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AN AUTOMATIC CELL COUNTER

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16. Abstract

A completely automatic cell counter using analog techniques and standard electronic TV components has been devised and is described here. It promises to perform with a counting error of around ±15 percent, and complete its operation in less than 2 min.

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SUMMARY

A completely automatic cell counter using analog techniques and standard electronic TV components has been devised and is described here. The basic idea behind its operation is first to gradually shrink the size of the spots representing the cell images. Then, when the spot size is within a certain range, it can be counted by a relatively simple procedure using special scanning technique. A simplified analysis of the proposed device is also given whose main result is that the expected counting error should be around ±15 percent. Finally, an account of a preliminary experiment is given which tested the principles behind the proposed spot shrinking procedure.

INTRODUCTION

In the following sections a detailed description will be given of an all electronic analog system that should be able to count biological cells on microscope photographs and also to group them into several size categories. It is assumed here that the microscope photograph can be processed so that there are only two widely separated levels of intensity. The cells are to appear uniformly black and the background is to be uniformly white. Thus, in a sense, it is actually the shadows of the cells that appear in the photograph. It is this photograph that is to provide all the input data to the instrument. Finally, the device, described below, fulfills the following requirements:

1. It is relatively inexpensive (i.e., costing less than $5000.00).
2. It is very simple to use.
3. It completes its operation in less than 2 min.
4. It has an accuracy comparable to that of a human operator.

The counter system consists of two independently functioning sections. The first section, which receives the input data from the microscope photograph, has the function of processing the data so that the cells, which come in various shapes and sizes, can be counted easily. This is accomplished by a type of processing where the sizes of all the black spots on the photograph (representing cells) are gradually reduced until they disappear. The smaller the spot the sooner it will disappear.

The second section, which receives the processed data from the first, has the function of counting the cells that appeared on the original photograph. The characteristics of the counting mechanism are such that only those cells will be registered whose size is roughly within a given range of values. The actual criterion that a cell image must meet, however, is more complex than this and is given in the form of probability statements, which will be discussed in detail in a subsequent section.

Thus, the overall operation of the automatic counter system is as follows: The input data from the original microscope photograph is fed to the processing section where the image is distorted by having the cell spots gradually shrink in size. This processed image, however, is at all times being projected into the counting section where a cell spot will be registered as soon as its size becomes reduced so that it falls within the determined range of values mentioned above. It will be seen that this type of counting procedure has the advantages of allowing the counting to be done by a relatively simple type of scanning procedure as well as providing for the classification of the cells into size categories.

PROCESSING SECTION

Preliminary Considerations

The processing section, as indicated above, is one of the two parts of the total counter device where the distortion of the cell image is accomplished before it can register a count. Figure 1 shows the distortion transformation associated with the processing procedure. Figure 1(a) represents two typical biological cells as they might appear on a microscope photograph (processed so that these are only two density levels as was mentioned above). After the cell images on the photograph have been treated by the processing section for a given time, they would have been transformed to have the shapes shown in Figure 1(b) (the dashed lines represent the original cell areas of Figure 1(a)). Note that the reduction takes place by having the points on the border move inward along the normals to the curve by a fixed distance. As will be seen, this fixed shrinkage distance, δ_s, increases uniformly with the processing (or it can be said to increase with time). Thus, if the processing treatment is allowed to progress further, the original cell image areas will have been transformed to the forms shown in Figure 1(c). Here it can be seen that the area on the right, which was originally the smaller one in Figure 1(a), is about ready to disappear with a little further processing; this processing, on the other hand, would only reduce the area on the left without removing it. The shrinking action shown in Figure 1(c) thus verifies the statement made above that the smaller areas disappear with processing before the larger ones.

