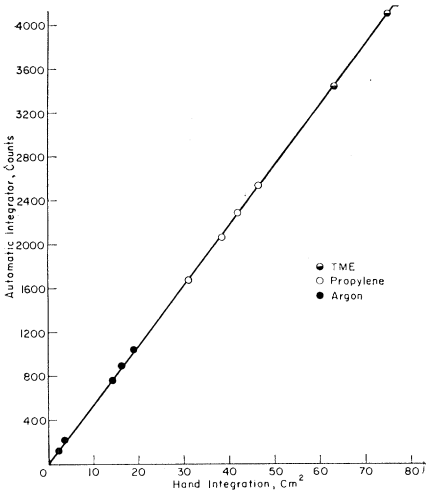

Fig. 3. Relationship between area of chromatogram and total pulse.

in this way, the analytic results could be printed out in digital form, vastly simplifying such work. It should also be noted that, if a situation should arise in which the data supplied by the integrator become a part of an elaborate and complicated calculation (as, for example, when a series of peaks from the same record are used to calculate the percentage composition of a complex mixture) then it would be possible to read out in punched-tape form, and the results could then be fed into a modern, high-speed calculating machine.

The total cost of material, labor, and so forth, exclusive of the cost of the recorder, is quite modest. This cost, however, could be reduced by a major fraction when the automatic printing and clearing features are not required. The instrument is, therefore, available to laboratories with very moderate budgets.

Accuracy and Repeatability

The accuracy and repeatability of the data are shown in Figs. 2 and 3, respectively. Figure 2 relates the number of pulses accumulated to the square area swept out on the chart; these data were obtained by using a scaling unit to totalize the number of pulses. The Speedomax was set with a millivolt source to run at a constant distance above the base line. The scaling unit and a timing clock were started and stopped simultaneously. During the elapsed time, a definite block of area was swept out on the chart as a function of the distance between the base line and the pen position. This area was plotted in arbitrary units against the total number of counts recorded in the 5-minute periods in Fig. 2. It can be seen that, on the basis of these square areas, the instrument gave perfect agreement. With the recorder used, it turned out that an arbitrary unit was nearly equal to 1 square centimeter, so that there is a close correspondence between the data plotted on Fig. 2 and on Fig. 3.

In Fig. 3, the results obtained from the automatic integrator for measurement of a number of calibration chromatograms are compared with the areas of the same peaks obtained using a B. K. Elliott hand planimeter. In these experiments, varying amounts of the several gases were passed through the column in a helium-carrying gas stream, and the response was recorded in the conventional manner. It should be emphasized that these data include results with three different gases: 2,3-dimethylbutane (tetramethylethane, or TME), propylene, and argon. The chromatographic peaks obtained for these were of vastly different shapes. The low-area argon peaks were extremely narrow and sharp. In fact, the areas are low because this gas was not held up in the column so that, even with a nearly full-scale deflection, the peak width at the base line did not exceed about 4 millimeters. The propylene peaks were still quite sharp, particularly on the front side, but were skewed by a moderately strong tail. Finally, the peaks for 2,3-dimethylbutane (TME) were very much broader than they were high. Thus, regardless of the shape of the peak, the automatic integrator appears to measure the area at least as accurately as hand integration.

This integrator can be made as an effective unit from known and available items, and we believe that its simplicity and convenience may make it useful to others.

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Automatic Particle and Bacterial Colony Counter

H. P. Mansberg

It has long been recognized that the counting of bacterial colonies on culture plates is one of the more tedious tasks performed in bacteriological laboratories. Many thousands of culture plates are counted daily in hundreds of dairy, food-industry, water-testing, pharmaceutical, and other laboratories throughout the country. In this era of electronics and automation it would appear surprising to many that automatic devices to perform this task have not been available.

Some of the standards and techniques that have evolved in bacterial assay tests are necessary compromises determined by the limitations of laboratory personnel in visual counting methods. These limitations include the inaccuracies of human counting, the inconsistencies between observers, and the high cost of both preparing and counting large numbers of samples. Quite possibly, if accurate automatic counting devices had been available before the evolution of bacterial assay standards, these standards would specify greater sampling quantities. For example, at present it is not at all unusual to find a fluctuation of 100 percent in the number of colonies from presumably identical aliquots of milk and cream (*1*). Where only one or two plates are now required by standard tests, with consequent poor sampling, the number of samples would be increased if the time and cost of preparation and evaluation were less.

