to obtain a new one.

IOP pulses are also used by the computer to determine when some action is required of it due to the completion of some event in the experiment sequence. This type of communication is carried out with the aid of the I/O SKP facility shown in the lower portion of Fig. (5). The I/O SKP facility is designed in such a way that when this terminal is driven to ground during the generation of an IOP, the computer skips the next statement in the program sequence.

The I/O SKP facility is used in conjunction with a device called a status "flag". The status flag is a circuit which provides a signal (a TTL voltage level) when some action is required of the computer. Under program control, the computer sends an IOP pulse to the flag circuits of each of the devices it must service. When a device flag is high, indicating that service is needed, the computer IOP pulse is combined by a NAND gate with its companion device selector pulse and the signal flag to drive the SKP I/O line to ground potential. This causes the computer to unconditionally skip the next program instruction and branch to a program sequence which is appropriat to the device being serviced. If the device flag is not raised, the computer either waits in a loop until the flag comes up or proceeds to check other devices which might need servicing.

3. The Role of Software in Computer Interfaced Experiments

The role of software in on-line computer applications is a sensitive function of the design of the experiment computer interface. Interfaces which are relatively monolithic and self controlling will often require little more from the computer than an IOP pulse to initiate some complex experimental sequence which is controlled by the interface until its completion. The software controlling such an interface will obviously be quite simple.

The danger of this approach is clear in regard to the prohibition of an active on-line application in which the computer alters the course of the experiment in attempts at optimization of the experiment. A much more fruitful approach is seen to be that in which the interface is made up of a number of simple devices, each of which controls one aspect or event in the experimental sequence. In such an interfacing situation, the possibility of writing software leading to an active on-line application is certainly much greater.

Support for the multiple device interface approach to interfacing is given by two software development principles put forth by Toren et al. (16). These seemingly disparate principles are: maximum system flexibility and minimum experimenter control. Adherence to the first principle results in software which gives the experimenter many options as to how the experiment is to be performed. A simple example of system flexibility can be found in the software controlling the computer interfaced photon counting spectrophotometer.

Because photon counting scans are time consumming and often take longer than planned, it is sometimes necessary to halt a scan prematurely. By including a software sequence in which the computer checks the position of a sense switch between data point acquisitions, orderly premature scan halts can be accomplished with no loss or distortion of data already acquired.

Adherence to the second principle of writing software for minimum experimenter control releases the experimenter for more productive efforts. An example of this would be a program sequence in which instrumental calibration data are automatically acquired by the system rather than being input by the experimenter.

The instructions that control a computer interfaced experiment are normally written in an assembly language which uses mnemonics to represent the actual binary instructions. The PDP8/I has eight basic instructions with which all computer functions must be performed. One of these instructions, the command to generate a coded IOP pulse has already been discussed. Another basic instruction causes operations on only the contents of the accumulator. Six other instructions are used to program all the remaining computer functions such as program branching and arithmetic operations.

Writing software in assembler languages is a difficult and time consumming task. It presents such a stumbling block that it is undoubtably the main hindrance to the widespread use of small laboratory computers. Programs involving complex arithmetic processing of data are especially difficult to write.

In an attempt to circumvent this hurdle, DEC has introduced the PS/8 Programming System (17). Using this system it is possible to write programs which are a combination of FORTRAN and assembler language statements. This represents a very real reduction in the problems associated with software preparation for on-line computer applications. Program sequences which interact with the ECI must still be written in assembler language. However, once the data words are in core they can be connected to FORTRAN variables and processed in that language. This programming system was used for all the software connected with the photon counting spectrophotometer.

C. Computer-Experiment Interactions Via the ECI

Information concerning three aspects of the experiment flows between the computer and the ECI. These are: (1) monochromator setting, (2) sample cell position and (3) photon counter pulse output rate. Each of these information flows requires an ECI circuit or circuits controlled by a program sequence. Because of the intimate relationship between the interface hardware and its controlling software, these two equally important aspects of the information flow process will be described jointly. The first and most simple interaction to be described is the one by which the computer controls the wavelength setting of the monochromator.

1. Monochromator Setting

Information flow between the computer and the ECI concerning the monochromator setting is strictly "one way". The monochromator can respond to commands but is not able to send any information to the computer concerning its current setting. This inability to communicate in a two way manner results in the experimenter having to initialize the monochromator before the scan begins by setting it at the starting wavelength. Other initialization includes telling the computer three scan parameters via the teletype: (1) the wavelength at which the scan begins, (2) the wavelength at which the scan ends and (3) the size of the increment between data points. Using this information the computer is able to control the monochromator setting from the initial to the final point of the scan.

The interfacing circuit through which the computer exercises control over the monochromator wavelength setting is shown in Fig. 6.

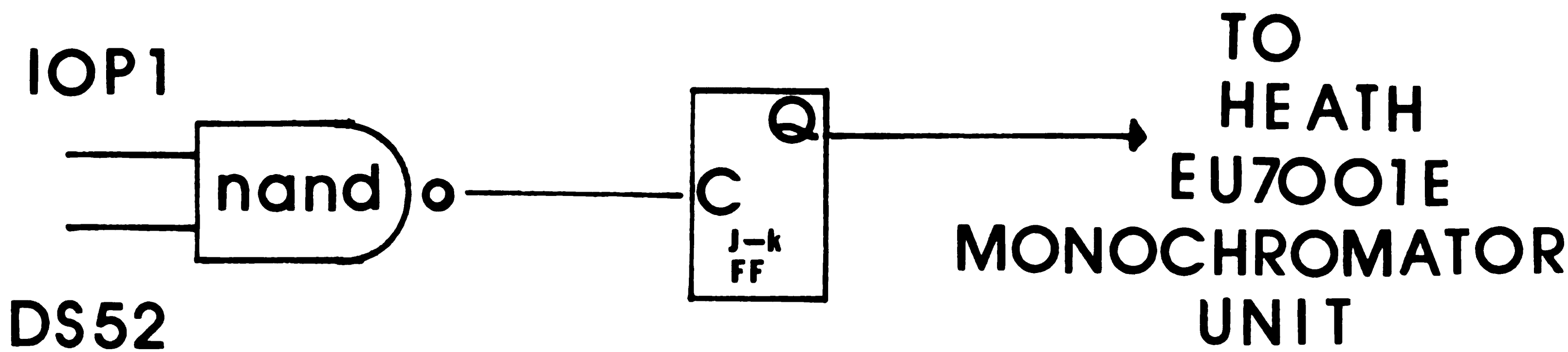


Figure 6. Monochromator Control Interface Circuit.

After generation by the computer and decoding, the pulses IOP1 and DS 52 are combined by a NAND gate prior to the clock input of a J-K flip-flop (FF). The waveform generated at the Q output of the FF is connected to the external programming jack of the Heath EU 700/E Monochromator Control Unit. Linked in this manner, the IOP1-DS 52 pulse combination serves as an oscillator signal and can be used to advance the Heath EU-700 monochromator in increments of 0.1 Å. The exact relationship between the number of computer pulses generated and the increase in the wavelength setting of the monochromator is determined by the SCAN RATE switch on the control unit. When this switch is set at 20 Å/sec, 60 computer pulses will increase the monochromator wavelength setting by one 1.0 A.

Knowing the number of pulses/Å required to advance the monochromator, it is a simple matter for the computer to advance the monochromator from one data point to the next. The computer need only multiply the number of angstroms between data points by sixty and use this number as a counter to control the number of times the command to generate the IOP is given.

This algorithm for monochromator control assumes that there is only one size increment between data points. This is not true when the number of angstroms covered by the scan is not an integral multiple of the scan increment. In this case, the increment between the last and next to last data points of the scan is some fraction of the specified increment and requires fewer pulses.

The flow chart of the algorithm adopted as providing the most flexible monochromator control is shown in Fig. 7. The initial and final wavelengths of the scan are used to calculate the total number

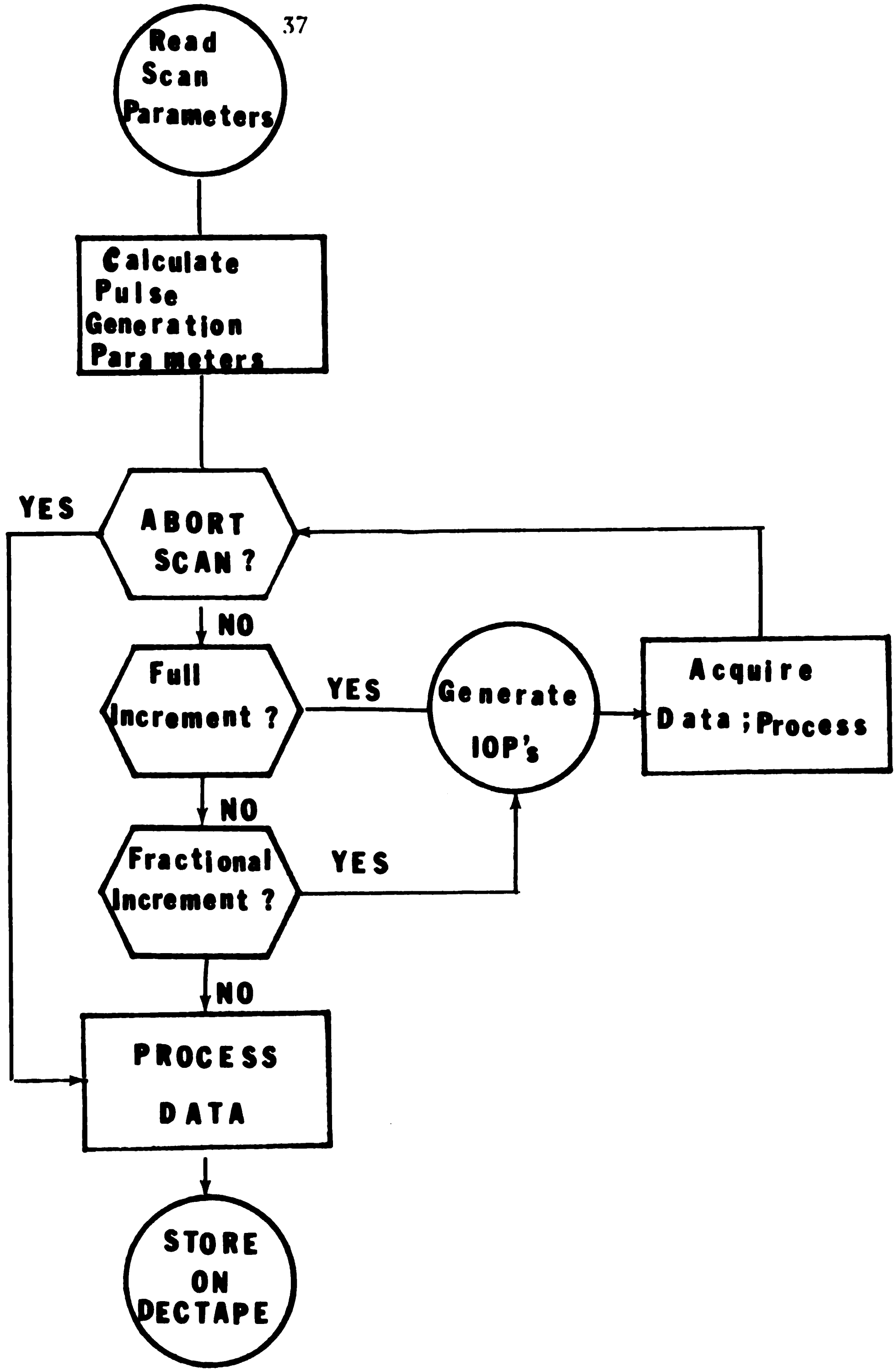


Figure 7. Flow Chart for Program Sequence Controlling the Monochromator.

of pulses required to step the monochromator through the desired spectrum. Similarly, the specified scan increment is used to calculate the number of pulses required to advance from one data point to the next. The number of complete scan increments necessary to advance the monochromator through the desired spectrum is equal to the integer portion of the quotient of these two numbers. The non-integer portion of this quotient, multiplied by sixty, gives the number of additional pulses necessary to complete the scan. This algorithm also allows the computer to determine when the scan has been completed by comparing the number of pulses generated with the total number required.

2. Sample Cell Position

The Heath EU-721-11 Alternating Cell Module is a member of the Heath EU-700 line of spectroscopy instrumentation. When used with the Heath EU-700 spectroscopy modules to construct an absorption spectrophotometer, its function is to move the sample and reference cells alternately in out of the optical path with a high degree of reproducibility. Within the module is a platform to which the cells are attached. The platform can assume two distinct positions which are labelled R and S for obvious reasons. By setting a mode switch on the module, the platform can be made to assume one of the two positions or to oscillate between them at a frequency of 2 or 4 Hz. When the module mode switch is set to PROGRAMMED, the cell assembly will oscillate at a 4 Hz rate until a low level TTL signal or ground is applied to the S or R pin of the programming terminal located on the back of the module. If the TTL LOW is applied to the S pin, the cell assembly will stop when the platform is in the S position and in a like manner the platform will stop the R position when that pin is LOW.

The external programming feature of the module allows the computer to exercise two options concerning platform position: (1) it can allow the platform to remain in its current position and (2) it can command the platform to move to the alternate position by applying a TTL LOW to the other programming pin. This degree of computer control is similar to that exercised by the computer over the monochromator. Under this type of control no information flows from the cell module to the computer. Software techniques to monitor platform position in this manner are easily applied. However, this technique of platform control places a heavy responsibility on the interface and instrumentation hardware. The platform must never fail to change position upon command from the computer and it must never change position except in response to a computer command.

This heavy responsibility placed on the hardware is lessened by taking advantage of another terminal on the Alternating Cell Module. This terminal, designated as the timing terminal, can be used by the computer as a source of information about the platform's position. Two pins, again labelled R and S, exhibit either a TTL HIGH or LOW voltage as a function of platform position. The timing terminal provides the means by which the computer can receive information from the module to answer two questions: (1) has the platform changed position in response to the command to do so?; and (2) what position is the platform currently in? The ability of the computer to answer these two questions is a major factor in the flexibility of the photon counting spectrophotometer.

The ECI circuits through which the computer communicates with the Alternating Cell Module are shown in Fig. 8. The twin pulses IOP4 and DS 52 or input to a NAND gate prior to the clock input of a J-K FF. A software statement to generate the coded IOP pulse toggles the FF and causes the Q and $\overline{Q}$ outputs to reverse states. This in turn reverses the TTL levels applied to the R and S pins of the programming terminal and causes the platform to change its position.

The interfacing circuit through which the computer determines platform position is also shown in Fig. 8. TTL levels present at the R and S pins of the timing terminal are wired to the bit 0 and 1 connections of a Gated Driver Card which is linked to the accumulator via the usual I/O Patch Card. Strobing the Gated Driver Card causes the status of the R and S timing terminal pins to be reflected in the first two bits of a word in the accumulator. Depending on the current platform position the binary word in the accumulator is either 101 111 111 111 or 011 111 111 111. How this platform status word can be used to determine if the platform has indeed changed position in response to a command is shown in the flow chart of Fig. 9.

Prior to ordering a platform move, the computer reads the platform status word and stores it in core. It then commands the platform to change position and waits for one second while the position change is accomplished. (The waiting time is controlled by the real time clock.) Following the wait period the computer again transmits to the accumulator the platform status word which it now compared with the platform status word obtained and stored prior to the move command. The comparison is done by means of a logical AND operation in which the first two bits of each word are compared to determine

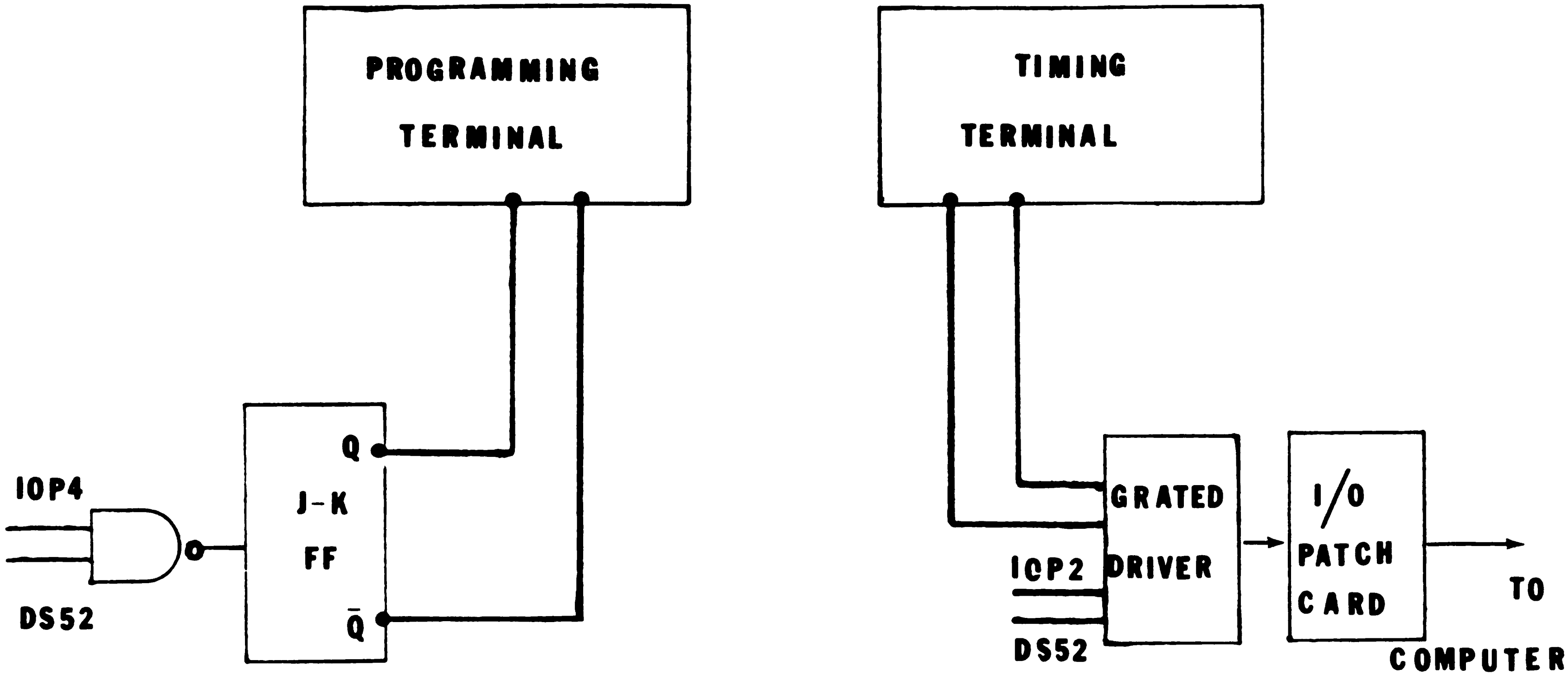


Figure 8. Alternating Cell Module Interface Circuit.