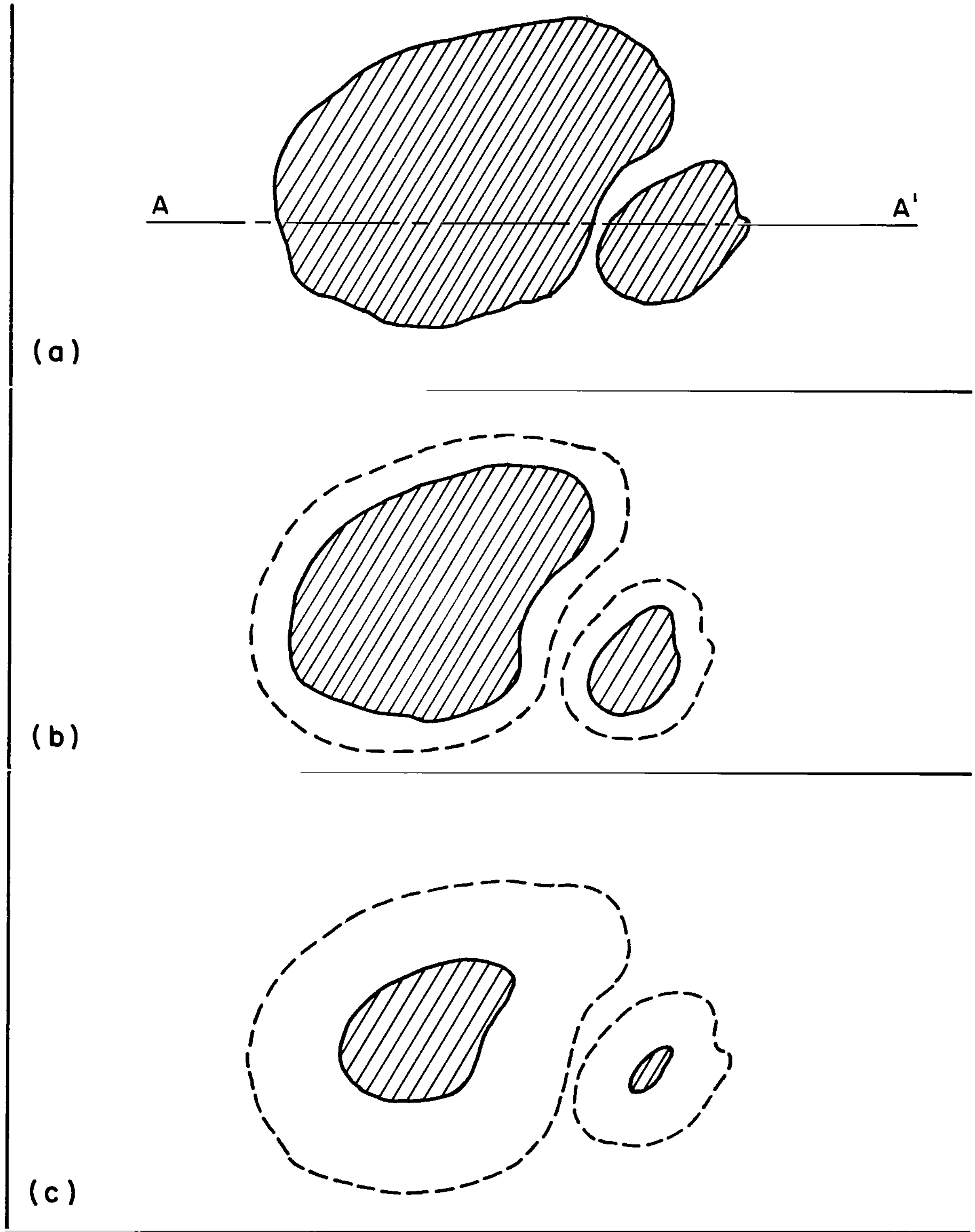

Figure 1.- Distortion transformation

Components and their Functions

The main components of the processing section are shown in Figure 2 and their functions are described as follows. The microscope photograph containing the input data is placed in the input chamber (labeled I.C. in Figure 2). It is briefly illuminated by a flash lamp so that its image is projected on the face of a special Vidicon camera tube. The light from the photograph passes through the half-silvered mirror, M_1, is reflected by the mirror, M_2, and is focused by the lens, L.