Although many mechanical and visual aids have been proposed and constructed

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for assisting the laboratory technician in performing these tasks, these aids generally do not provide a really significant reduction in counting time, nor do they reduce sufficiently the variability of human error. As a first attempt in this direction, workers at the Allen B. DuMont Laboratories developed the automatic bacterial colony counter described in this article (*2*) for the U.S. Army Chemical Corps.

Principles of Design

The primary objective sought in the development of the bacterial colony counter was a scanning device which would be faster, more reliable and consistent, and, if possible, more accurate than laboratory technicians. Throughout the development, tests were made using both simulated and live cultures on standard 100-millimeter plates. The final design of the machine is shown in Fig. 1. Petri dishes that are inserted into the counting chamber of the machine are scanned by a cathode-ray tube and optical system. The scanning action is similar to that used in television, using a linear raster, except that a field is scanned at a much slower rate (one field per second). A pattern of 1000 lines traverses the dish once per second to obtain a resolution exceeding that of conventional television. A schematic representation of the optical system is shown in Fig. 2.

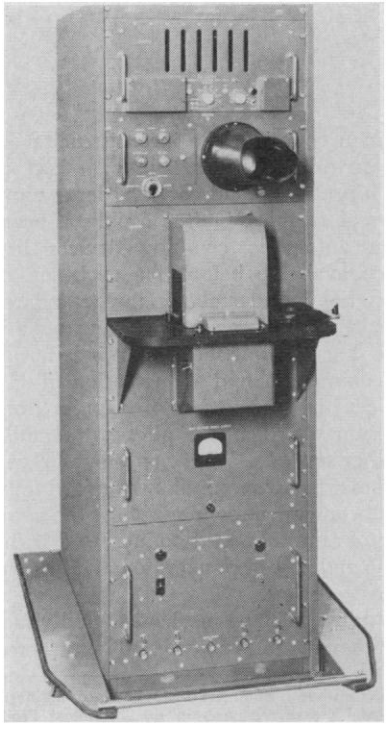

Fig. 1. Automatic particle and bacterial colony counter.

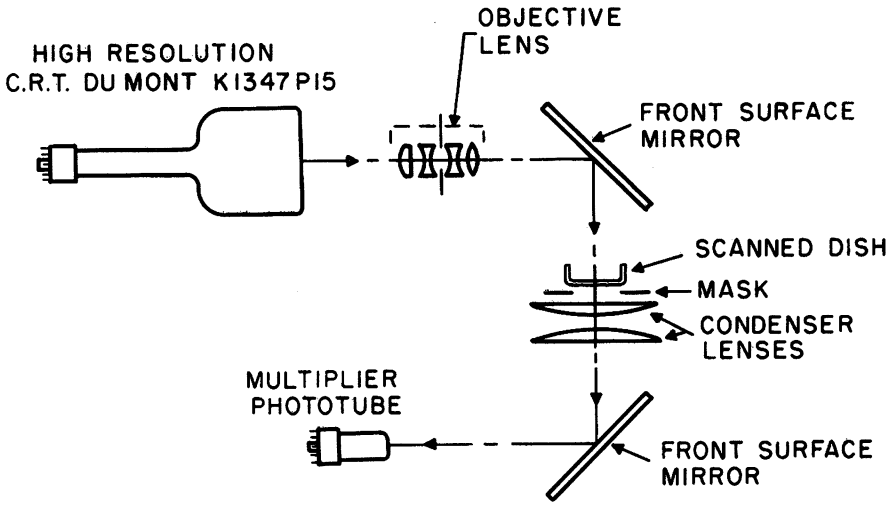

Fig. 2. Optical system.

The scanning spot produced by the cathode-ray tube is imaged, by means of an objective lens, on the agar surface of the culture dish. Light from this moving spot is transmitted through the dish and is collected by the condenser lenses, which in turn produce a disk of illumination on the photocathode of the multiplier phototube. The mirrors shown in Fig. 2 were used to fold the light path for convenience of mechanical design. Each time the scanning spot is occulted by an opaque or semiopaque colony (or any other particle), a pulse is produced by the phototube. The pulse width or duration is proportional to the chord length or intercept produced by traversing the colony. When a large number of closely spaced scanning lines is used, it is obvious that the number of pulses produced will be proportional to the sizes of the colonies, as well as the quantity. Since bacterial colony dimensions vary greatly with species, incubation time, and many other factors, an average colony diameter cannot be assumed. If such an assumption were valid, it would be a simple matter to calculate the quantity from the number of intercept pulses and the mean diameter.