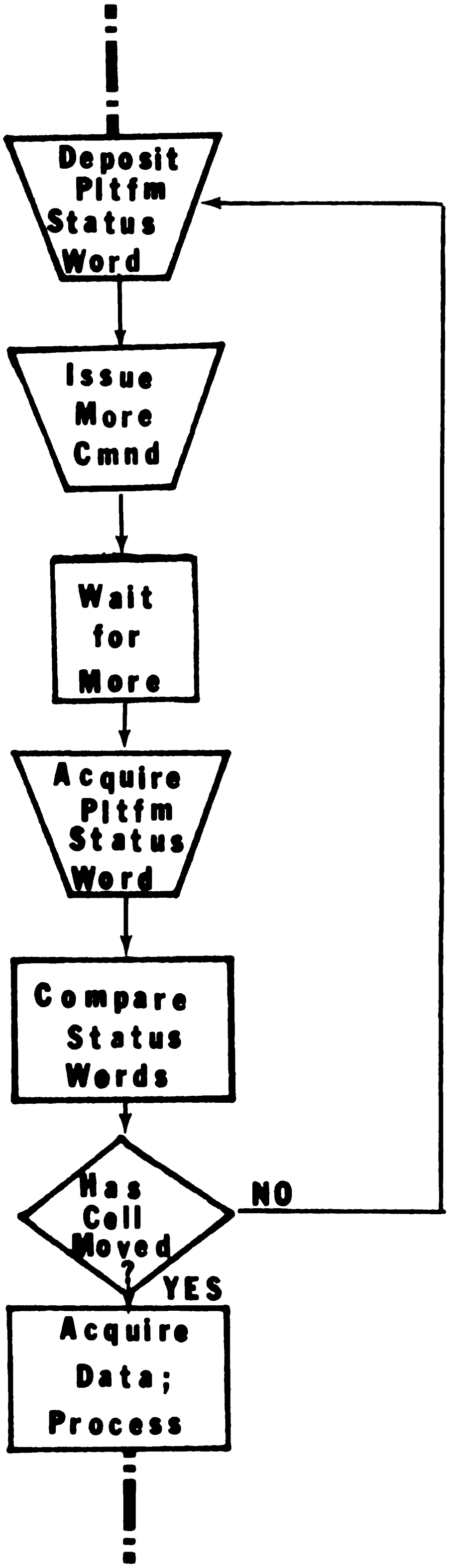


Figure 9. Flow Chart for Program Sequence Determining if Platform Has Responded to Move Command.

if they are the same or opposite. If they are opposite the platform has indeed changed position. If not, the computer reissues the move command until a successful platform position change is completed and verified.

The platform status word is also used to determine if the platform is currently in the R or S position. The flow chart for the program sequence controlling this decision is shown in Fig. 10. After the status word has been read into the accumulator, the state of the most significant bit is checked to determine if it is 1 or 0. Depending on the state of the first bit the computer jumps to one of two program sequences appropriate to the current platform position. Thus if absorption spectroscopy is being performed, the computer uses the first bit of the platform status word to determine if the sample or reference cell is in the optical path. If emission spectroscopy is being performed, the status word is used to determine if signal or background counts are being recorded.

3. Photon Counter Pulse Rate Measurement

Computer controlled measurement of the rate at which pulses are emitted by the photon counter is of course one of the most attractive features of an on-line photon counting spectrophotometer. As has been discussed, the volume and diversity of the rate measurements almost makes such computer control a mandatory feature of a photon counting system.

Measurement of the photon counter pulse rate is performed in the frequency mode by determining the number of pulses, N, that occur during a time interval ΔT. These two quantities are used to form the ratio

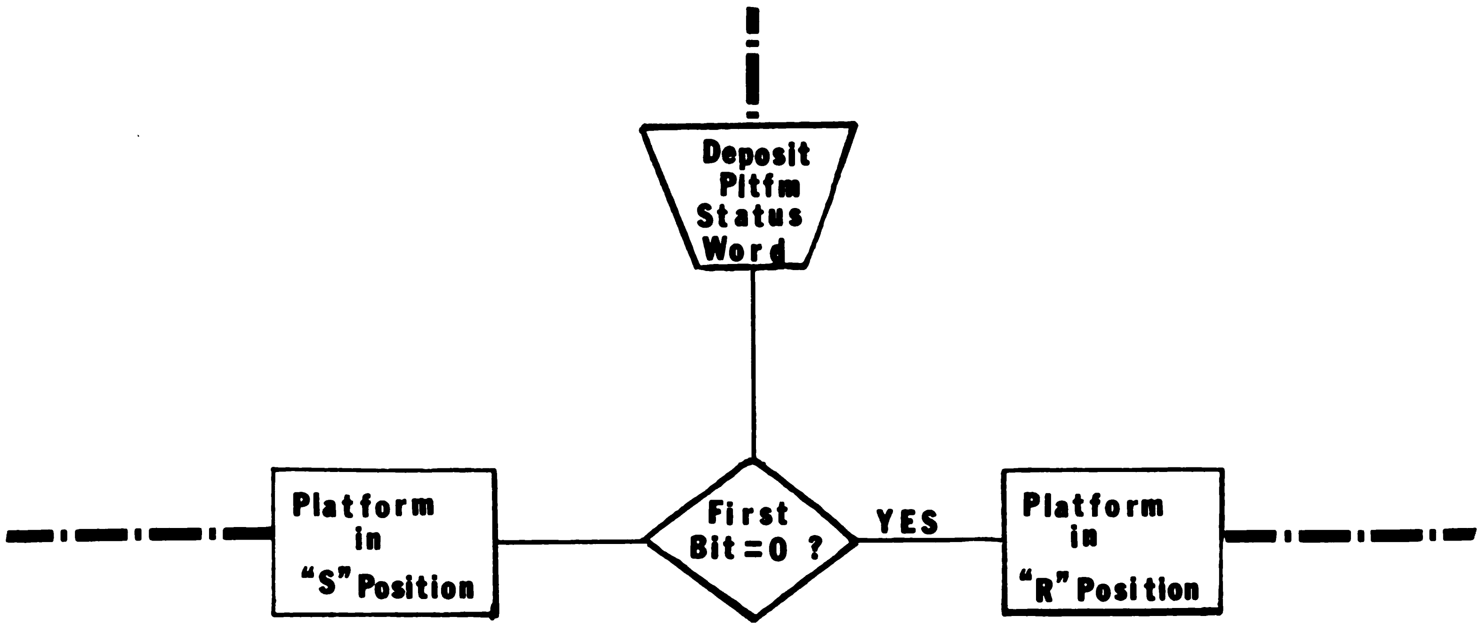
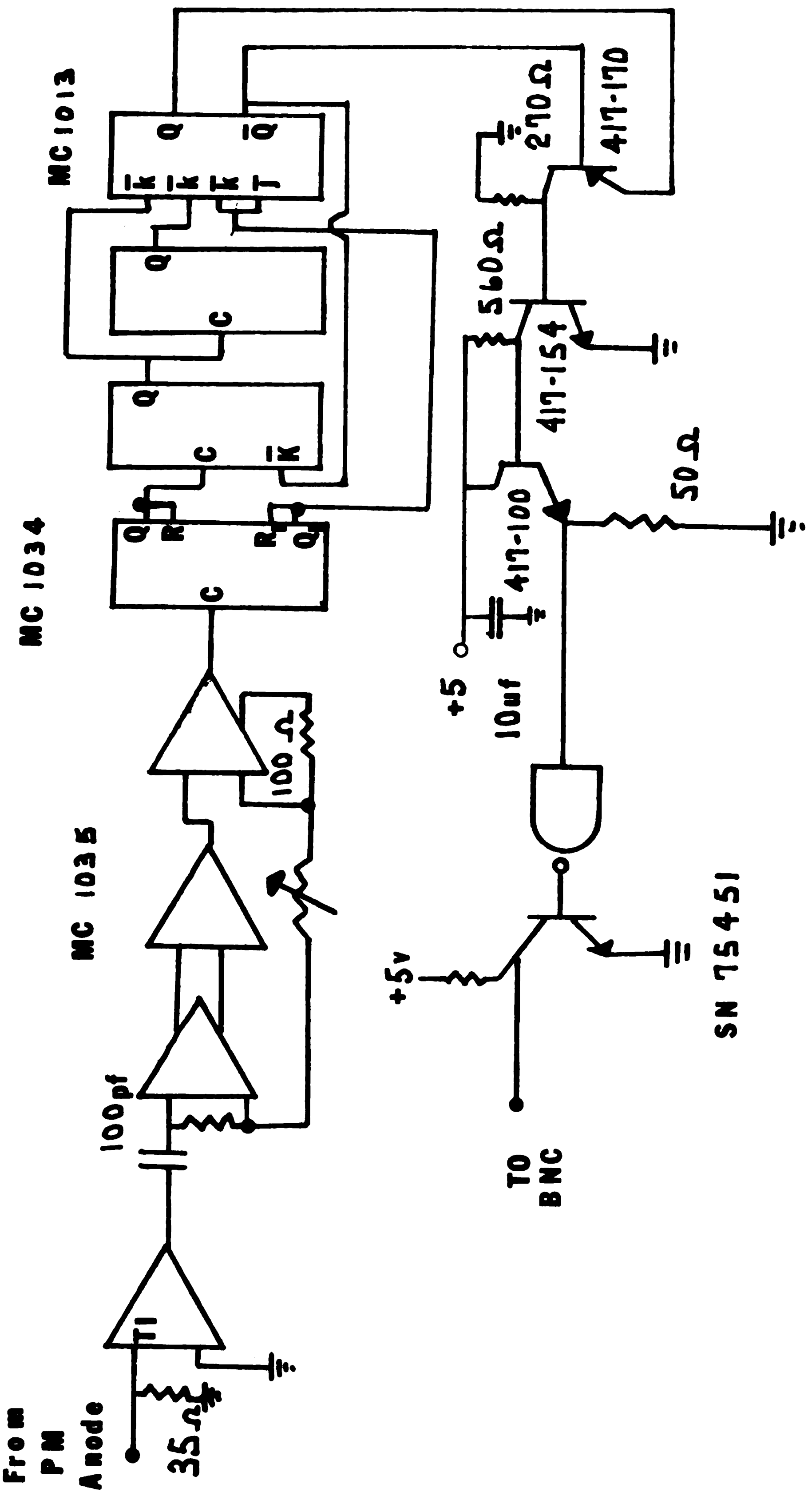


Figure 10. Flow Chart of Program Sequence to Determine Platform Position.

N/ΔT (pulses/sec) which is the average frequency with which pulses are emitted by the photon counter. For maximum system flexibility, the ECI circuits were designed to allow the computer to control the following two aspects of each frequency measurement: (1) initiation of the measurement and (2) the length of the measurement time interval ΔT. As will be shown, computer control of these two parameters is essential to the optimum performance of the system.

a. Photon Counter. A discussion of the manner in which frequency measurements are made on the photon counter output is best prefaced by a discussion of the photon counter itself. The photon counter currently being used in this instrument is a modification of a design used by R. Jarret (18). A circuit diagram is shown in Fig. 11. Charge pulses from the photomultiplier anode are converted to voltage pulses by connecting the anode to ground through a 35Ω resistor. The voltage pulses across the 35Ω resistor are amplified by a TI 5510 wide band amplifier and ac-coupled to the input of a Motorola MC 1035 Dual Schmidt Trigger/Triple Differential Amplifier integrated circuit which serves as a discriminator. The discriminator threshold level is adjusted by a potentiometer which introduces a hysteresis effect due to feedback between the output and input of the third differential amplifier. Increasing the resistance of the potentiometer causes a greater degree of feedback hysteresis and increases the amount of discrimination by raising the threshold voltage of the amplifier. Following the discriminator, the pulses are shaped by a MC 1034 Type D FF prior to passage through a divide-by-five frequency scaler made up of two integrated circuits - a MC 1032 100 MHz AC Coupled Dual JK FF and a MC 1013 85 MHz AC Coupled J-K FF.

Figure 11. Diagram of Photon Counter Circuit.



From PM Anode
35Ω
TI
100pf
MC 1035
100 Ω
MC 1034
MC 1032
MC 1013
+5
10uf
417-100
417-154
560Ω
270Ω
417-170
50Ω
+5v
SN 75451
TO BNC

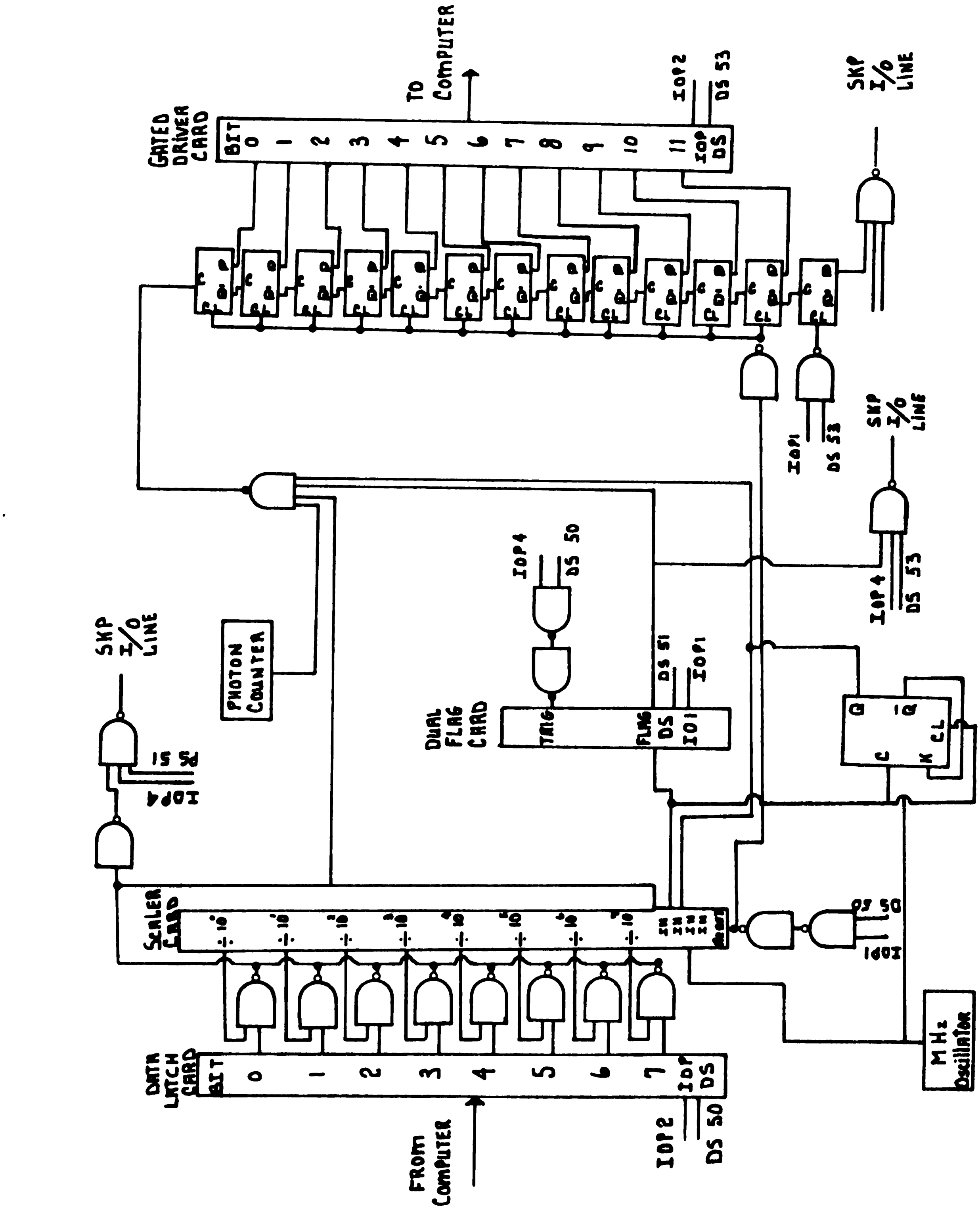
The four Motorola integrated circuits which make up the discriminator-scaler portion of the photon counter circuit utilize high speed emitter coupled logic (ECL). The use of such high speed integrated circuits maintains the frequency response of the photon counter by reducing dead time loss due to pulse pile up at the discriminator. Scaling the pulse train frequency by additional high speed ECL IC's preserves the frequency response of the photon counter by reducing the pulse rate to a level easily transmitted and sensed by the ECI's TTL IC's.