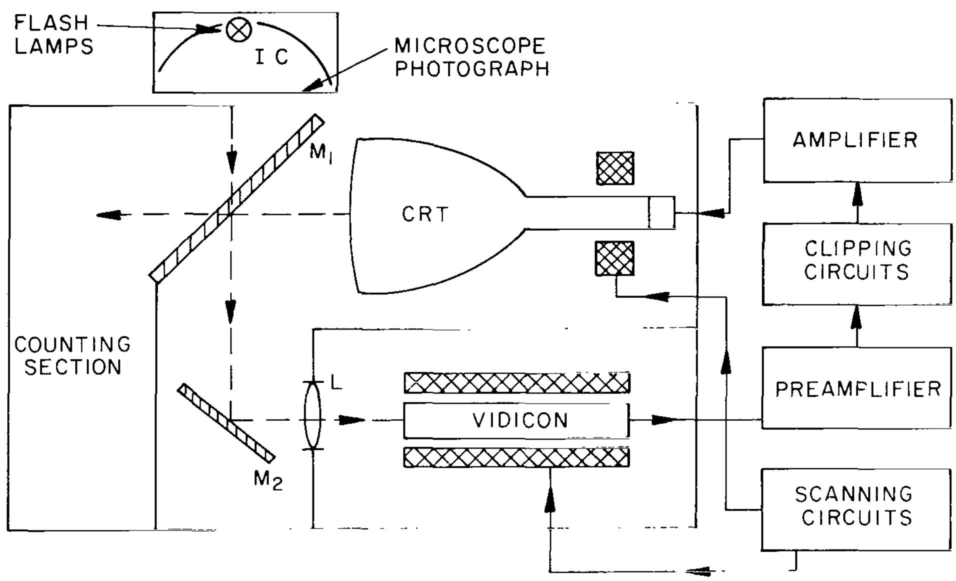

Figure 2.- Block diagram of main components of processing section

The photograph image, after being registered on the Vidicon face, is scanned by the Vidicon beam in a TV scan pattern (using around 500 lines) so that the optical information is converted into an electrical signal. This signal is amplified by the preamplifier and electrically processed by the clipping circuits. This signal is amplified again and applied to the intensity grid of a cathode ray tube (CRT) whose beam is scanning the face in exactly the same pattern as the Vidicon scan. The screen of the CRT has a long persistence phosphor (P12) which will retain an optical image for about 100 msec after it has been scanned. Thus, the optical image projected on the face of the Vidicon is in effect slightly distorted by an action to be described below and recreated on the face of the CRT.

As can be seen from Figure 2, the optical image from the CRT is projected on the face of the Vidicon by means of mirrors, M_1 and M_2, and the lens, L. Since M_1 is a half-silvered mirror, this image is also projected into the counting section where the cell images falling within the restrictions given above can produce counts.

The projected image from the CRT is allowed to store on the face of the Vidicon for a period of about 0.5 sec (at this point the CRT image intensity will have fallen to 1/100 of its original value). At the end of the storing period the image is scanned by the Vidicon beam to convert the optical information into an electrical signal. As is apparent from Figure 2, this signal is then applied to the CRT intensity grid after passing through the preamplifier, the clipping circuits, and the amplifier, just as was the previous signal corresponding to the original photograph image.

From the above description, it is apparent that the CRT and the Vidicon are part of what might be called a feed-back loop, with the CRT being electrically coupled to the Vidicon which is, in turn, optically coupled to the CRT. The action of this loop takes place in a series of successive cycles. The typical cycle would consist of the stored image on the Vidicon being scanned by its beam and thus converting it to an electrical signal. After being processed by the clipping circuits, this signal is used to recreate a slightly altered version of the image on the face of the CRT. The CRT image is projected on the face of the Vidicon where over a 0.5-sec time period it gradually creates a new stored image. The old one is erased as it is scanned by the Vidicon beam. The next cycle starts when the new stored image is scanned by the Vidicon beam.

It is apparent that the net effect of an action cycle is to slightly distort the optical image appearing on the CRT face. It will be shown that the distortion is such as to make the dark spots representing cell images slightly shrink in size in the manner described in Figure 1. By continuing the loop action over a sufficient number of cycles, all the cell-image spots can be made to disappear. The distortion action on the CRT image is now to be described in detail.