Techniques for scanning and counting blood cells, using apertures and slits of successively different sizes to obtain statistical counts, have been used by Lagercrantz and others (*3*). Another technique, described by Roberts and Young, for a flying-spot microscope, uses a dual-spot scan to prevent multiple counting (*4*).

The instrument described here, illustrated by Fig. 1 and the operational block diagram of Fig. 3, uses a memory system to prevent multiple counting, regardless of colony size. This is accomplished by storing all the pulses on each scanning line for the total duration of one scan-line interval and comparing these pulses with any produced by the next successive scanning line. The logic of the circuits is such that, when the scanning spot intercepts a colony for the first time, a count is produced; but when the spot, on its next scan, intercepts the same colony, the stored or delayed pulse from the first intercept locks out and prevents additional counts. The delayed and undelayed pulses are compared on a time-overlap basis so that all other colonies resolved by the system are properly counted. Although the complete functional block diagram is shown in Fig. 3, only a very brief explanation of circuit operation is given and the reader is referred to other detailed discussions of the design (*5*).

The pulses produced by interception of the scanning spot with a colony are amplified and shaped to correct for certain optical and electronic distortion effects and passed to a delay circuit. The undelayed signal and the delayed signal are then both coupled to an anticoincidence circuit which performs the logic of first-intercept-recognition mentioned previously. The output of the anticoincidence circuit consists of one pulse for each colony. Each of the pulses is fed to a digital electronic counter which totals the count for one complete field scan.

The rest of the circuits shown in Fig. 3 provide for deflection of the scanning cathode-ray tube and of a monitor-display cathode-ray tube. The monitor-display tube presents a picture of the culture plate to the operator and facilitates proper adjustment of the machine in accordance with the types of specimens scanned. For example, certain types of culture medium may be extremely dense optically, so that a compensating adjustment of the sensitivity of the phototube or the brightness of the scanning spot may be required. Frequently the culture plate contains very small contaminants or dirt particles which cause extra counts. Under certain conditions

the display monitor also facilitates the making of compensating adjustments for this condition. Finally, the visual display monitor allows the operator to determine, at a glance, whether all the colonies are being detected and counted. The display monitor produces an image of each colony on the screen with a small bright checking dot superimposed at the top of each colony as it is electronically counted. A typical display of a scanned culture dish is shown in Fig. 4.

Evaluation of Machine Performance

Before presenting evaluation data on the performance of the machine, it would be well to describe briefly the basic capabilities and limitations determined by the present design. The size of the smallest colony that can be detected is determined primarily by the diameter of the scanning spot. In scanning a 100-millimeter diameter culture dish, colonies down to 0.5-millimeter diameter can be detected and counted reliably. Optical defects in the glass or agar, or contaminants, if large enough to be resolved, cannot be distinguished from colonies by the machine. If present, these defects and contaminants increase the count. It should be pointed out that a human observer, too, is frequently inaccurate in his count because of such factors.

Colonies which are contiguous and clump together are recognized by the machine as individuals only if they are oriented so that they present individual tangencies to the intercepting scanning lines. This effect can be observed on the picture display of Fig. 4. Note that colonies that are vertically contiguous produce only a single bright counting mark, while other contiguous colonies are registered properly. Here again, the technician performing visual counts may err in his judgment concerning clumped colonies, although not to the extent of the machine, which has no judgment.

Correction factors for contiguity can be derived by statistical methods when large samples of particular species of bacterial cultures are run. Care in the inoculation of culture plates and a reduction in incubation time, where possible, minimize contiguity. Care should also be exercised to prevent spreading the inoculant to the extreme edges so that colonies grow on the meniscus or sides of the culture dish. The glass-bead rim of molded petri dishes necessitates optical masking of the extreme edge of the dish in the machine. With poured plates this factor cannot be controlled, but statistical correction factors can be applied.

Finally, with regard to performance capabilities inherent in the technique, it should be realized that the machine is not subject to fatigue and that it is therefore far more consistent on repeated sampling than are human observers.

Before acceptance of the machine by the Chemical Corps, extensive evaluation tests were performed in their laboratories. The data obtained during these tests are not available for publication, but it may be stated that the results have been very satisfactory for their particular requirements. The major limitation to counting accuracy has been contiguity, as anticipated, but correction factors for some bacteria species have been derived.

Performance tests have also been made at the DuMont laboratories and at two dairy laboratories. Typical test data obtained at the DuMont Laboratories with cultures of *Serratia marcescens* on peptone agar are shown in Table 1. In performing these tests, the machine scans and registers a count every 2 seconds, while it takes a technician approximately 2 minutes to obtain a count by normal methods.