The output of the ECL type IC's is not compatible with the TTL components of the system. An ECL-to-TTL interface is performed by three transistors after which the pulses are transmitted to the photon counter output by a TI SN 75451 Dual Open Collector Line Driver.

To minimize noise the photon counter circuit is enclosed in a metal chassis which is kept at ground potential. Input and output connections are made through BNC terminals.

b. ECI Circuits and Software. The interface circuits through which the computer measures the frequency of the photon counter output are shown in Fig. 12. These circuits can be divided into three groups: (1) those which enable the computer to select and measure a time interval ΔT, (2) circuits through which the computer receives information concerning how many pulses N were emitted by the photon counter during the time interval ΔT and (3) control circuits which allow the computer to initialize the interface prior to a measurement and initiate the measurement.

Figure 12. Interface Circuits for Measuring Photon Counter Output.


TO COMPUTER
GATED DRIVER CARD
BIT
0
1
2
3
4
5
6
7
8
9
10
11
IOP
DS
IOP2
DS 53
SKP I/O LINE
IOP1
DS 53
SKP I/O LINE
IOP 4
DS 53
IOP4
DS 50
PHOTON COUNTER
DS 51
IOP1
DUAL FLAG CARD
TRIG
FLAG
DS
IO1
SKP I/O LINE
IOP4
DS 51
SCALER CARD
MHz Oscillator
DATA LATCH CARD
FROM COMPUTER
IOP2
DS 50

The measurement process is conceptually very simple. Upon command from the computer, pulses of a known frequency from a crystal oscillator and pulses from the photon counter are simultaneously fed into two separate electronic counters. After a computer-determined number of pulses from the crystal oscillator have been accumulated, electronic gates to both counters are simultaneously closed preventing any additional pulses from being counted. The closing of the counter gates also serves to signal the computer that the measurement has been completed. The computer now examines the contents of the counter in which pulses from the photon counter were accumulated and determines the number of pulses N. Knowing the number of accumulated oscillator pulses, the computer need only have the frequency of the oscillator clock signal in order to calculate the frequency of the photon counter output.

The ECI circuits which gate and count the oscillator clock pulses are shown in the left side of Fig. 12. A clock signal of known frequency is supplied by a Heath EU-800-KC 1 MHz Crystal Time Base Card. This card contains a crystal oscillator and produces TTL level clock signals with frequencies from 1 MHz to 0.1 Hz. The long term accuracy of the signals are one part per million.

During a measurement the oscillator clock pulses are accumulated in a Heath EU-800-CA Scaler Card. This card contains a series of seven decade counting units (DCU) which divide the frequency of an input signal in successive tenfold or decade steps. Waveforms illustrating this frequency reduction by a DCU are shown in Fig. 13. Note that ten pulses applied to the input of the DCU results in one pulse at the output. Note also that the DCU output goes from a LOW

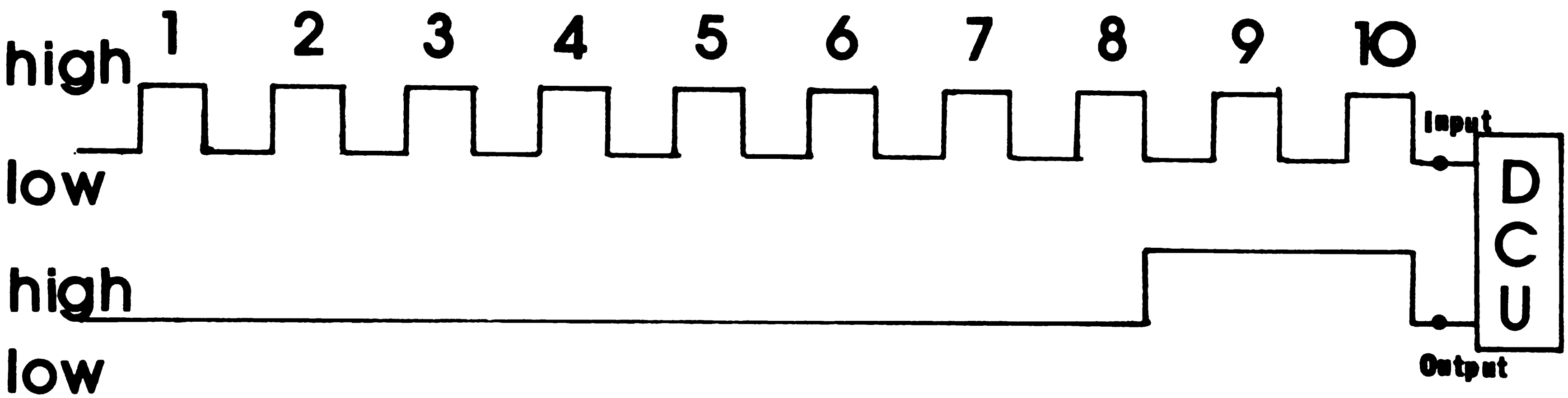


Figure 13. Decade Counting Unit Wave Forms.

to HIGH after eight pulses have been applied to the input. Using this logic level change in the output, the DCU can serve as a counter capable of indicating when eight pulses have been accumulated. A counter capable of indicating when eight and/or eighty pulses have been accumulated can be easily constructed by linking the output of one DCU to the input of another. Since each DCU divides the input frequency by ten, ten pulses applied to the input of the first DCU will result in one pulse at the input of the second DCU. This means that eighty pulses applied to the input of the first DCU will result in eight pulses being applied to the second DCU causing its output to go HIGH. Since the Scaler Card has seven DCU's linked in series it can be used as a counter to indicate when 8×10^0, 10^1, 10^2, 10^3, 10^4, 10^5, or 10^6 pulses have been accumulated.

The ability to indicate when some decade multiple of eight pulses has been accumulated allows the Scaler Card DCU's to be used to measure a precise time interval. For example, following the application of a 1 MHz signal to the input of the first DCU, exactly eight sec will pass before the output of the seventh DCU goes HIGH. This corresponds to 8×10^6 microsecond pulses passing through the first DCU and eight pulses being accumulated by the seventh DCU.

The computer uses the Scaler Card to measure time intervals in exactly this fashion. A command from the computer opens an electronic gate and applies the clock signal of known frequency to the input of the first DCU. The computer chooses the length of the time interval it wishes to measure by selecting which DCU output is allowed to signal the end of the measurement time interval. Thus if a 1 MHz signal is applied to the first DCU input and a time interval of 0.8 sec is desired, the computer will wait for the output of the sixth DCU to go HIGH.

Selection of which DCU output will indicate the end of the measurement time interval is made by the computer via a control word held in a Data Latch in the ECI as is shown in Fig. 12. Each of the first seven bits of the control word is linked individually through a TI SN 7401 Open Collector NAND gate to one of the seven DCU outputs. (When the output of an Open Collector NAND gate is HIGH it exhibits an infinite resistance to ground. When it is low it acts as a short circuit to ground.) To specify which DCU output will signal the completion of the time interval, the computer generates a control word such that when exported to the Data Latch, the most significant non-zero bit is input to the same NAND gate as the DCU output of interest. Thus, to observe the transition from LOW to HIGH of the sixth DCU output, the computer generates and exports the control word 000 000 111 111. The sixth most significant bit of this work is a "1" which, when combined with the HIGH or "1" of the sixth DCU output via the NAND gate, will drive the SKP BUS to ground and signal the computer that the measurement has been completed. By rotating the most significant non-zero bit of the control word either right or left, the computer can select the DCU output it wishes to observe and thus the time interval it wishes to measure.

The computer command which begins the frequency measurement by opening the gate of the Scaler Card also simultaneously opens the gate of the counter in which pulses from the photon counter are accumulated. The photon counter register is made up of twelve J-K FF's linked together as shown in the right side of Fig. 12 to form a basic binary counter. In a counter such as this, each successive FF represents a binary digit and the sum of the values of the FF outputs represent the cummulative count at any instant. The operation of the basic binary counter is shown in Fig. 14.

A binary counter composed of twelve FF's exhibits 4096 different states which means it can count from 0 to 4095 before returning to its zero state on the 4096th pulse. Obviously a counter capable of counting only 4095 pulses is not adequate for the vast majority of photon counting measurements. Fortunately it is a simple matter to extend the capacity of this counter to well over a million by instructing the computer to record not only the number of pulses present in the counter at the end of the measurement interval, but also the number of times the counter overflows during the measurement. The computer records the number of overflows with the aid of a 13th J-K FF attached to the output of the 12th counter FF. After 4095 pulses have been counted, the output of the 12th FF goes LOW as the counter returns to its zero state. This change of logic levels clocks the 13th J-K FF causing its Q output to go HIGH and in the process alerts the computer via the SKP BUS that an overflow has occurred. Noting the overflow, the computer clears the 13th flip-flop and increments an overflow counter word held in core and thus keeps a running total on the number of overflows occurring during each measurement.

When the measurement has been completed, multiplying the number of overflows by 4096 and adding the contents of the binary counter at the time the measurement interval ends, gives the total number of pulses emitted by the photon counter during the measurement. The

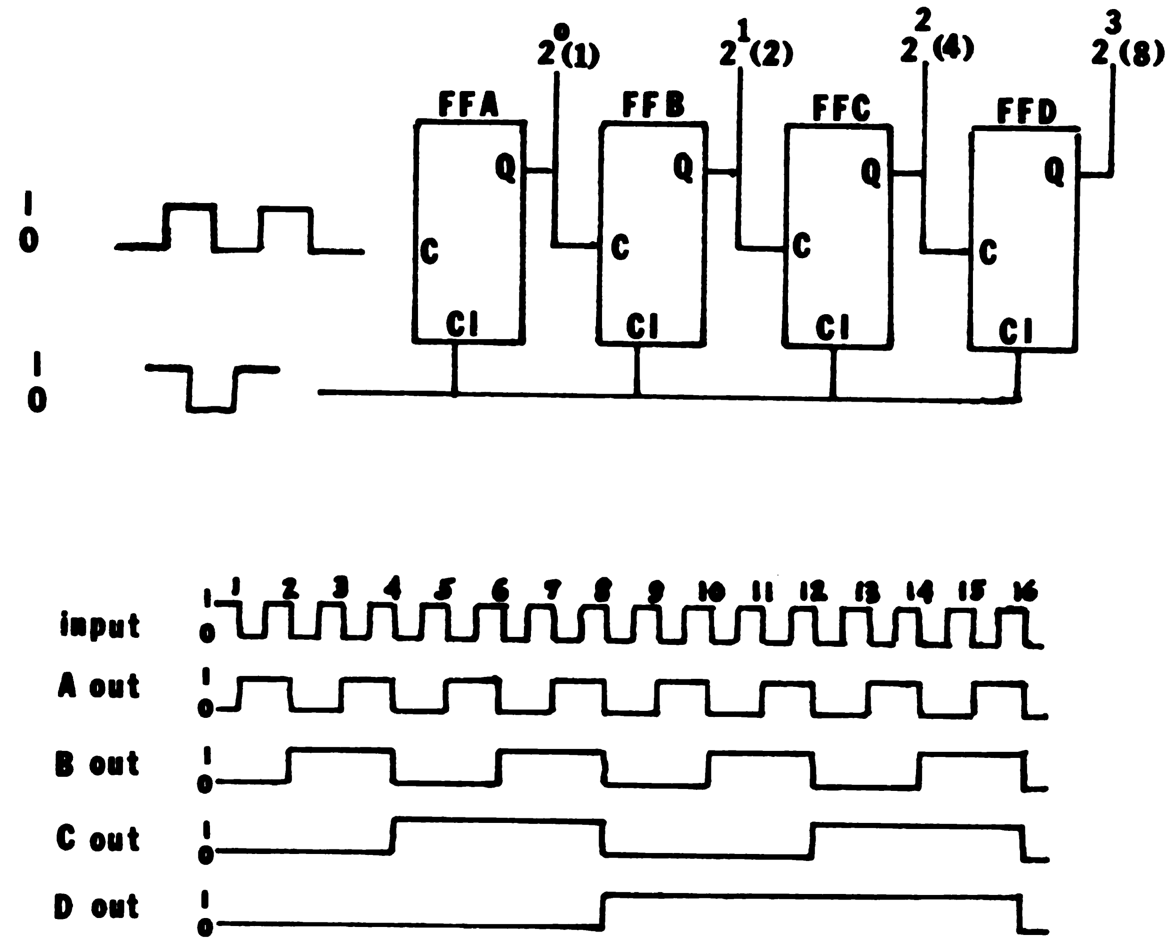


Figure 14. Basic Binary Counter and Waveforms.

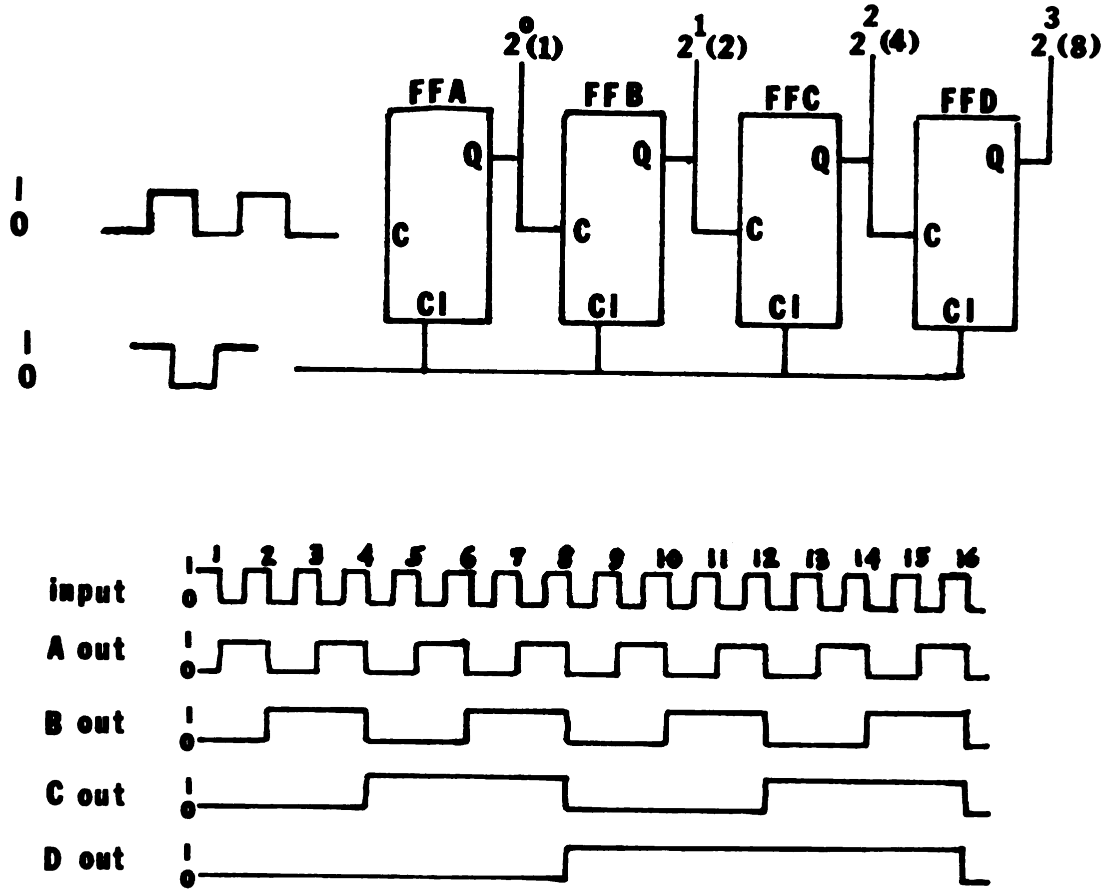


Figure 14. **Basic Binary Counter and Waveforms.**

number of counts present in the binary counter at the end of the frequency measurement time interval is represented by the logical states of the Q outputs of the 12 J-K FF's making up the counter. Through a Gated Driver Card, all the FF states are transmitted to the computer to form a binary word in the accumulator. Decoding this word gives the computer the decimal number of pulses in the counter at the end of the measurement.