This distortion can best be understood by referring to Figure 3. In Figure 3(a) the solid line represents the one-dimensional intensity distribution that would result if the light intensity were plotted versus the distance, X, along the section line, AA^1, of Figure 1(a) (the points A & A^1 of Figure 3(a) corresponds to the points A & A^1 of Figure 1(a)). An important feature of the operation of the system shown in Figure 2, which has not been mentioned so far, is that the Vidicon lens, L, is slightly defocused so that the AA^1 intensity distribution of the image projected on the Vidicon face would be given by the dashed lines in Figure 3(a), where it is seen that the sharp edges of the original distribution of Figure 1(a) have been "rounded off." If the Vidicon beam were to scan along the line AA^1, it would actually generate an electrical signal versus time pattern identical to the one given by the dashed curve of Figure 3(a). By assuming that this type of scan has taken place, it is possible to illustrate just how the clipping circuits function.

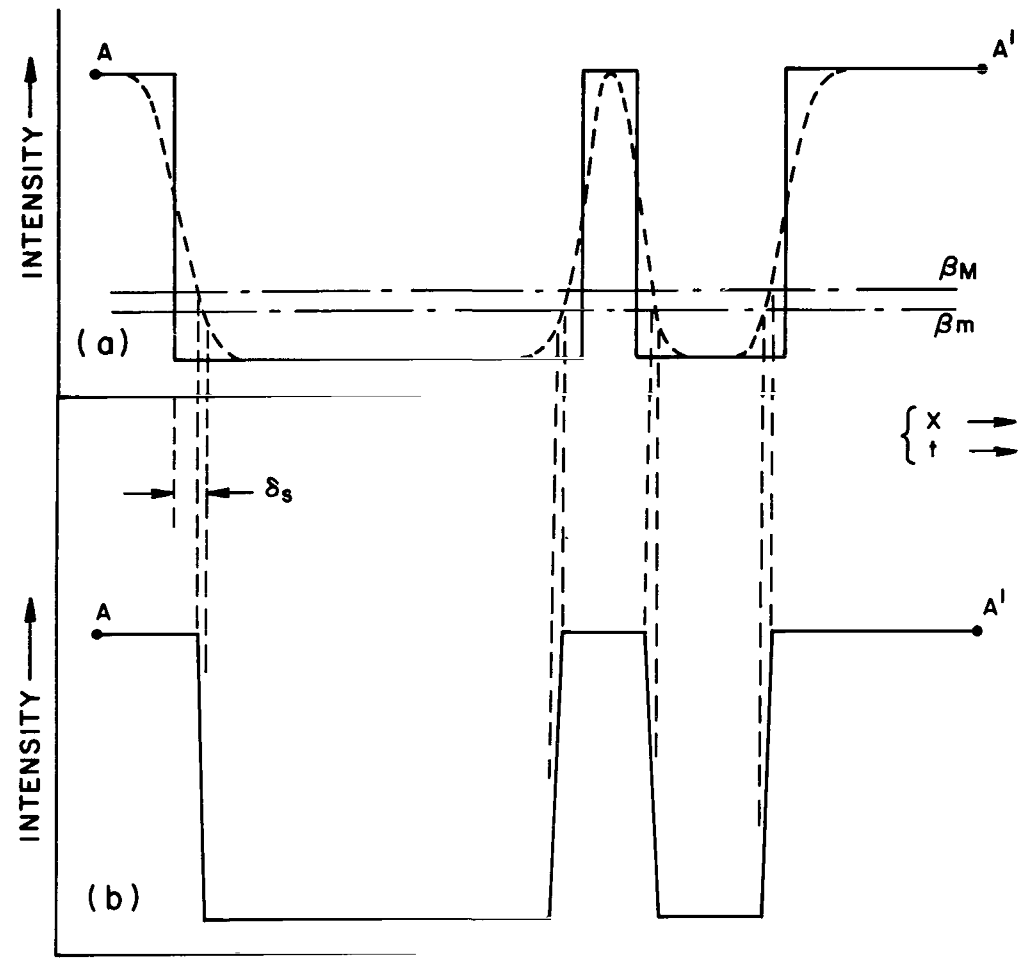

Figure 3.- Distortion action on CRT image

The main component of the clipping circuits is a special kind of amplifier system which is normally biased beyond cutoff and which saturates at a low signal level when in a conducting state. The result of this type of operation is that, when an electrical signal having a time pattern of the dashed curve in Figure 3(a) is applied, signals below the level, B_m, will be below cutoff and thus not be able to pass through. Signals above the level, B_M, will saturate the amplifier system. Thus it follows that the output signal from the clipping circuits would have a time pattern of the form given by the curve in Figure 3(b).