Table 1. Typical machine versus visual counts.

Plate No.	Visual count	Machine count
1	24	24
2	39	40
3	42	45
4	44	48
5	49	55
6	19	23
7	17	20
8	40	42
9	16	20
10	23	24
11	45	47
12	40	48
13	30	33
14	109	107
15	84	86
16	122	120
17	140	135
18	181	160
19	194	151
20	247	241
21	166	150
22	492	437
23	529	439

Data such as those given in Table 1, obtained from routinely prepared plates, show that the relationship of machine count to technician's count is a function of plate population density. At low densities the automatic count tends to be higher than the technician's count, whil at high densities the machine count runs 80 to 90 percent of the visual count. Study has shown that the causes of this bias are as follows: (i) High counts at low densities are caused by a few contaminants and defects in the glass and agar which are significant at low densities. (ii) Low counts at high densities are primarily caused by a large percentage of contiguous colonies. (iii) Low counts at high densities are caused, to a lesser extent, by the presence of colonies at the periphery of the dish which are not scanned by the machine.

Another condition which should be recognized is that it is common practice in counting high-density plates for a technician to count only a portion of the total area and then to estimate the total by the ratio of areas. The repeatability of technicians' counts is therefore very poor, and the variation between different technicians counting the same high-density plate is substantial. Typical variations and spread in technicians' count versus spread in machine count for repeated tests are shown in Table 2. These data

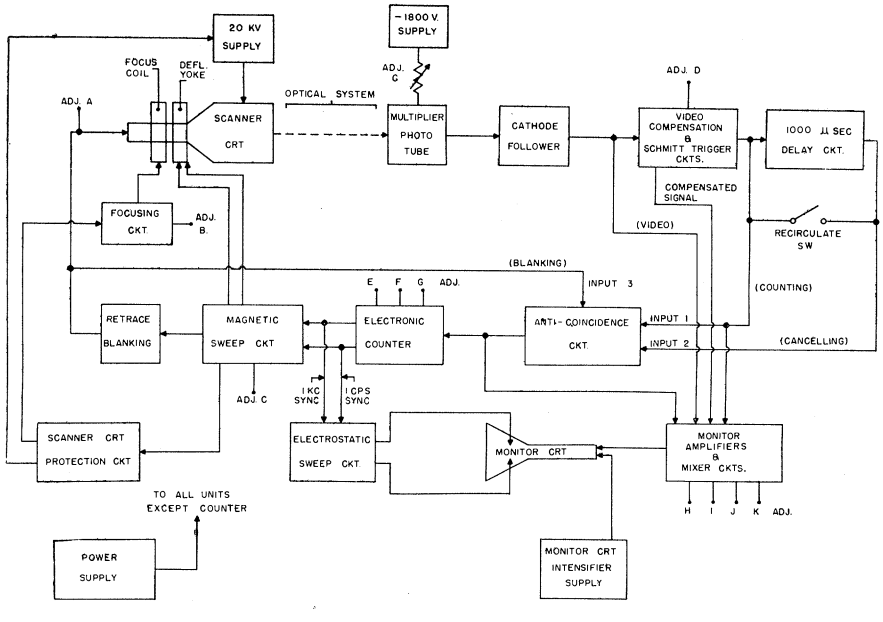

Fig. 3. Electronic scanning and counting system.

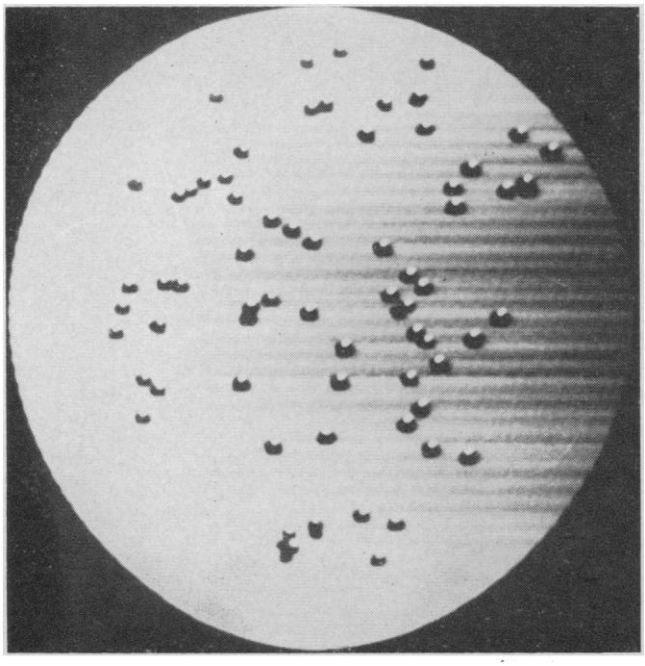

Fig. 4. Monitor picture display of a culture plate being counted.