In the center of Fig. 12 are shown the circuits through which the computer initializes the interface prior to a measurement and then initiates the measurement. Interface initialization involves setting the interface counters to zero and clearing all flip-flops. This is done by strobing the CLEAR inputs of the various IC's with IOP's. To initiate a measurement, the computer uses the FLAG output of a Dual Flag Card to simultaneously open the NAND gates which preceed the two counters. To remove triggering error or jitter, the JK FF in the lower center of Fig. 12 synchronizes the opening of both gates with the arrival of an oscillator pulse. This means that no fractional oscillator pulses are counted by the Scaler Card as it determines the measurement time interval.

c. Software. A flow diagram of the software controlling each frequency measurement is shown in Fig. 15. After initializing various program constants, the computer formulates the control word which determines the length of the measurement interval and exports it to the Data Latch Card contained in the ECI. Next the computer initializes the interface by clearing all flags and setting the counters in their zero states. Upon command from the computer the electronic gates open and the measurement time interval is begun. As a safeguard, the

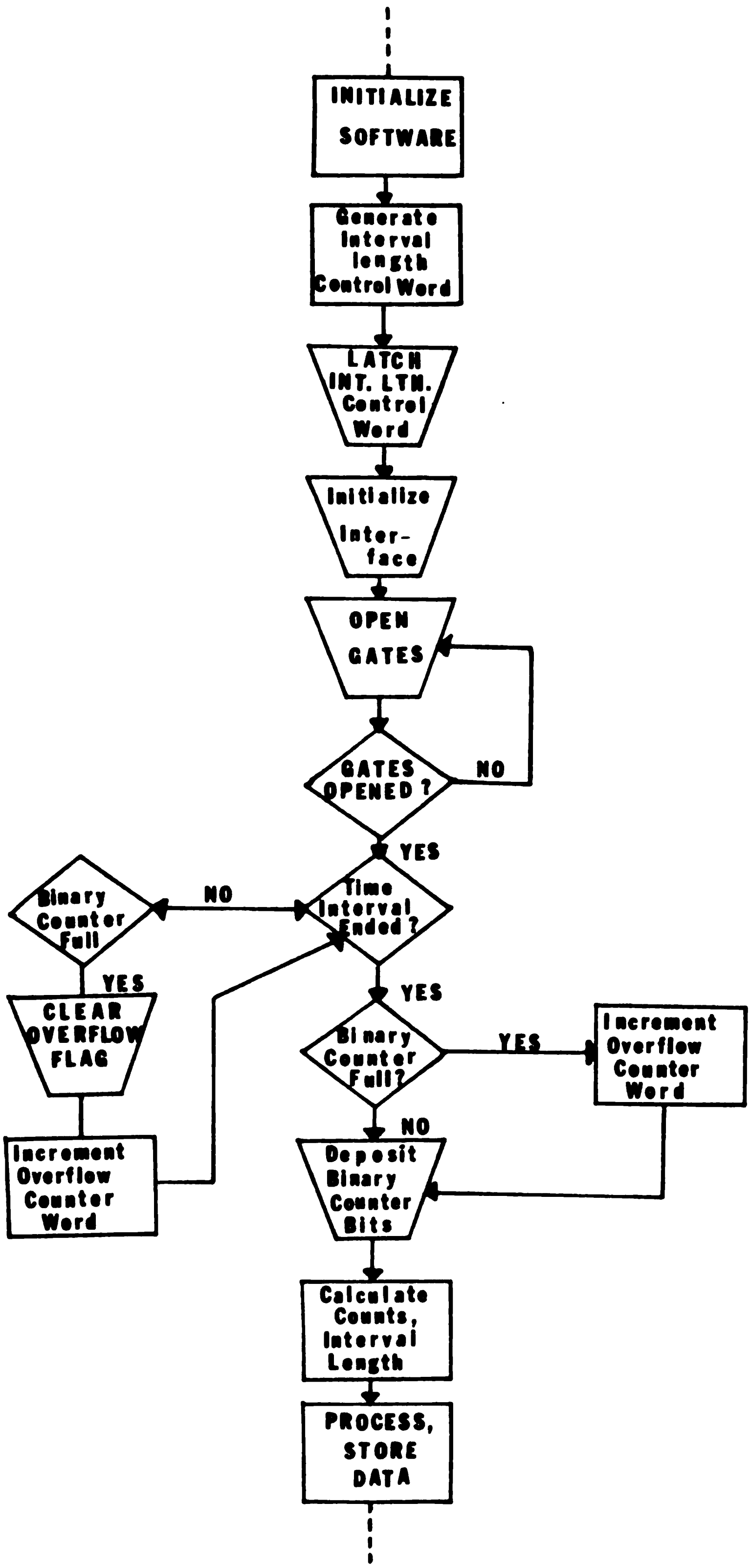

INITIALIZE SOFTWARE
Generate Interval length Control Word
LATCH INT. LTH. Control Word
Initialize Inter-face
OPEN GATES
GATES OPENED ?
NO
YES
Time Interval Ended ?
NO
Binary Counter Full
YES
CLEAR OVERFLOW FLAG
Increment Overflow Counter Word
YES
Binary Counter Full?
YES
Increment Overflow Counter Word
NO
Deposit Binary Counter Bits
Calculate Counts, Interval Length
PROCESS, STORE DATA

Figure 15

computer checks to make sure that the gate opening command was obeyed. After initiating the measurement, the computer must be aware of two events. It must know when the binary counter accumulating counts from the photon counter has overflowed and it must know when the selected DCU output has gone HIGH to signal the end of the measurement. Thus, as the flow diagram indicates, the computer must constantly check the condition of the flags whose rising indicates the occurrance of these two events. Each time a flag is raised indicating an overflow, the computer must clear this flag, increment the overflow counter word and then check to see if the measurement time interval has ended while it was performing these tasks.

After the DCU output has gone HIGH indicating to the computer that the measurement has ended, the computer performs one last check on the counter overflow flag to determine if an overflow occurred just prior to end of the measurement time interval. Following this check the computer transfers the contents of the binary counter into the accumulator and combines this count with the overflow count data to compute the total number of pulses emitted by the photon counter during the frequency measurement. After decoding the interval length control word to determine the length of the measurement interval, the computer proceeds to a program sequence which processes the newly acquired frequency data.

D. Evaluation of Computer Experiment Interactions

Prior to performing spectroscopic measurements, the operation of each of the interfaced components was evaluated individually. Of special interest in the evaluation was the long term reliability of the interfacing circuits. This operational aspect was stressed because of the anticipated long time periods required to obtain low intensity spectral data.

1. Monochromator Control

No problems were encountered in the computer control of the monochromator setting. The simplicity of the interfacing circuits and the reliability of the Heath instrumentation precluded any major difficulties in either the hardware or software. Advances in the monochromator wavelength setting involving hundreds of increments were carried out with a perfect correspondance between the actual wavelength and that assummed by the computer.

2. Platform Position Control

Problems were initially encountered in controlling the sample cell platform position by the computer. It was found intermittantly that commands from the computer to move the platform were not obeyed. This caused the computer to process data out of sequence and thus effectively ended the scan at that point. This was especially annoying when it occurred early in an overnight scan. As has been discussed, this problem was solved by opening a new information channel through which the computer can determine if its move command has been obeyed. Subsequent control of the sample cell position by the computer has been flawless.

3. Photon Counter Output Measurement

Two aspects of the computer controlled measurement of the photon counter output needed evaluation. First it had to be demonstrated that the computer controlled interface could perform accurate frequency measurements on a clock signal and secondly it had to be demonstrated that the photon counter output rate was accurately reflecting the light intensity striking the detector. The second was decidedly more difficult than the first.

To evaluate the operation of the interface as a frequency meter, measurements were made on signals of accurately known frequencies obtained from a crystal oscillator. After each measurement the computer printed out the measurement time interval and the number of oscillator counts recorded. Very good results were obtained. It was found that the measurements were accurate to within $\pm$ 1 count. This is the theoretically expected uncertainty due to the fact that the beginning of the measurement interval is not synchronized with the signal being measured. The long term stability of the oscillator supplying the time base for the frequency measurements was found to be within the manufacturer's specification of 1 PPM.

Considerable more difficulty was encountered in the initial operation of the photon counter. During scans lasting several hours spuriously high count rates were observed. The problem was solved by reducing the value of the resistor connecting the photomultiplier anode to ground. The original value of this resistor had been 50Ω which seemed very reasonable in view of the fact that this value resistor produces a voltage pulse of about one millivolt assumming that the peak current of an average pulse is about 10^{-5} amps. However, upon inserting resistors having still lower values, it was found that the problem of random high pulse rates disappeared. A value of 35Ω was ultimately chosen and is currently in use.

Following this modification the photon counter has operated very well in conjunction with a RCA IP28 photomultiplier tube contained in the Heath EU-701-30 photomultiplier module. When operated at 950 volts, the photon counter showed a typical IP28 tube to have a dark count from about 350-500 counts/sec depending on the discriminator level. To test for spurious noise pulses the output of photon counts was observed after power to the photomultiplier tube had been shut off. No noise pulses were observed. To determine the range over which the count rate is a linear function of the photocurrent, the log of the count rate was plotted against the log of the photocurrent. Such a plot is shown in Fig. 16. Good linearity is obtained for photocurrents up to about 10^{-7} amps. For photocurrents larger than this, the degree of non-linearity due to pulse overlap increases.

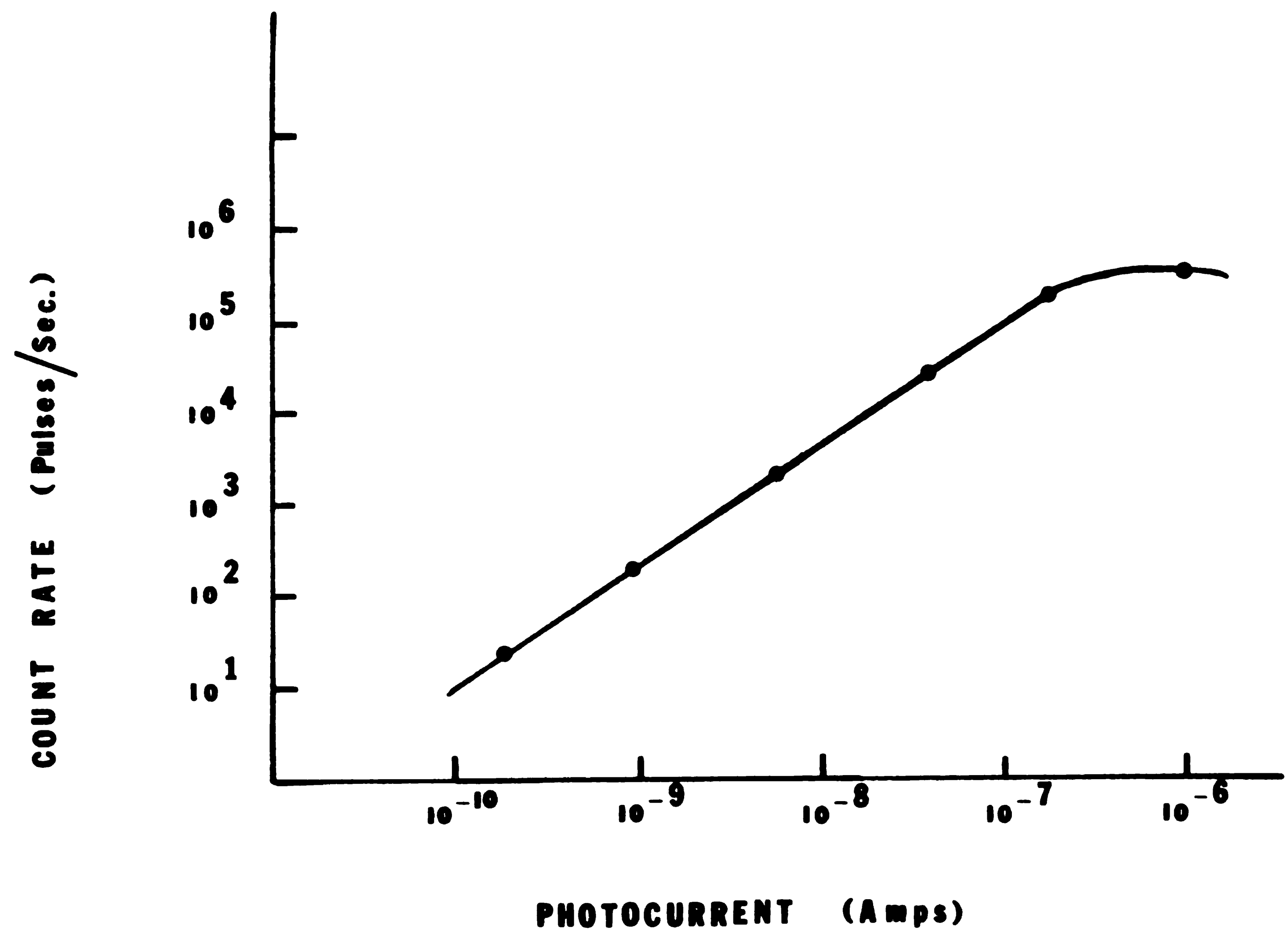


Figure 16. Plot of Count Rate vs Photocurrent.

IV. SYSTEM NOISE FILTERING CAPABILITIES

Earlier discussions have proceeded under the assumption that the only noise to be contended with in photon counting measurements was the inherent quantum noise caused by the nature of light itself. Based on this assumption, simple expressions were derived showing how the S/N of any photon counting measurment could be improved by simply increasing the length of the measurement time interval.

Unfortunately, this assumption concerning the absence of any noise except photon noise is not completely accurate. Other noise sources in addition to quantum noise do exist and by their existence invalidate to a certain extent the previously derived S/N expressions. For lack of a better term, the label "excess noise", N_{ex}, has been applied to the noise which exists in the photon counter output in addition to quantum noise.

Since quantum noise is random or frequency independent, excess noise is that part of the noise which is frequency dependent. The origins, effect on a measurement S/N, and rejection of excess noise are some of the subjects of this chapter.

A. Discussion of Excess Noise

1. Evidence for Existence

Excess noise in the form of drift in the photon counter output can be verified by observing the standard deviation among the means of a large number of averaged photon counting measurements. From statistics it can be shown that for random events the standard deviation of the means of sets of averaged measurements is equal to the standard deviation of the individual measurements divided by the number of measurements in each set. Since for phenomena governed by Poisson statistics the standard deviation of a single measurement is equal to the square root of its mean, it is a simple matter to calculate the expected standard deviation of the mean of sets of averaged repetitive photon counting measurements. This is shown in eq (15) in which N is the number of photon counting measurements per set and $<X>$ is the average number of counts acquired in each individual measurement.

$$\sigma_m = \frac{\sigma}{\sqrt{N}} = \frac{<X>^{1/2}}{\sqrt{N}} \tag{15}$$

To show that excess noise is present in the photon counter output it is only necessary to show that the standard deviation of the mean of sets of averaged repetitive photon counting measurements is greater than that given by eq (15). Such evidence was acquired by monitoring the photon counter background count rate for a period of about ten hours. During this time, 1200 sets of measurements were accumulated with each set being the average of eight individual measurements having a measurement time interval of 0.8 sec. During the ten hours test period the average number of counts acquired in each of the 0.8 sec measurements was about 330.

To evaluate the acquired data for indications of excess noise, the 1200 measurements were themselves averaged in groups of sixty to obtain a total of twenty mean measurements. From eq (15) it can be seen that the theoretical standard deviation among the twenty mean measurements should be slightly less than one count:

$$\sigma_m = \frac{(330)^{1/2}}{(8 \times 60)^{1/2}} \approx \frac{18.2}{21.9} \approx 1.0$$

A plot of the twenty mean counts vs time is shown in Fig. 17. As can be seen, the scatter in the means is far greater than would be expected for a purely random Poisson phenomenon. The wide scattering of the means as a function of time indicates quite clearly that excess noise in the form of drift is contributing heavily to the photon counter output.

2. Origins of Excess Noise

There are a number of possible sources of excess noise in the photon counting detection system. There may be after pulses in the photomultiplier tube which result in one photon generating several counts. Despite shielding, random pulses may be generated by electrical pick up of stray fields which may occur in short bursts. The gain and dark count of the photomultiplier tube may vary with time, due to temperature, previous exposure to light and other effects. The electronic components of the system such as the high voltage power supply, discriminator and pulse amplifier may also vary with time, either as a slow drift or in sudden changes. Fluctuations in the dicriminator threshold level are especially critical. At low discriminator levels, excess noise due to such fluctuations can be relatively unimportant. At high discriminator levels however, where the rate of change of the observed count rate with discriminator

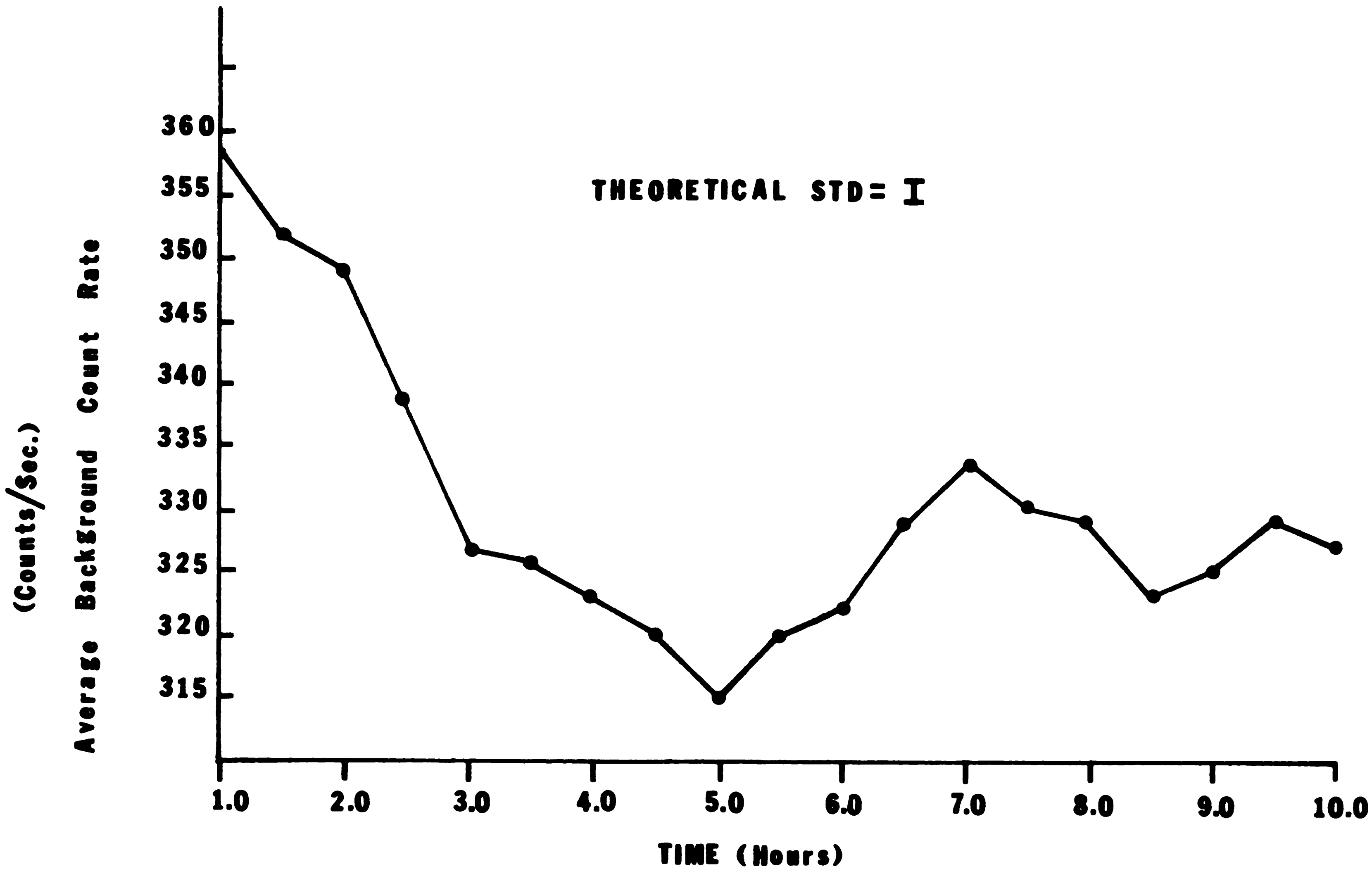


Figure 17. Plot of Average Background Count Rate vs Time.

level is large, fluctuations in the discriminator threshold level can be a major source of excess noise.