Note that the square "wells" in the curves of Figures 3(a) and 3(b) represent the dark cell spots and their widths represent the actual width of these spots along the line, AA^1. Inspection of Figures 3(a) and 3(b) reveals that the width of the "wells" (and therefore the dark spots) in Figure 3(b) has been reduced in comparison with that of the solid curve in Figure 3(a). This is a result of the combined action brought about by the defocusing of the Vidicon lens, L, and the processing of the Vidicon electrical signal by the clipping circuits. It can also be seen that the width of each "well" in Figure 3(b) has been reduced by approximately the amount, $2\delta_s$ (where δ_s is the shrinkage distance). Thus, from the above description, it is apparent that the defocusing of the lens, L, and the action of the clipping circuits are together able to produce the type of cell spot shrinkage that is illustrated in Figure 1.

The salient features of the processing section have now been qualitatively described. There are still some important details of operation and construction to be considered, and this is done in the following section.

Some Operational Considerations

Choice of δ_s.- One of the most important problems in the operation of the processing section is the choice of value for the shrinkage parameter, δ_s (which can be controlled by varying the defocusing of the lens, L). If the maximum cell spot area that the counting section will register is α_M and the minimum area is α_m, it is desirable that δ_s be chosen so that in going from one action cycle to the next all the cell spots having areas in the range α_M to α_m be reduced to areas less than α_m (or be made to disappear). Otherwise, some of the cells may be counted twice. The preceding condition would seem to limit the minimum value of δ_s.

Another condition to be satisfied is that in going from one action cycle to the next, δ_s must be such that none of the cell spots having areas greater than α_M be reduced to a value less than α_m. Otherwise, some of the cells will miss being counted. This condition would seem to limit the maximum value of δ_s.

In general, where the cell image spots would have a large variety of sizes and shapes, it will probably not be possible to choose a δ_s so that no cell will ever miss being counted or be counted twice. The best that can be done here is to choose δ_s so that these two effects will be minimized (or perhaps made to be equal on the average since they counteract each other). No meaningful quantitative statement, however, can be made on the probable counting error as a function of the value of δ_s until a mathematical investigation is made using probability theory. This is done in a subsequent section of this report.

Vidicon requirements.- In studying the performances of the CRT and the Vidicon of the system of Figure 2 during an action cycle, it can be seen that it is necessary that the Vidicon beam completely (or at least very nearly) erase the stored image as it scans it. Otherwise the image on the CRT face will be constructed from electrical signals corresponding to previous cycles as well as its own cycle and this will, in general, be an error signal. This means that the Vidicon utilizing a photoconductive target plate cannot be used in this system, since it requires around 10 scans over any given line to obtain sufficient erasure (which has been taken to be such that there is less than a 1 percent residue).. However, a Vidicon (No. C23136) has been developed which has a target face consisting of an array of silicon diodes and which can achieve adequate erasure with two scans. Using this camera tube appears to be feasible for the present system. The beam scanning pattern produced would be somewhat different from the TV pattern. The pattern would be such that each line would be scanned twice before the beam went to the next line. The electrical signal would be taken only from the first scan of a line with the signal from the second scan being gated out. Thus the scanning time for a frame would be 1/15 sec instead of 1/30 sec.

One other point should be mentioned in connection with Vidicon scanning. Almost as soon as the Vidicon beam reaches any given point in the image frame (i.e. within 1/2 μsec) that point is starting to be illuminated by light from the CRT with an intensity corresponding to that associated with the given point on the image of the next action cycle. Since the lens, L, is slightly defocused, a point in the image on the CRT face will produce a circular spot on the Vidicon face whose diameter could be around 10 scanning lines. This means that, when the Vidicon beam is at a given point on a given line, the CRT illumination corresponding to that given point is extending over 10 lines lying nearby, five of which are soon to be scanned by the beam and erased. The net effect is that part of the signal associated with the next action cycle is being fed into the present action cycle and thus producing an error in processing. However, with the present design this error signal will constitute a negligible fraction of the total signal. This is obvious from the fact that the greatest error will come when the beam scans the fifth line in advance where the CRT light has been storing for $(5 \times 2)/(500 \times 30) = 0.7 \times 10^{-3}$ sec. However, because of the persistence of the CRT screen, it will be emitting light for effectively 120×10^{-3} sec, so that the portion being prematurely erased by the beam is $(0.7 \times 10^{-3})/(120 \times 10^{-3}) = 1/180$, and this can be considered negligible here.