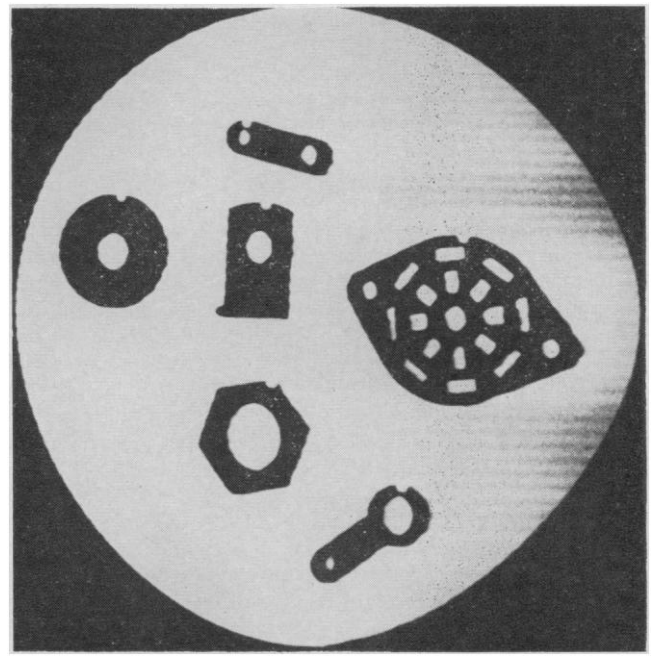

Fig. 5. Monitor picture display of assorted pieces of hardware being counted.

were obtained by visually counting incubated culture plates using four different observers and then counting each plate in the automatic counter using four different orientations with respect to the scan direction.

While the average machine count is frequently considerably less than the average visual count for normally prepared culture plates, it was found to be much more consistent. The automatic colony counter was sensitive to orientation of the culture plate because of the effect of colony contiguity. Since the colonies are randomly oriented with respect to clumping, no orientation could be found which would yield the true count. The machine was very consistent in repeated counts when the plate was kept in a fixed position.

Tests with simulated colonies on a plastic base, where no glass defects or contiguity were present, gave extremely accurate results. As an example, a specimen was prepared using a photoengraver's half-tone screen containing 2500 opaque dots 0.5 millimeter in diameter. The machine was able to produce counts of 2500 consistently for many hours. The addition of a single particle of material into the dish produced a count of 2501. The inherent accuracy of the machine is therefore 100 percent under ideal conditions. This suggests the desirability of improving the techniques of culture-plate preparation in order to realize fully the advantages of automatic colony counting. On the other hand, it is realized that the use of greater care in the preparation of cultures may offset the economies achieved by automatic counting.

An evaluation of the instrument's usefulness in routine dairy laboratory bacterial colony counting was undertaken at the Sealtest Supplee Laboratory of Philadelphia and at the New Jersey Dairy Laboratories of New Brunswick (*6, 7*). Present dairy laboratory counting procedures require that extremely small, pin-point colonies be counted in order to minimize incubation time and to supply rapid sample evaluation. Dairy culture plates usually contain a very large percentage of colonies 0.1 millimeter in diameter. Since the machine was designed to count colonies larger than 0.5 millimeter in diameter, the results of these tests were quite poor. In addition, routinely prepared plates in most laboratories contain optical defects in the medium and scratches in the bottoms of the petri dishes; both produce false counts. It was found that when the dish bottoms were treated with oil and a layer of water was poured on the surface of the agar, these errors were reduced. An experimental modification of one machine was made, to reduce the area of the dish scanned, so that a smaller scanning spot could be realized. Subsequent tests showed that, with this modification, colonies 0.1 millimeter in diameter could be detected and counted. However, the same difficulties previously noted with clumping or contiguous colonies occurred.

Table 2. Variations in visual count of four technicians versus variations in machine count in four orientations.