3. Ramifications of Excess Noise

Excess noise has been shown to exist in the photon counter output in the form of a slow drift in the averaged observed count rate. The primary consequence of the presence of excess noise is to invalidate to a certain extent the Poisson description of the count rate statistics. Poisson statistics are invalidated because a primary prerequisite for a phenomenon governed by Poisson statistics is the existence of a mean which is stable over the measurement period and around which the magnitude of the phenomenon always varies in a random manner. The presence of drift in the photon counter output adds a time dependence to the average count rate and thus the distribution of fluctuations in the observed count rate is something other than Poisson.

Excess noise can be best understood by considering the noise power which it induces in the photon counter output. Denoting the total noise power of the photon counter output by $N(\omega)$ and letting N_p and $N_{ex}(\omega)$ represent the noise power of quantum and excess noise respectively, and remembering that N_{ex} is that part of the total noise which is frequency dependent, the simple equation shown below can be written (13).

$$N(\omega) = N_p + N_{ex}(\omega) \tag{16}$$

Long experience with drift or 1/f noise has shown that the noise power of drift noise can be expected to increase rapidly as the frequency decreases. This supposition is born out by a crude frequency analysis of the drift rate observed in the output of the photon counter which shows a noise power maximum at a frequency of approximately 0.005 Hz. Because of the very low frequency at which the excess noise becomes important, its effect on the S/N of a photon counting measurement is not felt until long counting times are used.

The effect of excess noise on photon counting measurements is most cogently described in terms of the S/N expressions governing the measurements. The deviation of these expressions is based on the assumption that signal and background count rates can be described by Poisson statistics. If this assumption is not valid, the standard deviation or noise in a photon counting measurement can not be simply equated with the square root of the mean count rate with the result that the previously derived S/N expressions are incomplete. As eq (17) shows, an additional noise term is needed in the denominator to account for the increased measurement standard deviation due to excess noise.

$$S/N = \frac{N_S + N_B - N_B}{(N_S + 2N_B + N_{ex})^{1/2}} \tag{17}$$

From eq (17) it is seen that the effect of excess noise in decreasing the S/N of photon counting measurement is greatest for low intensity measurements in which the number of signal counts is less than or equal to the number of background counts. Because this is exactly the light intensity region in which photon counting techniques are most effective, it is imperative that some technique be found for rejecting the excess noise.

Fortunately, the long experience with drift noise which has shown that the noise power of drift noise is greatest at low frequencies has also given rise to techniques for filtering out drift noise. These filtering techniques, called modulation or synchronous detection techniques, rely on the frequency dependence of the excess noise as a means of rejecting it. This filtering can be done, as the next section will show, with such a high degree of effectiveness that excess noise is virtually eliminated as a problem.

B. Synchronous Detection Techniques for Excess Noise Reduction

1. Description of Synchronous Detection Techniques

The basic principles of synchronous detection techniques as exemplified by the lock-in amplifier are well known and need not be discussed (19). Suffice it to say that synchronous detection techniques are an example of frequency domain filtering in which an attempt is made to make the signal bandwidth as narrow as possible so as to detect as little noise as possible with the signal. This is accomplished by modulating or chopping the signal of interest so that the information it contains is made to appear in some relatively noise free band of frequencies $\Delta\omega$ centered around the modulation frequency ω_o. A narrow signal bandwidth is achieved by tuned amplifications at the modulating frequency ω_o followed by demodulation and passage through a low pass filter.

Synchronous detection techniques are able to greatly increase the S/N of measurements performed on modulated signals buried in noise for two reasons. The first is that they are able to locate the information in the signal away from frequency ranges which exhibit a great deal of noise. The second reason for the effectiveness of a synchronous detector is its ability to provide very narrow bandpass filtering of the signal. In effect, a synchronous detector strives to create a filter whose bandpass is equal to $\Delta\omega$, the frequency range containing all the desired information. By narrow bandpass filtering in a region of low noise, a synchronous detector is able to greatly improve the efficiency of transmission and recognition of weak signals.

One measure of the noise filtering capabilities of a synchronous detector is the effective bandpass of the filter it simulates. As the simulated bandpass decreases the degree of improvement in the S/N of the measurement increases because less and less noise is passed along with the signal information. Using state of the art electronics, lock-in amplifiers are able to achieve effective bandpasses of less than 0.001 Hz. Of course, an effective filter bandpass of less than $\Delta\omega$ would also reject some of the signal information and should be avoided.

Synchronous detection techniques are especially effective in rejecting drift or 1/f noise. This success stems from the fact that almost all the signal power contained in drift noise is located in a very low frequency region. By proper selection of a modulating frequency it is relatively easy to locate the desired signal information at a frequency far removed from the region in which the power of drift noise is greatest. Thus to eliminate drift noise located at low frequencies, a modulation frequency of 25 Hz could be chosen, provided, of course, there is no other noise source displaying measurable power at that frequency.

2. <u>Application of Synchronous Detection Techniques to Photon Counting</u>

Synchronous detection techniques are applied to the digital output of photon counting systems and the analog output of conventional dc systems in much the same way. In each case the recognition of information contained in a signal is enhanced by passage through a noise-rejecting narrow bandpass filter created by modulating and demodulating the signal. The primary difference between the application of synchronous detection techniques to digital and analog signals is that the digital application is simpler and more effective.

As in the case of analog signals, the synchronous detection of the digital output of a photon counter begins with the modulation of the signal being observed. In the photon counting systems modulation is achieved by alternately openning and closing a shutter placed between the source whose intensity is being measured and the photomultiplier tube - photon counter combination.

Modulation of the signal at this point does not lead to filtering of any excess noise sources located between the shutter and the excitation source. Excess noise due to drift in the excitation source is not modulated. However, the by far most important source of excess noise, drift in the photon counter output, is modulated by opening and closing the sample cell shutter.

The net result of opening and closing such a shutter is that the photon counter output is equal to the sum of the signal and background counts when the shutter is opened while, when the shutter is closed, the photon counter output is made up of only background counts.

Demodulation and with it a simple form of synchronous detection is achieved by subtracting alternate signal and background measurements. As in analog applications, the net result of this digital form of synchronous detection is the creation of a filter through which the signal is passed and, hopefully, noise rejected.

A description of the filtering achieved through digital synchronous detection is best given in terms of the power transfer function of the filter created by subtracting alternate signal and background measurements. Robben (13) has shown that the power transfer function of such a filter is given by eq (18) in which t is equal to the measurement time for each phase of a measurement cycle and ω is the modulating frequency.

$$H(\omega)H^*(\omega) = \left(\frac{4}{\omega_t}\right)^2 \sin 4 \left(\frac{\omega_t}{2}\right) \tag{18}$$

From the trigonometric portion of equation (18) it can be seen that the filter created by digital synchronous detection has a bandpass centered at $\omega_t = \pi$ with harmonics appearing at odd integral multiples of π. The width of the filter bandpass is determined by the term $(4/\omega_t)^2$. As a large number of synchronous detection cycles are averaged, the summed measurement time t becomes very large causing the term $(4/\omega_t)^2$ to become very small. After summing a large number of cycles the filter bandpass becomes very narrow with a fractional width proportional to the inverse of the number of synchronous detection cycles.

3. Comparison of Analog and Digital Synchronous Detection

Digital synchronous detection as described above is superior to analog synchronous detection because of the infinitely small filter bandpasses which can be obtained by summing a very large number of measurement cycles. Because the alternate signal and background measurements are obtained in digital form, they can be summed in registers as numbers for as many cycles as desired with absolutely no loss of information. What this amounts to is that the demodulation process is optimized in digital synchronous detection. This optimal demodulation is not true of analog synchronous detectors which suffer from a slight but significant degree of instability in the demodulation process. Because of this demodulation instability, the maximum signal integration times available in analog synchronous detectors are definitely bounded. As a result, the signal filtering capabilities of analog synchronous detectors are inferior to their digital counterparts.

C. Computer Controlled Digital Synchronous Detection

1. System Operation As a Synchronous Detector

Synchronous detection of the photon counter output is a task easily accomplished by the computer interfaced photon counting spectrophotometer. Modulation of the signal is accomplished by computer controlled movement of the sample cell platform. A lever arm attached to the platform opens and closes a shutter as a function of platform position. When the platform is in the REF position, the lever arm opens a shutter allowing light to fall on the photomultiplier tube and a signal measurement to be made. When the platform is in the sample position, the shutter is closed which prevents light from striking the photomultiplier tube and allows background measurements to be made.

The demodulation step of digital synchronous detection is even more easily accomplished by the computer interfaced system since demodulation only involves the simple arithmetic operations of addition and subtraction. As the results of alternate signal and background measurements are transferred to the computer, their difference is found and summed with the results of previous measurement cycles. Obviously this process can continue indefinitely under computer control.

A flow chart of the computer controlled operations which make up a synchronous detection measurement are shown in Fig. 18. After performing the first phase of the measurement cycle, the computer determines if the newly acquired datum is a signal or background measurement and stores it accordingly. The computer then checks to determine if the second phase of the measurement cycle has been completed. If not, the computer reverses the shutter position and proceeds to take the second measurement. Having completed both phases of a measurement cycle the computer finds their difference, sums this difference with the results of previous measurements and then proceeds to a decision making program sequence.

A timing diagram for one cycle of a synchronous detection measurement is shown in Fig. 19. Because the actual sampling time is made smaller than the shutter open and closed times, the problem of chopper synchronization is avoided.

2. Evaluation of System Operation As a Synchronous Detector

It will be recalled that synchronous detection techniques were adopted as a means of filtering out drift noise in the photon counter output. Recall also that excess noise in the form of drift can be deduced by the departure of the photon counter output from Poisson statistics. With these facts in mind it is apparent that an evaluation of the system's performance as a synchronous detector must examine the statistical nature of the photon counter output for a return to Poisson statistics via synchronous detection.

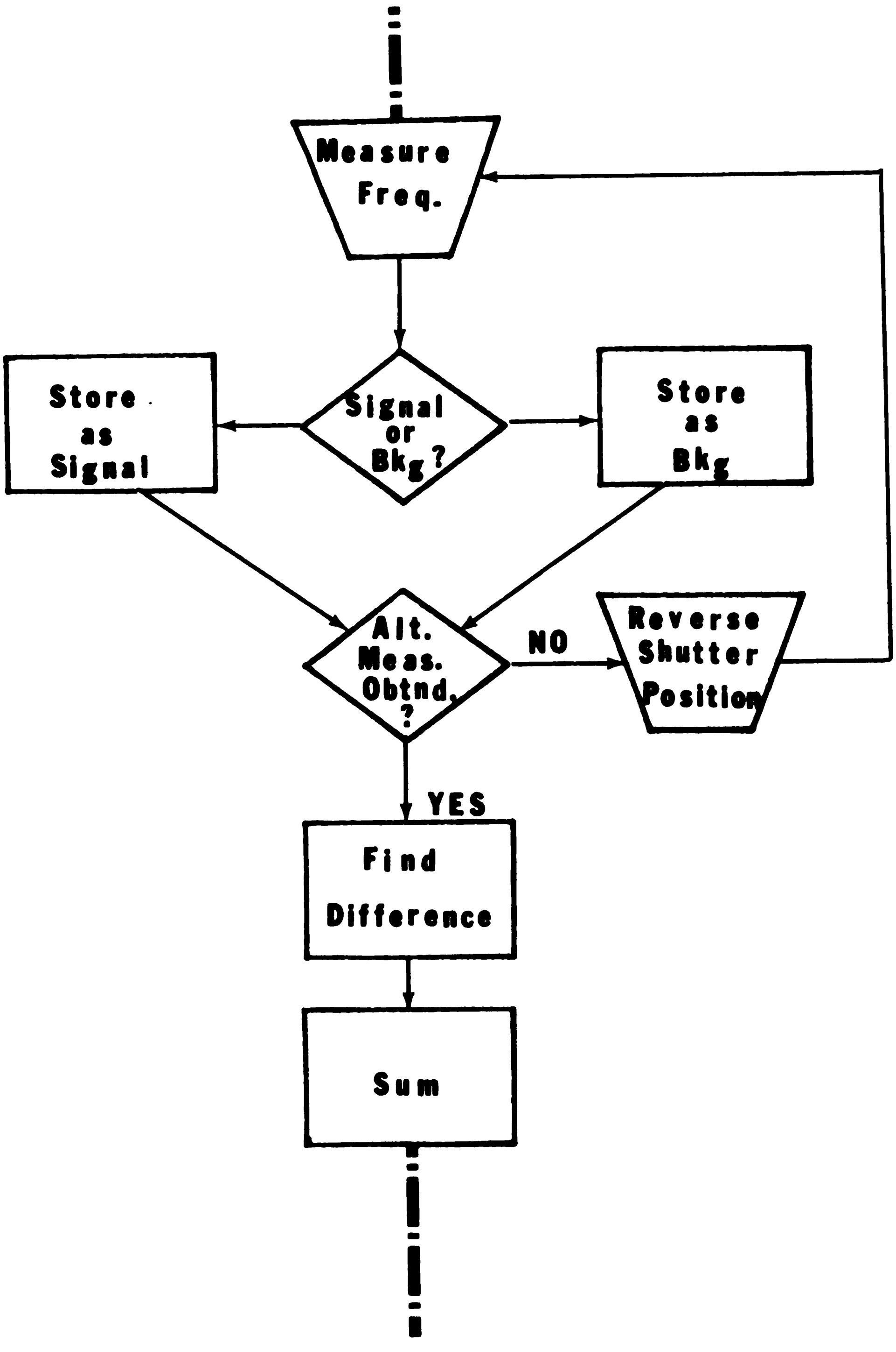


Figure 18. Flow Chart of Program Sequence for Performing a Digital Synchronous Detection Measurement.

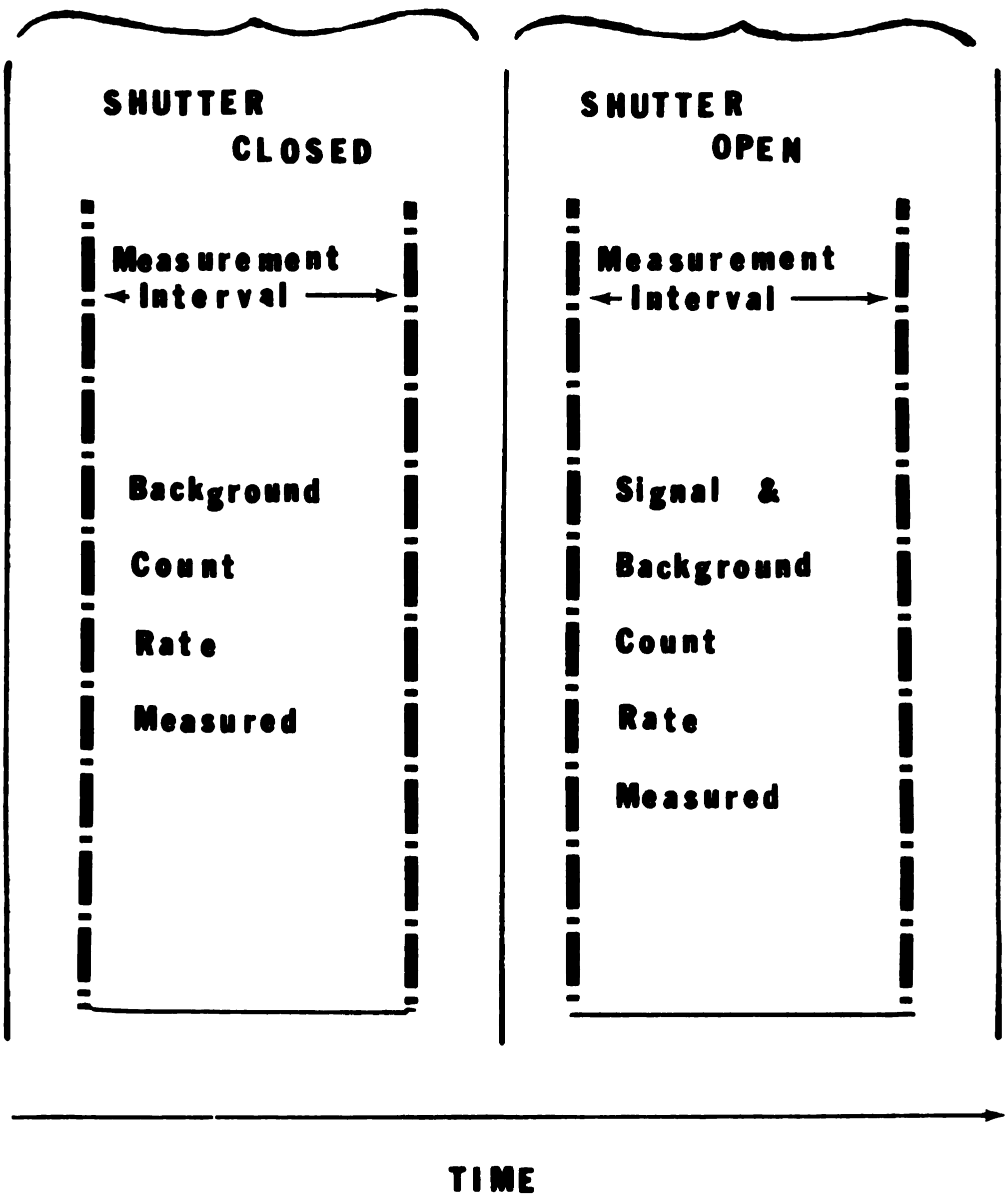


Figure 19. Timing Diagram for a Digital Synchronous Detection Measurement.