The Vidicon lens.- The defocusing of the Vidicon lens, as well as the shape of the lens aperture stop, are crucial elements in the processing action. It is desirable that the slope of the intensity curve (i.e., the "rounding off" effect illustrated by the dashed curve in Figure 3(a)) after defocusing be as great as possible at the values where the clipping circuits take effect (i.e., at the intensity values B_m & B_M in Figure 3(a)). This slope can be significantly increased if the aperture stop is such that the light transmission area is a narrow circular ring instead of a circular disk. A new type of aperture stop can be constructed by simply placing a black opaque circular disk with a radius somewhat less than the lens radius at the center of the lens.

Just how much the slope is enhanced by using the new aperture stop can be determined by a mathematical analysis using geometric optics theory. This is another task that could be undertaken in connection with the development of the Automatic Counter.

Image frame size and positioning.- A brief treatment is to be undertaken here of the important condition that CRT optical image frames from two successive action cycles must be projected on the Vidicon face so that the frame borders exactly coincide. If there is a slight displacement in positioning or in size, this error will be repeated in going from one action cycle to the next and the effect will be accumulatively magnified. Thus, if the error is in positioning, the image frame will gradually move off the edge of the Vidicon face at the end of a large number of action cycles. If the error is in size for a large number of action cycles, the frame will either grow so large as to go off the Vidicon face or it will shrink to an unmanageable small size.

It is hoped that careful manual adjustments made at reasonable intervals will be enough to counteract the effects mentioned above. If a large number of action cycles are desired (i.e., more than 25), it may be necessary to develop some sort of servo system to counteract the positioning and size errors.

Experimental Testing

It is pertinent to this report to relate the results of a certain experiment which tested the principles underlying the processing action described above. The experiment equipment consisted of a TV camera, a TV monitor kinescope, and a Land photographic camera. An artificial test pattern was constructed by cutting out various shaped spots from black paper and pasting them in a random pattern on a white paper background (8-1/2" x 11"). The test pattern was viewed by the TV camera and reconstructed on the face of the TV monitor which was viewed by the camera. The Land camera was then used to photograph the monitor image.

The action cycle discussed earlier in "Components and their Functions" was simulated by first placing the photograph before the TV camera to be viewed. Next, the lens on the camera was slightly defocused from optimum. The contrast control on the TV monitor was turned up all the way, and the light level control was turned down to provide a crude simulation to the clipping circuits. At this point, a new image appears on the TV monitor which is slightly different from the previous one due to the TV lens defocusing and the special setting of the monitor controls. The next action cycle begins when the new image is photographed and placed before the TV camera. Just as before, a new image with further distortions appears on the TV monitor. This is photographed to begin the third action cycle. In this way, any number of consecutive cycles can be performed and corresponding to each cycle, there is a photograph of the TV monitor.

When the experimental procedure described above was actually performed, it was found that practically all of the image spots had disappeared after 10 cycles. Even though there was no accurate simulation of the clipping circuits, at least qualitatively it appeared that the "shrinking" action was taking place on all spots with the small spots disappearing on earlier cycles than the large spots. Significant magnification of the image field was taking place in the later cycles due to the image size error effect.

The above experimental results would seem to partially verify that the desired processing action takes place in the actual operation of the system in Figure 2 and is not just a theoretical possibility. Whether or not the "shrinking" action was exactly like that of Figure 1 could not be determined due to the crudeness of the experiment. However, from theoretical considerations, it seems probable that it would be so in the system in Figure 2.