Visual count spread	Machine count spread
43–48	48–53
49–55	43–49
110–118	104–108
191–239	190–196
249–309	233–243
375–477	333–347
444–498	403–419

As a result of these dairy laboratory tests, the following conclusions were reached. (i) Substantial improvement in scanner resolution is required in a colony counter intended for dairy laboratory use. (ii) Satisfactory use of an automatic colony counter in the dairy laboratory requires that more care be used to avoid scratching petri dish bottoms and to reduce defects in the agar medium. (iii) More even dispersion of sample dilutions in the medium is required for automatic counting. (iv) Certain types of colonies, called "spreaders" and "pulls," would be extremely difficult or impossible to count automatically. (These types of colonies also confuse technicians, who must undergo a period of training before they can differentiate between the colonies of "pulls" and those which are not portions of such groupings.)

New techniques now being developed in the preparation of cathode-ray tube phosphors definitely show that greatly reduced spot size can be achieved. Improvement in scanner resolution (item i) therefore appears to be realizable. The

difficulty in handling described in item ii can be overcome by the use of oil treatment of the glass or by improved handling techniques. A step in the direction of solving the problem of item iii has been taken by the New Jersey Dairy Laboratories, which uses mechanical rotation of dishes during preparation to improve distribution. Most laboratories employ manual rotation of petri dishes, but the colony distribution so produced is poor. Building sufficient logic into a scanning machine so that it can count "spreaders" and "pulls" properly appears, at present, to be prohibitively expensive.

Other Applications

The scanning and information processing techniques used in the automatic particle and bacterial colony counter are applicable to many problems involving the analysis of a small or large visual field. For example, any objects that can be placed in a petri dish may be counted, provided that they are sufficiently large to be resolved (0.5 millimeter in diameter). Figure 5 shows an assortment of electrical hardware components being scanned and displayed on the monitor. The bright counting dot at the top of each item shows that it was properly counted and that the instrument was not "confused" by its shape or the presence of holes. Modified versions of the instrument have also been used with equal facility to count bacterial colonies on opaque membrane filters and pinholes in opaque metal foils. Although demonstrations of these capabilities have been impressive, no single version of a flying-spot scanning device can be versatile enough to handle all of the problems requiring optical scanning. Some applications require the scanning of very large areas, and others require the scanning of microscopic areas. Furthermore, many scanning applications require logic circuitry to provide size and area, density, spectral, and other types of information. This means, of course, that different optical and electronic systems must be developed for the basic flying spot scanner-phototube combination to apply it to these problems.

A whole new field of industrial instrumentation involving the use of flying-spot scanners for automatic flaw inspection of large-area sheet products such as paper, glass, metal, and foils appears to be imminent (*8*). At the other extreme, with respect to size of area scanned, flying-spot microscopes are being developed in this country (and are commercially available in England) to perform such functions as the counting and sizing of microparticles. One of the most significant contributions to biological research, in the field of cell and tissue studies, has been the development of an ultraviolet flying-spot microscope. This instrument, developed at the University of Texas Medical School, has permitted, for the first time, the unlimited study of living cells with ultraviolet light (*9*).

It is believed by many that the flying-spot microscope, when equipped with special information-processing circuits such as those of the automatic colony counter, will provide a very powerful tool for industrial and biological research laboratories. Accordingly, it may be destined to follow a path of development, requiring evolution of techniques and accessories, analogous to the path of the development of the electron microscope. The automatic particle and bacterial colony counter should be considered, therefore, to be one step in the development of flying-spot, optical-electronic transducer instrumentation.

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Servo Control of General Anesthesia

J. Weldon Bellville and George M. Attura

The automatic control art with its associated servo machines offers a finer control of physical variables than can normally be accomplished by human operators. This holds true for complex military devices as well as for those automatic control systems that have been introduced into the field of medicine. We have developed a servo control mechanism that will control a single physiological process, depth of anesthesia.

Regardless of its application, the quantity to be controlled by a servo must fulfill certain criteria. It must be measurable by some data-sensing element which translates the quantity in test into an electric voltage, and motor means must be available to control this quantity.

In the field of anesthesia, we have a situation that will fulfill these criteria. The depth of anesthesia is the quantity that must be controlled. For our purposes, we can define depth of anesthesia in terms of the cortical potential that is recorded by an electroencephalograph. The deeper the level of anesthesia, the less the electric cortical potential. If increasing concentration of anesthetic in the blood parallels increasing depth of anesthesia, then this premise is valid, since it has been shown that there is an inverse relationship between the concentration of ether or cyclopropane in the blood and the electroencephalograph potential (*1, 2*). The depth of anesthesia is usually controlled by the amount of anesthetic agent administered to the patient per unit time, whether the agent

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