The statistical nature of the photon counter output following synchronous detection can be determined by examining the variance among the count differences which result from each measurement cycle (13). Let Y be the difference between the number of signal counts X_1 and the number of background counts X_2 acquired in one synchronous detection measurement cycle.

$$Y = X_1 - X_2 \tag{19}$$

Since the variance in Y, Var(Y), is equal to the sum of the variance in X_1 and X_2, eg (19) can be written

$$Var(Y) = Var(X_1) + Var(X_2) \tag{20}$$

If it can be further assummed that Poisson statistics govern the count rates X_1 and X_2, the variance of those signals can be equated to their means $\langle X_1 \rangle$ and $\langle X_2 \rangle$ as is shown in eq (21)

$$Var(Y) = \langle X_1 \rangle + \langle X_2 \rangle \tag{21}$$

Equation (21) provides the basis for a simple yet comprehensive evaluation of the performance of the system as a synchronous detector (13). The evaluation consists of the following steps:

1. After incapacitating the modulation process, a large number of repetitive synchronous detection measurements are acquired and stored. (The lack of modulation means that no difference exists between the signal and background count rates.)

2. The variance among the individual difference measurements Y_i is calculated and divided by the sum of the average number of signal

and background counts acquired in each measurement. This ratio is termed the normalized variance $Var(Y)/(\langle X_1\rangle + \langle X_2\rangle)$.

If the system is operating perfectly as a synchronous detector the normalized variance should be equal to unity. This can be seen by examining eq (21) and the assumptions leading up to it. Fundamental to eq (21) is the assumption that Poisson statistics govern the fluctuations in the equivalent signal and background count rates. Stated another way, a normalized variance value of unity means that all excess noise has been filtered from the photon counter output. Only the inherent quantum noise is responsible for variance among the synchronous detection difference measurements.

An evaluation of the performance of the system as a synchronous detector also involves finding the conditions under which optimal excess noise filtering is obtained. As has been discussed, optimal filtering is achieved by locating the center frequency of the synchronous detection filter in a frequency region displaying minimal excess noise power. If too low a modulation frequency is used (i.e. too long a measurement time t), the synchronous detection filtered bandpass is not shifted far enough away from the excess noise region with the result that drift noise is present in the photon counter output.

The normalized variance can aid in selecting an optimal modulating frequency because it is a sensitive function of excess noise. To find a modulating frequency at which drift noise is rejected, it is only necessary to observe the behavior of the normalized variance as a function of the measurement time interval t.

Two programs were written to aid in the evaluation of the computer interfaced photon counting spectrophotometer as a synchronous detector.

The first of these, GPSTAT, performs a large number of repetitive synchronous detection measurements and records on magnetic tape the number of counts acquired in each phase of a measurement cycle. The program was written to allow the experimenter to control the center frequency and the bandwidth of the synchronous detection filter by allowing the experimenter to control the measurement interval time t, and also the total number of averaged measurement cycles.

The synchronous detection data acquired and stored by GPSTAT is analyzed by another program called PSTAT. A main task of this program is to calculate the normalized variance of the data acquired by GPSTAT. In addition, PSTAT checks the stability of the demodulation process as a function of time by calculating a quantity known as the fractional difference. To calculate the fractional difference, PSTAT divides a series of synchronous detection measurements into groups of a size determined by the experimenter. For each group, the program totals all the acquired signal and background counts, subtracts the two totals and then divides the difference by the sum of the totals. This quantity, $\langle Y \rangle / (\langle X_1 \rangle + \langle X_2 \rangle)$ can be plotted as the group number to show any drift or bias in the demodulation process.

Some of the data acquired in evaluating the operation of the system as a synchronous detector are shown in Table I. The data were taken with the photomultiplier tube both shuttered and exposed to light. Of special interest are the data analyzing the statistical nature of the background count rate since when low intensity spectra are taken, the background statistics will dominate the S/N of a measurement.

The data in Table I indicate the importance of selecting the proper modulating frequency (measurement time interval) when applying digital synchronous detection techniques. The normalized variance values obtained for measurements made with relatively short measurement intervals of 0.8 and 1.6 sec are closer to the theoretically expected values of unity then are the measurements made with the 8.0 sec interval. The very definite trend toward a poorer normalized variance value with increasing length of the measurement time interval indicates that a significant amount of excess noise power is passed at the lower modulating frequencies.

The data of Table I also indicate the increased filtering effectiveness obtained by averaging the results of individual synchronous detection measurements. This is very apparent when the data of sums I and II are compared. Although the measurement time intervals are identical, the effect of summing five synchronous detection cycles is to markedly improve the signal filtering as is demonstrated by the normalized variance value of 1.13.

The data of Table I related to the statistics of background count rates are especially encouraging. The normalized variance values close to unity indicate that Poisson statistics apply quite well to background count rates. As a result of this knowledge, very low intensity measurements in which background statistics dominate can confidently be made.

Because of the large number of variables involved in the day to day performance of a photomultiplier tube - photon counter combination it is difficult to speculate as to the exact source of the departures of the normalized variance values from unity. One source of error is

Table I. Statistical Analysis of Synchronous Detection Data.

Run No.	Type of Pulses	No. of Measurements Per Run	Measurement Interval (sec)	No. of Measurement Cycles Summed Per Point	Ave. No. of counts Per Measurement	Ave. Fractional Difference	Ave. Normalized Variance
I	Background	1200	0.8	5	2895	$+0.11 \times 10^{-2}$	1.12 ± 0.03
II	Background	1200	0.8	1	320	$+0.48 \times 10^{-3}$	0.79 ± 0.04
III	Light, 5000 Å	1200	1.6	1	19434	-0.30×10^{-3}	0.88 ± 0.01
IV	Light, 5000 Å	350	8.0	1	32619	-0.16×10^{-4}	0.70 ± 0.01
V	Light, 2500 Å	1200	8.0	1	87482	$+0.28 \times 10^{-4}$	0.74 ± 0.01

offered by Robben (13) who explains normalized variance values greater than unity in terms of after pulsing in the photon multiplier tube. According to his work, a RCA IP28 photomultiplier tube, the type currently in use in the photon counting spectrophotometer can be expected to show about 2% after pulsing leading to normalized variance values which are 4% too large.

The average fractional difference values show that the system exhibits very good stability and freedom from bias. In Fig. 20, a plot of fractional difference values vs time is given for a run of twelve hundred measurements. As can be seen no drift or bias exists.

In general the system seems to perform very well as a synchronous detector. Through the use of measurement time intervals in the range of 1-8 seconds and by summing a large number of synchronous detection cycles, very good noise rejection can be achieved. On the basis of these results it should be possible to measure very low level light intensities.

Perhaps the most important aspect of this evaluation of the photomultiplier - photon counter is the evaluation procedure itself. By using the programs GPSTAT and PSTAT in conjunction with the computer controlled experiment interface it is possible to evaluate any detector-photon counter combination, thoroughly and with ease. With the continual advent of new detector and high speed photon counting circuits, new improved photon counting systems can be expected to appear almost continually. The ability to statistically analyze any new system for excess noise and other non-ideal behavior is an attractive and important feature of the computer interfaced photon counting system.

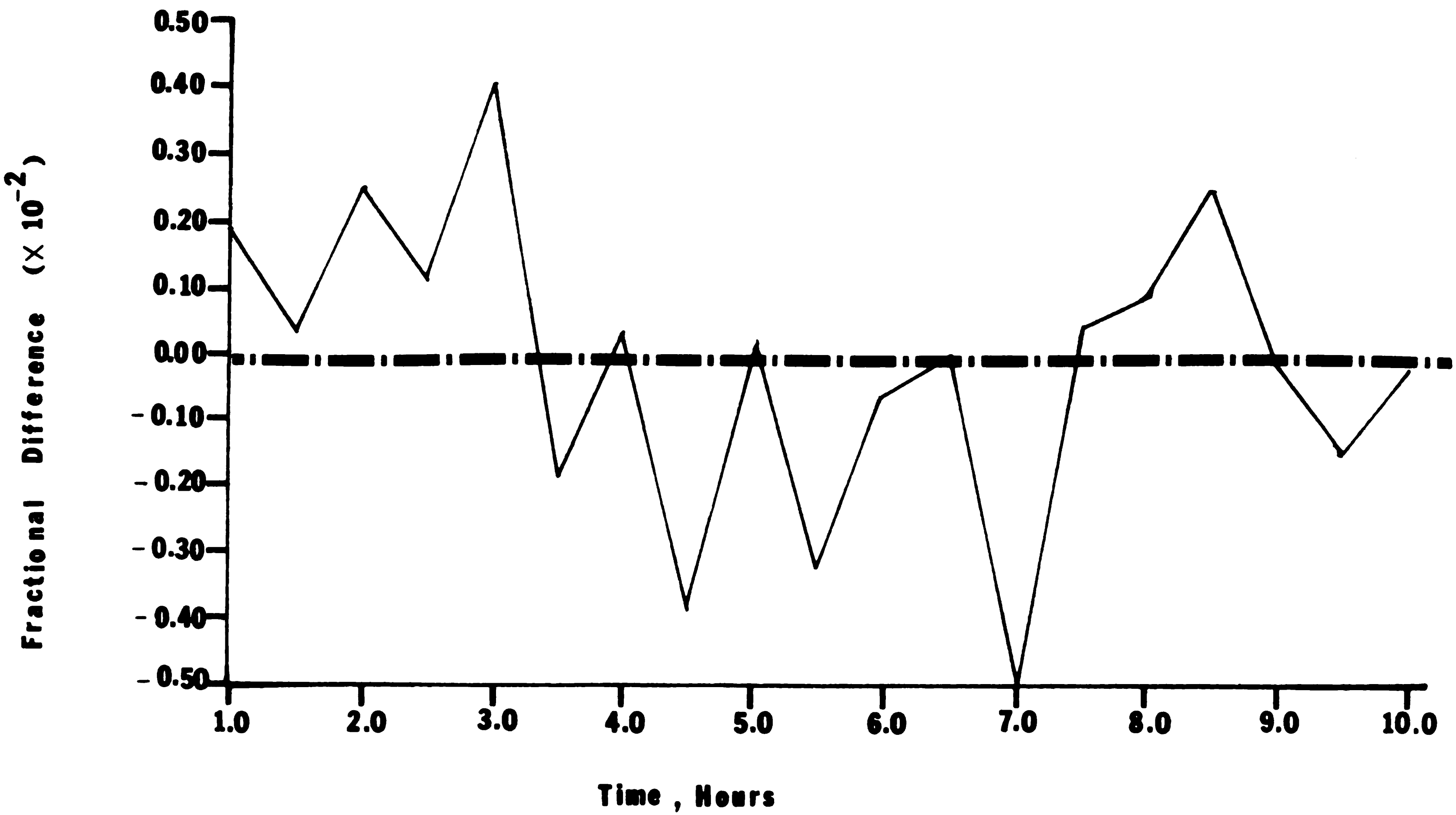


Figure 20. Plot of Fractional Difference vs Time Showing Lack of Drift or Bias in Synchronous Detection Measurements.

V. SYSTEM PERFORMANCE AS AN EMISSION SPECTROPHOTOMETER

Following extensive testing on an individual basis, the system components were assembled together to form an emission spectrophotometer and tested as a unit. The performance of the system hardware in its role as an emission spectrophotometer was both gratifying and anticlimacic. Previous testing had shown conclusively that the computer interfaced components were perfectly capable of performing the functions of monochromator control, synchronous detection, etc. There was however, one aspect of the systems performance as an emission spectrophotometer that exceeded expectations. This aspect was the degree of experiment optimization and efficiency achieved by making full use of the computer's ability to interact independently with the individual system components.

A. Hardware Configuration

The manner in which the individual system components were linked to form a typical right angle luminescence spectrophotometer is shown in Fig. 21. Radiation from a Heath EU-701-50 Light Source Module passes through a Heath EU-700E Monochromator and impinges on one face of an emission sample cell mounted on the platform of the Heath EU-721 Alternating Cell Module. The cell is mounted on the platform in such a manner that when the platform is in the REF position. radiation emitted from the cell at a right angle to the exciting radiation falls on the entrance slit of a second Heath monochromator.

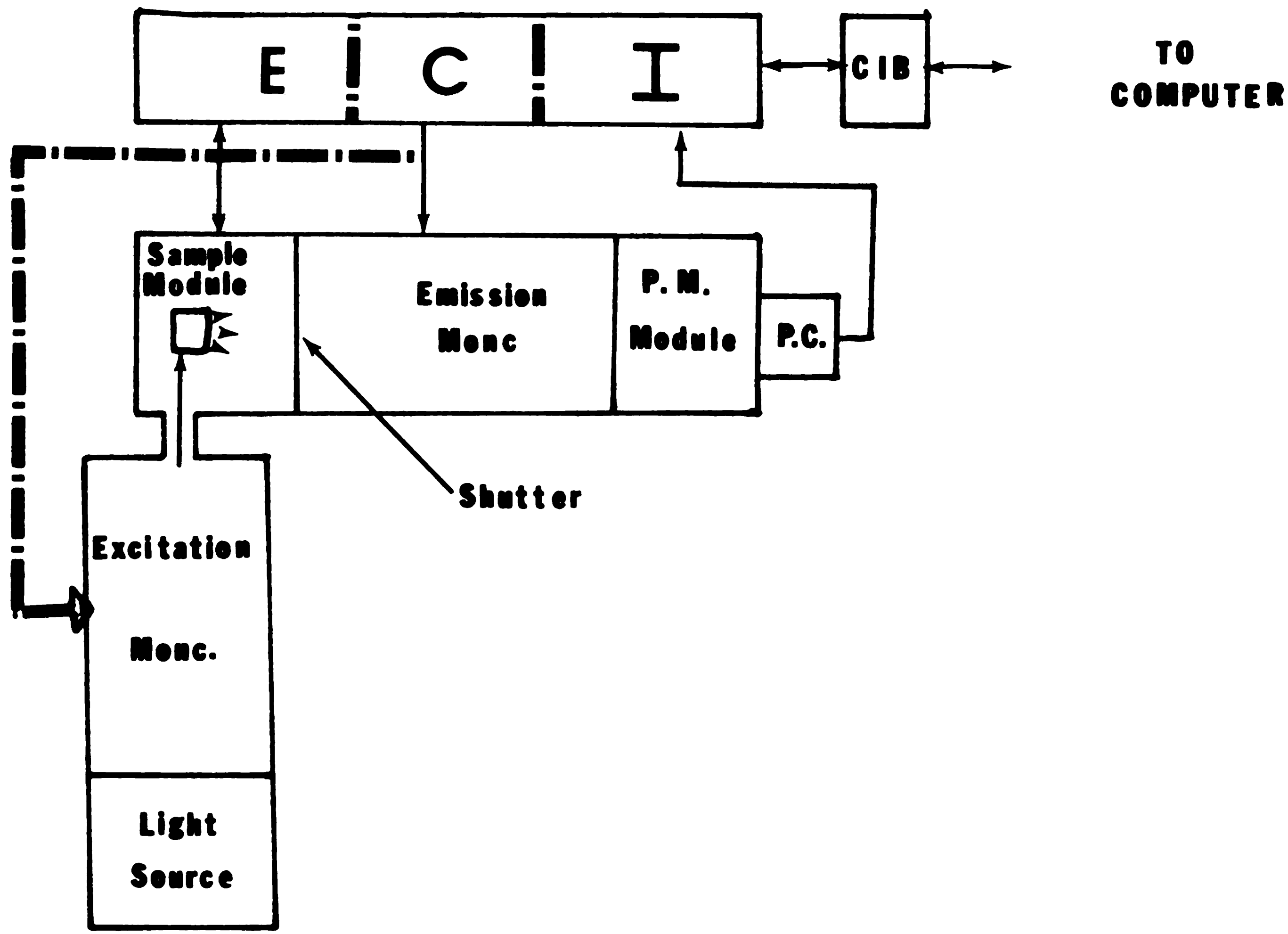


Figure 21. Block Diagram of Components of Computer Interfaced Photon Counting Emission Spectrophotometer.

After passing through the second Heath monochromator, the emitted radiation falls on an RCA 1P28 photomultiplier tube contained in the Heath EU-701-30 Photomultiplier Module to which the photon counter is connected.

Synchronous detection of the emitted radiation is performed by attaching a lever to the sample cell platform holding the emission cell. When the platform is in the REF position, the lever opens a shutter contained in the sample cell module and allows radiation being emitted from the cell to exit the cell module. When the platform is in the SAMPLE position, the lever arm closes the shutter which prevents any radiation from leaving the cell module. Signal and background measurements can then be made as a function of platform position which is, of course, under computer control.