COUNTING SECTION

Preliminary Remarks

The detailed description of the Counting Section is now discussed. As will be seen, the counting procedure used here depends on having the microscope photograph image stored on the face of a Vidicon and then having it scanned with the electron beam in a special manner. The electrical signal from the Vidicon during the scanning can then be processed to give the desired count information. In this report, one method of scanning will be treated which, at this time, seems the most promising in terms of the trade-off between counting accuracy and simplicity of implementation. Understand that this approach is only a first attempt. It is possible that in the future even more desirable scanning methods can be devised. One of the main objectives of this report is to illustrate the basic fundamentals in the counting procedure when it is performed by a scanning technique for the type of counting which is of interest here.

Components and Operational Description

The main components of the Counting Section are illustrated in Figure 4. It is apparent that the section of the main chassis in Figure 2 labeled "Counting Section" has now been supplied with details. Essentially, the counting system is a TV camera tube recording the successive CRT images of the Processing Section and the associated circuitry to analyze the electrical signals from the camera tube. In addition to external circuitry, camera tube, and its deflection coils, the components in Figure 4 are: the mirror M_3 to provide a 90° deflection of the CRT image and L_C, and the lens for the Vidicon. Functions of the various circuitry blocks in Figure 4 become apparent in the subsequent sections which provide a rough qualitative description of the Counting Section operation.

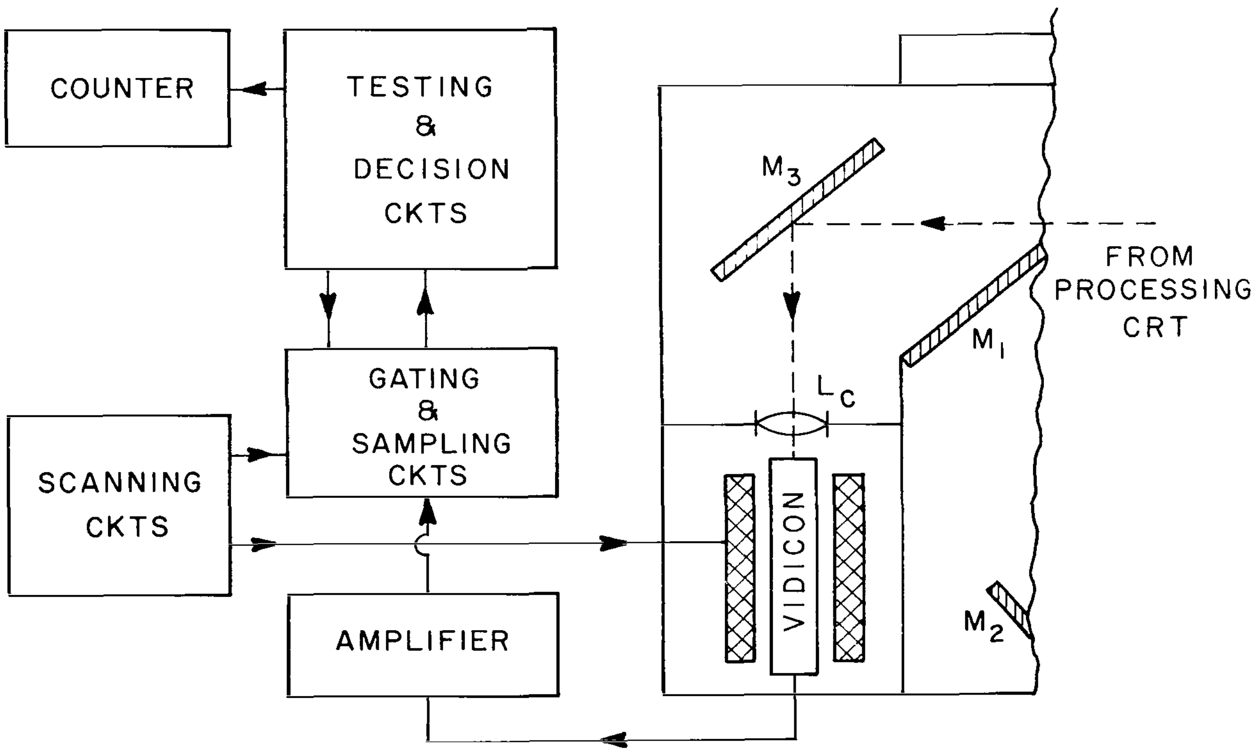

Figure 4.- Counting section block diagram

To understand this operation, assume that an optical image associated with one of the action cycles has just been created on the face of the CRT in the Processing Section. This image is then projected on the Vidicon, Figure 4, where it is stored by creating an electric charge image on the camera tube face. When this charge image is scanned with the electron beam, it generates an electric signal which is then amplified by the circuitry of the amplifier block in Figure 4. The exact type of scanning employed and the exact form of signal being generated is now considered in detail. The essential features of the scanning mode is illustrated in Figure 5.