The experimental configuration shown in Fig. 21 can be used to obtain either excitation or emission spectra. Excitation spectra are obtained by setting the monochromator viewing the sample emission at one wavelength while scanning the monochromator which controls the wavelength of the exciting radiation. Emission spectra are obtained in just the opposite manner. Because of the computer's ability to control either monochromator, both types of spectra are obtained with equal ease.

The spectroscopic characteristics of the equipment used to assemble the emission spectrophotometer are not optimal for luminescence work in which signal strength is always a problem. A more sensitive photomultiplier tube and a monochromator configuration having a higher optical speed would be advantageous. Certainly the tungsten or deuterium lamp of the Heath Light Source Module would seldom be used as an excitation source because of their extremely low intensities. In most luminescence work, high intensity sources are used in an effort to stimulate as much emission as possible from weakly emitting samples. For purposes of evaluation however, the less-than-optimal spectroscopic characteristics were ideal since they combined to simulate the very low level signal conditions under which the system was designed to be most effective.

B. Software

1. Description

The modular nature of the computer interfaced photon counting spectrophotometer extends to the software as well as the hardware. As has been discussed, each computer-hardware interaction is controlled by an almost independent software package. As a result, programs for controlling the computer interfaced photon counting spectrophotometer during the acquisition of spectral data can be assembled from the individual software packages in much the same manner as the spectrophotometer is assembled from the individual modules. Happily, the flexibility and versatility which are characteristic of modular hardware are also characteristic of modular software to the extent that major alterations in program design consist of simply resequencing the individual software packages.

A central theme in the synthesis of a total software package to control the computer interfaced photon counting spectrophotometer was the desire to exploit as much as possible the computer's ability to optimize the data acquisition process by making real time decisions. The current result of this desire for the most active on-line application possible is the program DCN1. Under the control of this program the computer attempts to obtain the best spectral data in the shortest possible time by continually making decisions concerning the most efficient manner in which each spectral data point can be acquired. Forming the heart of the decision making process is the computer's ability to calculate and increase the S/N of any data point measurement.

The computer is able to calculate the S/N of any synchronous detection measurement by evaluating eq (22) after each measurement cycle.

$$S/N = \frac{R_S t^{1/2}}{(2R_S + R_B)^{1/2}} \tag{22}$$

To increase the S/N of any measurement the computer needs only extend the total measurement time t by summing a number of repetitive synchronous detection cycles.

The most primitive means of exploiting the computer's ability to measure and increase the S/N of spectral data points is to instruct it to acquire each data point at a minimum preset S/N. This is one of the features of the program DCN1. In an initial dialog with the computer, the experimenter specifies the minimum acceptable S/N with which each data point is to be acquired. Then as the computer acquires each data point, the S/N is measured and the measurement time is increased, if necessary, until the minimum S/N specified by the experimenter is attained.

While the ability to preset the S/N of spectral data offers obvious advantages, it is not without hazard. The most serious complications are due to the large variations in signal intensities which can be encountered when making a spectral scan. Consider what happens when the experimenter specifies that a relatively high measurement S/N must be obtained at each spectral point. As soon as a region of low intensity is encountered ($R_S << R_B$) very long integration times are required to obtain the preset S/N. The net result is that the vast majority of the total scan time may be spent on a few unimportant low intensity spectral points lying at the beginning or end of the scan or in between higher intensity information-containing bands.

While a scan could be performed in this manner, the data acquisition process would be much more efficient if the computer could be instructed to obtain the very low intensity data points at a much lower and thus far less time consuming S/N.

By specifying in an initial dialog the maximum amount of time to be spent on any one data point, the experimenter is able to endow the computer with the ability to decide whether or not a data point can be economically obtained. To reach this decision the computer first performs a few preliminary measurements on each data point to obtain representative values of the signal and background count rates. By substituting these count rates and the preset S/N desired by the experimenter into the S/N expression of eq (22), the computer is able to estimate the total measurement time required to measure the data point at the preset S/N. If the estimated total measurement time is less than the maximum allowed measurement time the computer proceeds to average readings at the data point until the preset S/N is reached. If, however, the estimated total measurement time is greater than the maximum allowed measurement time, the computer stores the results of the preliminary low S/N measurement and proceeds to the next data point in the scan. This ability of the computer to determine after only a few synchronous detection cycles that an excessively long total measurement time would be required to reach the preset S/N results in an enormously increased data acquisition efficiency.

Another potential source of inefficiency in the data acquisition process, this time associated with the measurement of high intensity signals, exists. This inefficiency is revealed by considering the form of the photon counting S/N expression in the limit of high count rates ($R_S >> R_B$). In this limit the S/N expression assumes the form

$$S/N \approx R_S^{1/2} t^{1/2} \tag{23}$$

As can be seen from eq (23), the S/N of a photon counting measurement under conditions of large signal count rates is, to a very good approximation, not a function of the background count rate. The operational implication of eq (23) for synchronous detection photon counting measurements is clear: under the condition of high signal count rates, measurement efficiency can be increased by temporarily suspending synchronous detection and measuring only the signal count rate.

The computer interfaced photon counting spectrophotometer is easily programmed to suspend synchronous detection when the signal count rate is much larger than the background count rate. At each spectral point, the computer is programmed to perform one synchronous detection measurement and then evaluate the ratio of the measured background count rate to the measured signal count rate. The decision to either suspend or continue synchronous detection for the current data point is reached by comparing the magnitude of this ratio with a maximum value set by the experimenter. If the signal count

rate is not sufficiently larger than the background count rate as evidenced by a small value of the ratio, synchronous detection is continued. However, for those spectral data points at which the signal intensity is sufficiently great, the total measurement time is reduced by 50% by temporarily suspending synchronous detection and measuring only the signal count rate.

2. Flow Chart

A flow chart for the program DCN1 is shown in Fig. 22. Following start-up, an initialization dialog is carried out between the computer and the experimenter. A typical dialog in which replies by the experimenter are underlined is shown below.

TYPE OUTPUT DEVICE FOR NORMALIZED SPECTRUM DTAØ

TYPE OUTPUT FILE FOR NORMALIZED SPECTRUM RBN1

TYPE OUTPUT DEVICE FOR UNNORMALIZED SPECTRUM DTAØ

TYPE OUTPUT FILE FOR UNNORMALIZED SPECTRUM RBU1

TYPE IN DEVICE FOR TEMP STORAGE OF SPECTRA DTAØ

TYPE IN FILE FOR TEMP STORAGE OF SPECTRA TRBU1

INITIALIZE THE SPECTROMETER

SET MONC. CONTROL TO 2ØA/SEC, EXTERNAL CLOCK

CHOOSE CORRECT LIGHT SOURCE

INITIALIZE MONOCHROMATER

WHEN INITIALIZATION COMPLETE, PRESS CONT

TYPE IN INITIAL WAVELENGTH 37ØØ.

TYPE IN FINAL WAVELENGTH 6ØØØ.

MAX SCAN INCREMENT IS 33A

MIN SCAN INCREMENT IS 1.1

Figure 22. Flow Chart of Program DCN1.

TYPE IN THE SCAN INCREMENT 3.

INITIAL WAVELENGTH = 37ØØ.Ø

FINAL WAVELENGTH = 6ØØØ.Ø

TYPE IN THE DESIRED SIGNAL TO NOISE RATIO 75

TYPE IN READING INTEGRATION TIME(SEC) 8.

TYPE IN MAX COUNTING TIME ALLOWED(SEC) 1ØØ.

The first information received by the computer concerns storage of the spectral data. The experimenter specifies on which DEC TAPE unit the spectrum is to be stored and under which file name. The experimenter must make three such specifications since for ease of subsequent processing and display the raw spectral data stored in the temporary file are reordered and stored and then normalized to unity and stored again.

Following the dialog governing storage of the spectral data, statements are issued by the computer reminding the experimenter that the hardware must also be initialized. Following these reminders the computer halts while the experimenter makes any necessary hardware adjustments.

The next information requested by the computer concerns the scan parameters. In response to queries, the computer is told the desired initial and final wavelengths of the scan. After digesting the information the computer informs the experimenter of the maximum and minimum allowed scan increments. Upon being told the desired scan increment size, the computer calculates the parameters with which the monochromator will be controlled and as a safety measure echos the initial and final wavelengths of the scan.

During the remainder of the dialog the computer receives information concerning the data acquisition process. The experimenter indicates the S/N desired for each point and the maximum amount of time to be spent acquiring data at any one point. Also indicated by the experimenter is the length of the synchronous detection measurement time interval. Following completion of the dialog, the computer prints headings for a table in which each datum point will be displayed as it is acquired and then it begins the scan.

As the first step in the acquisition of each datum point, the computer performs one synchronous detection measurement cycle, calculates the S/N of the measurement and compares it to the preset value determined by the experimenter. If the S/N of the initial measurement is greater than the preset value, the computer considers the data acquisition process to be complete and after storage and display of the measured signal count rate, continues on with the scan.

If the S/N of the initial measurement is less than the preset value, the computer utilizes the decision making process described earlier in an effort to determine the most efficient way in which the measurement S/N can be increased. The first decision to be made is whether or not the signal count rate is sufficiently greater than the background count rate to suspend synchronous detection. If the computer finds that the signal count rate is 200 times as great as the background count rate, synchronous detection is suspended and the data acquisition S/N is increased to the preset level by summing repetitive measurements of the signal count rate only.

Upon finding that the signal count rate is not high enough to suspend synchronous detection, the computer determines if the signal count rate is so low that the required measurement S/N can not be attained within the maximum time allowed to measure one point. As has been described, this decision is reached by using the results of preliminary synchronous detection data to estimate the total time required to complete the data point acquisition at the required S/N. If the estimated time is greater than the maximum allowed by the experimenter, the computer records and prints the results of the preliminary measurements and proceeds to acquire the next point. If, however, the estimated time required to complete the acquisition of the data at the preset S/N is less than the maximum allowed measurement time, synchronous detection is continued until data acquisition at the preset S/N is attained.

In the program DCN1, two additional decision making steps preceed the decision of whether or not acquisition of the current datum point can be completed within the maximum allowed time. In one of these steps, the computer ensures that the preliminary synchronous detection data used to estimate the total measurement time are of a high enough quality to give a good estimation. To gain this assurance, the computer requires that the preliminary data have at least a S/N equal to five before they are used to estimate the total measurement time. Unfortunately, in the case of very weak or non-existant signals, the computer could conceivably spend an excessive amount of time in attempting to acquire the preliminary data at a S/N of five or greater. To limit this time, a second decision making step is introduced in which the computer monitors the number of synchronous detection cycles used to acquire the preliminary data. Should they exceed a maximum number, the attempt to acquire preliminary data is halted and, after displaying and storing the results of the preliminary measurements, the scan is continued.

After acquiring, displaying and storing a data point, regardless of how it is accomplished, the computer checks to see if the experimenter desires to prematurely halt the scan. The experimenter can communicate this desire by setting a sense switch on the front panel of the computer.

Finding no signal from the experimenter to abort the scan, the computer examines the monochromator control parameter to determine if the scan is complete. If not, the monochromator is advanced and acquisition of the next point begun. After acquiring the last data point in the scan the computer normalizes and restores the spectral data and then exits the program DCN1 to return to MONITOR.

C. Evaluation of System Performance

The evaluation of the system's ability to function as a computer interfaced photon counting emission spectrophotomer consisted, quite naturally, of the measurement of a number of emission spectra. During the evaluation, two aspects of the system's performance were subjected to particular heavy scrutiny: the first of these was the performance of the system hardware when assembled as an emission spectrophotometer. The second and equally important focal point of the system evaluation was the efficiency of the data acquisition process under the control of the program DCN1. The outstanding success with which the system met all performance requirements is best illustrated by discussing two spectra recorded by the system.

1. Fluorescein

The emission spectrum of a 0.1 μg/ml alkaline solution of fluorescein was obtained with the system. The system which agrees well with previously published (19) spectra is shown in Fig. 23 The spectrum was obtained using the configuration of Fig. 21 with the exception that a Bausch and Lomb 75 Watt Xenon Lamp was used as the source of the exciting radiation. The excitation monochromator was set at 480 nm with a slit width of 2000 μm. The emission monochromator slit width was set at 1500 μm. Spectral data points were acquired every 1.0 nm between the initial and final wavelengths of 480 nm and 600 nm respectively. Total scan time was about one hour.

Data acquisition was carried out under the following conditions:

DESIRED S/N: 500

SYNCHRONOUS DETECTION MEASUREMENT INTERVAL: 8 sec

MAXIMUM ALLOWED MEASUREMENT TIME (PER POINT): 120 sec

The conditions under which the fluorescein spectrum was obtained provided a good test of the ability of the program DCN1 to optimize the data acquisition process. Because of the high intensity source and the high quantum yield of fluorescein, peak emission count rates of about 9000 counts/sec were obtained. In an effort to utilize these peak values to test the ability of the program DCN1 to efficiently measure high count rates, the decision making process was adjusted so that synchronous detection was suspended for signal count rates over 7000 counts/sec.

By specifying a desired S/N of 500 and a maximum allowed measurement time of 120 sec. the ability of the program DCN1 to prematurely

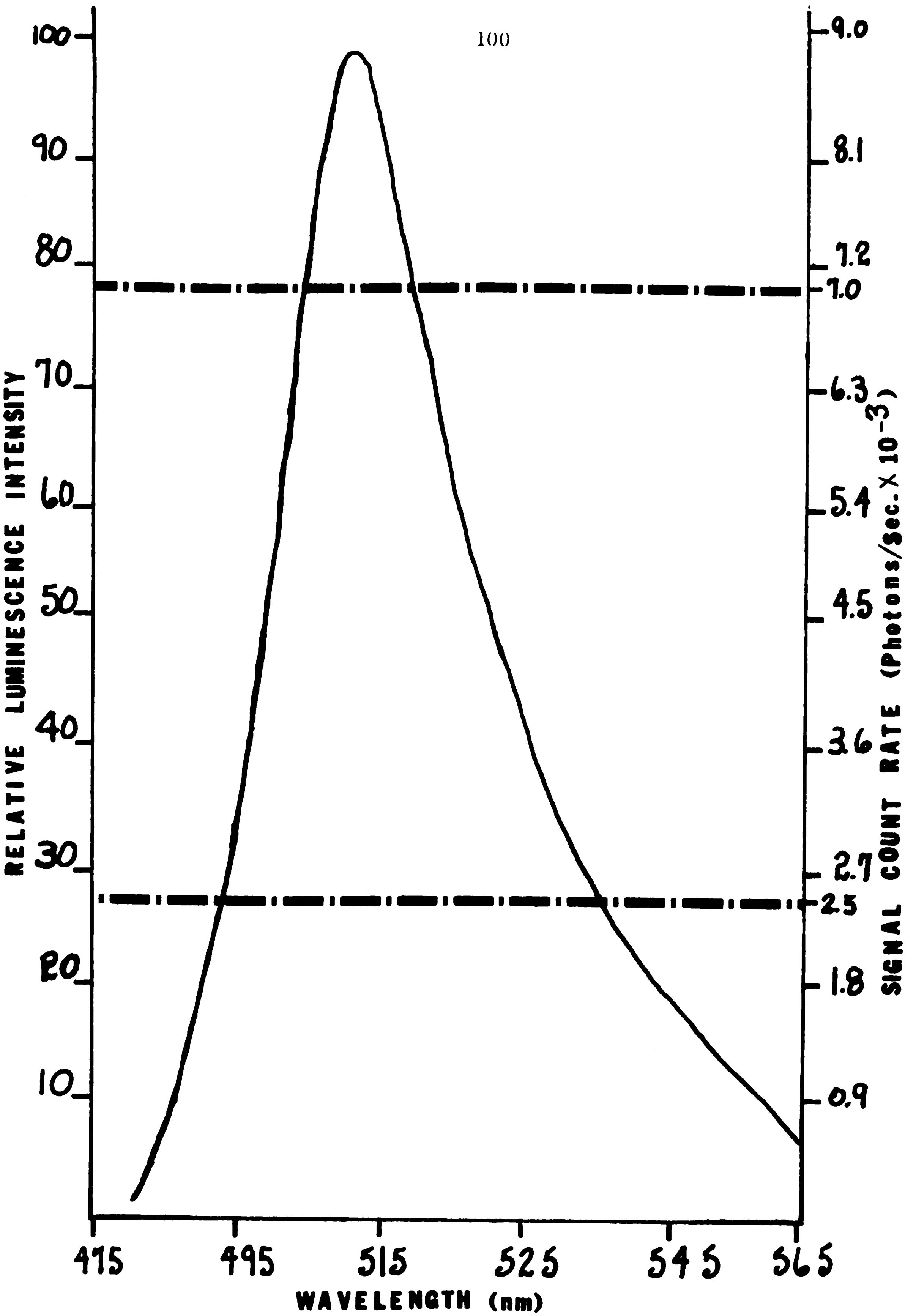


Figure 23. Emission Spectrum of 0.1 μg/m Alkaline Solution of Fluorescein.

terminate data acquisition on low intensity points was also tested. Assumming a dark count rate of about 300 counts/sec, a simple calculation shows that the measurement of all data points displaying a signal count rate of less than 2500 counts/sec should be prematurely suspended since acquisition of these data points would require total measurement times greater than 120 sec.

Observation of the system during the scan showed that the program DCN1 was controlling the data acquisition process exactly as intended. Under control of this program three distinct modes of data acquisition were observed. For signal count rates less than about 2500 counts/sec, data acquisition for each point was suspended after a few preliminary synchronous detection cycles. For signal count rates greater than 2500 counts/sec and less than 7000 counts/sec, the system was observed to sum as many synchronous detection cycles as was necessary to obtain a measurement S/N of 500. For signal count rates greater than 7000, synchronous detection was observed to be suspended after one cycle and only signal count rates were summed in the process of attaining a S/N of 500 for each datum point measurement. Each of the three data acquisition regions is shown in Fig. (23).

The very satisfactory performance of the computer interfaced photon counting spectrophotometer in obtaining the fluorescein spectrum of Fig. 19 was repeated for other spectra exhibiting relatively high count rates. Having gained assurance from these experiments that the system was operating properly, attempts were made to record very weak emission spectra.

2. Anthracene

Displayed in Fig. 24 is the emission spectrum of a 10^{-5} M solution of anthracene in ethanol as recorded by the computer-interfaced photon counting spectrophotometer. The total time required to obtain the spectrum was slightly over thirty hours.

The system configuration was identical to that shown in Fig. 21 Exciting radiation was provided by the Heath Deuterium Lamp. The excitation monochromator was set at 250 nm with a slit width of 2000 μm. The slit width of the emission monochromator was also 2000 μm. The scan was from 370 nm to 463 nm with a data point taken every 0.5 nm.

Data acquisition was carried out under the following conditions:

DESIRED S/N: 100

SYNCHRONOUS DETECTION MEASUREMENT INTERVAL: 8 sec

MAXIMUM ALLOWED MEASUREMENT TIME (PER POINT): 2000 sec

The anthracene spectrum of Fig. 24 is an excellent example of the degree of signal recovery that can be attained with the computer interfaced photon counting spectrophotometer. Due to the very low intensity of the exciting source, every signal count rate measured during the scan was less than the background count rate of approximately 330 counts/sec. Under these conditions the signal can be truly said to be buried in noise. In view of the noisiness of the signal, the ability to record the anthracene spectrum at a S/N of 100 is a clear demonstration of the almost unlimited signal recovery capabilities of the system. Every data point in the anthracene spectrum could have been measured with a S/N of 100 at the expense of a longer total scan time.

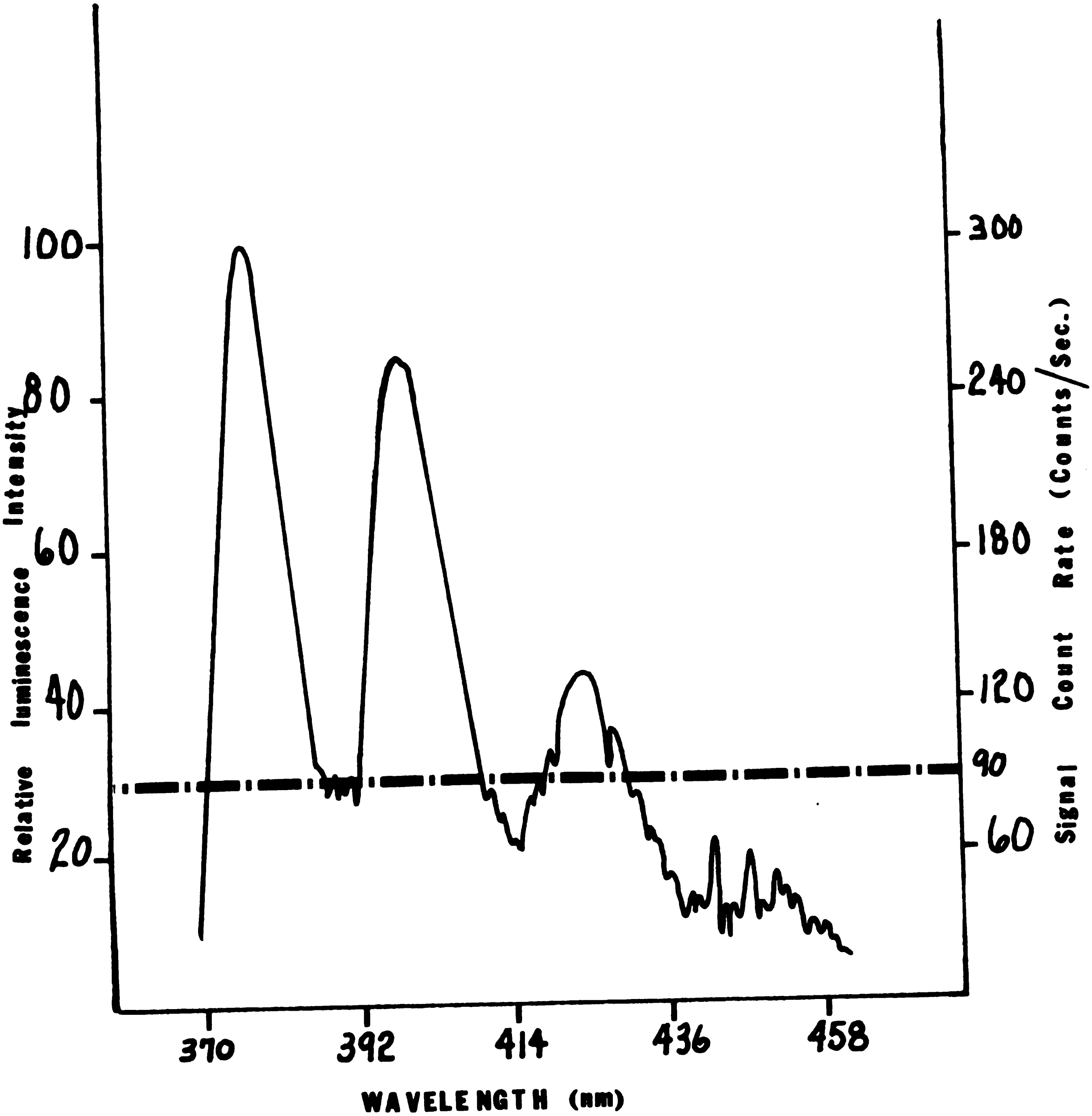


Figure 24. Emission Spectrum of 10^{-5} M Solution of Anthracene in Ethanol.

The use of extremely long scan times to enhance the measurement S/N of very weak signals requires long term system stability and reliability. Certainly the acquisition of the anthracene spectrum over a period of thirty hours demonstrates the long term stability and reliability of the computer interfaced photon counting spectrophotometer. Additional evidence for the stability and reliability of the system was amply provided during scans consuming almost three consecutive days. No operational problems were encountered during these extended runs.

The anthracene spectrum shown in Fig. 24 agrees well with the accepted spectrum shown in Fig. 25. The spectrum obtained with the photon counting system clearly shows three of the four accepted anthracene emission peaks. The fourth and weakest of the anthracene emission peaks can be vaguely discerned in the noise on the low energy side of the experimental spectrum shown in Fig. 24.

The reason for the poor resolution of the fourth peak is of course the fact that the signal count rates in this area of the spectrum were too low to be measured at the preset S/N of 100 within the maximum allowed measurement time per point. Under the data acquisition conditions specified for the scan, the minimum signal count rate which can be measured with a S/N of 100 is about 90 counts/sec. Since the signal count rates making up the weak fourth emission peak of anthracene were on the order of about 40-60 counts/sec they were measured at a much lower S/N of about 5. A line drawn through the spectrum indicates the 90 count/sec cutoff point for synchronous detection.

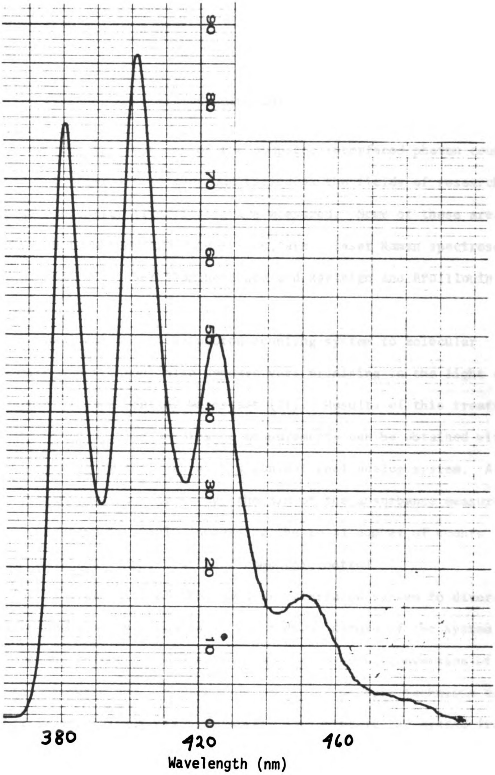


Figure 25. Accepted Emission Spectrum of Anthracene in Ethanol (Ref. 20).

VI. Conclusion

Potential applications of the computer interfaced photon counting spectrophotometer are as many and varied as the fields of research in which low intensity light levels are measured. Some of these areas, in addition to molecular luminescence, are: laser Raman spectroscopy, chemiluminescence, thermoluminescence and Rayleigh and Bruillouin scattering.

The application of the photon counting system to molecular absorption spectroscopy also appears very promising in the light of a theoretical treatment by Malmstadt (21). Results of this treatment show that more precise absorbance measurements can be obtained with a photon counting system than with a conventional analog system. Also, as in luminescence spectroscopy, the S/N of the absorbance measurement can be increased by simply increasing the total number of counts - a process easily accomplished under computer control.

The applicability of the computer interfaced system to diverse research problems is enhanced by the modular nature of the system hardware and software. Due to this modular nature, conversion of both the hardware and software from one spectroscopic technique to another is exceedingly straightforward. To convert the system from emission spectroscopy to absorption spectroscopy requires only a repositioning of the hardware components and a resequencing of the individual software packages controlling the individual system

functions. Similarly, it is easy to convert the system into a dual wavelength spectrophotometer capable of obtaining highly accurate derivative spectra. In this spectroscopic technique, two monochromators are synchronously scanned in such a manner that a small constant increment is maintained between their wavelength settings. Such scans can be easily performed with the computer interfaced system by including two monochromator control sequences in the program instead of one.

In addition to resulting in a very useful instrument, the design and operation of the computer interfaced photon counting spectrophotometer has proved instructive concerning some aspects of the relatively new field of interfacing small computers with laboratory instrumentation. One of the benefits of such interfacing, as is amply demonstrated by the photon counting system, is the increased data collection efficiency and experiment optimization which result from being on-line. Also illustrated is another less obvious though equally important benefit of being on-line. This is the potential of the computer interfaced instrument for extracting the maximum possible amount of information from the experimental data through use of signal processing techniques.

This potential is exploited to a great degree by the computer interfaced photon counting spectrophotometer. Here the computer functions as a filter through which the experimental data is passed in an effort to reject information - concealing noise. During its information retrieval effort, the computer filters the photon counting data in three domains - frequency, time and digital. Through synchronous detection and the averaging of repetitive synchronous detection cycles, the computer filters the photon counting data in the frequency and time domains. Digital filtering is accomplished by a program described in the appendix which applies the digital smoothing techniques of Savitzky or Golay (22) to the spectral data. By filtering in three domains, the computer interfaced instrument is able to extract information which is unattainable with conventional instruments.

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APPENDIX

DISCRIPTION OF PROGRAMS FOR DATA DISPLAY

Three programs are available for displaying spectra stored on the DEC TAPES. These programs are named POT, POTX and SCOPE2 and are used to display spectral data on an analog plotter, the teletype and a CRT respectively.

A number of similarities exist between the programs. Each assumes that the data points of each spectrum to be displayed have been normalized to unity. Also, all of the programs are capable of displaying data in any of three different modes: relative luminescence intensity, absorbance and transmittance. The particular display mode desired by the experimenter is specified in a dialog statement preceeding the display.

Each of the three display programs is capable of displaying all or any part of a spectrum. The experimenter specifies the spectrum segment to be displayed by typing in the wavelength at which the display is to begin and end.

Another common feature of the display programs is their scale expansion capabilities. To scale the spectral data, the experimenter need only type in the upper and lower scane limits of the scale on which the data is to be displayed. The data is then scaled such that these limits become full scale on the displaying device.

In addition to these similarities, each program offers particular data display advantages. These are described below in a brief description of each program.

POTX

This program displays spectra at the teletype in histogram form. A typical dialog and display are shown here.

```
TYPE IN SPECTRUM NO.  1.52
TYPE IN DEVICE HOLDING SPECTRUM FOR PLOT  DTAØ
TYPE IN FILE HOLDING SPECTRUM FOR PLOT RBNB
TYPE A, T, OR L FOR DESIRED PLOTTING MODE  L
TYPE IN THE WAVLTH AT WHICH THE PLOT BEGINS  37ØØ.
TYPE IN THE WAVLTH AT WHICH THE PLOT ENDS  4ØØØ.
TYPE IN THE LOWER SCALE LIMIT  Ø.
TYPE IN THE UPPER SCALE LIMIT  15.


PLOT OF REL LUM INTENSITY VS WAVLTH IN A


REL LUMINESCENCE INTENSITY
 Ø.ØØ  1.5Ø  3.ØØ  4.5Ø  6.ØØ  7.5Ø  9.ØØ  1Ø.5Ø  12.ØØ  13.5Ø  15.ØØ
  I     I     I     I     I     I     I     I      I      I      I
WAVELTH#XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
37ØØ.Ø     READING OFF HIGH END OF SCALE    1ØØ.ØØØ**
37Ø4.Ø     READING OFF HIGH END OF SCALE     77.582**
37Ø8.Ø     READING OFF HIGH END OF SCALE     57.229**
3712.Ø     READING OFF HIGH END OF SCALE     43.632**
3716.Ø     READING OFF HIGH END OF SCALE     33.262**
```

```
3720.0        READING OFF HIGH END OF SCALE     23.537**
3724.0        READING OFF HIGH END OF SCALE     18.739**
3728.0***********************************************************
3732.0************************************************
3736.0*****************************************
3740.0***********************************
3744.0*****************************
3748.0***********************
3752.0*********************
3756.0**************************
3760.0*************************
3764.0************************
3768.0***********************
3772.0**********************
3776.0************************
3780.0****************************
```

As can be seen from the display, the program prints the value of the point when it is off scale. A drawback of this display technique is that because of the relatively slow printing speed of the teletype, spectra containing many points require a long time to print out.

POT

This program retrieves spectra stored on tape and displays them on an analog strip chart recorder. The spectra of Figs. 23 and 24 were obtained with the program POT. A typical dialog is shown below.

---ANALOG PLOTTING PROGRAM---

TYPE IN SPECTRUM NO. 1.2

TYPE IN DEVICE HOLDING SPECTRUM FOR PLOT DTA!

TYPE IN FILE HOLDING SPECTRUM FOR PLOT AT

TYPE A, T, OR L FOR DESIRED PLOTTING MODE L

TYPE IN THE WAVLTH AT WHICH THE PLOT BEGINS 37ØØ.

TYPE IN THE WAVLTH AT WHICH THE PLOT ENDS 4625.

TYPE IN THE LOWER SCALE LIMIT Ø.

TYPE IN THE UPPER SCALE LIMIT 1ØØ.

TYPE IN DESIRED NO. OF A INCH FOR PLOT AXIS 1ØØ.

ACTUAL NO. OF A/INCH ALONG PLOT AXIS ARE 1ØØ.ØØ

WILL PAUSE BEFORE PLOT, PRESS CONT WHEN READY

SCAN PRINT OUT COMPLETE

DO YOU WISH ANOTHER PLOT, TYPE ,Y, OR ,N, N

SCOPE2

This program displays spectra on an oscilloscope interfaced to the computer. This display technique is useful for evaluating displays before obtaining them in hard copy form. A typical dialog is shown below.

TYPE IN SPECTRUM NO. 1.Ø

TYPE IN DEVICE HOLDING SPECTRUM FOR DISPLAY DTAØ

TYPE IN FILE HOLDING SPECTRUM FOR DISPLAY RBN3

TYPE A, T OR L FOR DESIRED DISPLAY MODE L

TYPE IN THE WAVLTH AT WHICH THE PLOT BEGINS 48ØØ

TYPE IN THE WAVLTH AT WHICH THE PLOT ENDS 55ØØ

TYPE IN THE LOWER SCALE LIMIT Ø.

TYPE IN THE UPPER SCALE LIMIT 1ØØ.

DO YOU WISH ANOTHER DISPLAY,TYPE ,Y, OR ,N, N

DISCRIPTION OF PROGRAMS FOR DATA PROCESSING

SMUTH

This program digitally smoothes spectral data using the smoothing functions developed by Savitzky and Golay (22). The experimenter is given the choice of applying a 5.7 or 9 point smoothing function to the data. A typical dialog is shown below.

TYPE IN DEVICE HOLDING SPECTRUM FOR SMOOTH DTA!

TYPE IN FILE HOLDING SPECTRUM FOR SMOOTH AT

TYPE IN DEVICE TO HOLD SMOOTHED SPECTRUM DTA!

TYPE IN FILE TO HOLD SMOOTHED SPECTRUM AT!

TYPE IN THE NO. OF POINT SMOOTH DESIRED 9

DO YOU WISH ANOTHER SMOOTH? Y OR N N

SET SR(Ø) to Ø, PRESS CONT

NORM

This data processing program will add, subtract or multiply two spectra. The subtraction feature is especially useful when it is necessary to subtract a background spectrum from an emission spectrum. A typical dialog is shown below.

PROGRAM: NORM

TYPE IN DEVICE HOLDING MAJOR DATA DTAØ

TYPE IN FILE HOLDING MAJOR DATA RBU

TYPE IN DEVICE HOLDING MINOR DATA DTAØ

TYPE IN FILE HOLDING MINOR DATA RBUB

TYPE IN DEVICE TO HOLD PROCESSED DATA DTAØ

TYPE IN FILE TO HOLD PROCESSED DATA RBNC

PROCESSING DESIRED; 1=ADD, 2=SUB, 3=MULT 2

NORMALIZATION DESIRED? 1=YES, 2=NO 1

TYPE IN DEVICE TO HOLD NORM PROCESSED DATA DTAØ

TYPE IN FILE TO HOLD NORM PROCESSED DATA RBNCN