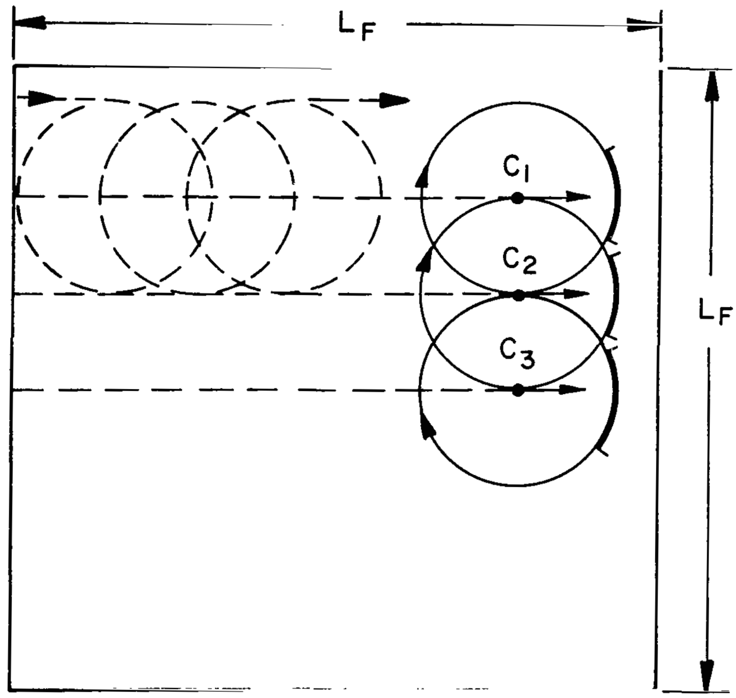

Figure 5.- Scanning mode

The actual trace of the scan is shown by the heavy dotted lines and is a hypocycloid. This is formed if it is imagined that the beam trace is travelling clockwise in a circle around a center point with constant velocity and that this center point is travelling in a horizontal line (from left to right), also with a constant velocity. The radius of the circle, r is about 1/20 of the optical field length, L_F. The velocity of the center point is to such that it will have advanced a distance of around r/10 in the time interval between successive maximum vertical positions of the beam trace (i.e., the period of a complete rotation). In Figure 5, C_1 is the center point of the top trace. When the trace has made a complete (left to right) horizontal sweep of the field (i.e., when point C_1 has travelled a distance L_F), a new horizontal scan is then initiated by deflecting the beam to the left side of the field and moving the center point vertically downward a distance of r. The center point would then be represented by point C_2 in Figure 5. The circles associated with center points C_1 and C_2 are shown in Figure 5 by the full lines with the arrows indicating the directions of rotation. Note that there are segments of the C_1 and C_2 circles which are emphasized by heavy lines. These are called detection arcs and their function will be explained below. When the C_2 circle has horizontally scanned the field, a new trace is then initiated which is similar except that it is deflected downward a distance of r. This new trace would have center point C_3 shown in Figure 5.

From the above description, it should now be obvious how the field is scanned. The scanning trace consists of a series of horizontal hypocycloids each displaced vertically downward from the previous one by a distance of r. A number, (L_F/r), of these horizontal traces should be able to cover the whole optical field.

To understand the process of how an image spot is to be detected, tested, and how the count decision is made, reference must be made to Figure 6. In Figure 6(a), two image spots, S_I and S_{II}, have roughly the same area and the same shape. The only difference (other than the horizontal positions) is that S_{II} is slightly displaced downward from S_I. As mentioned above, the scanning trace of the electron beam is a hypocycloid which can be generated by the beam moving in a circle (of radius r) whose center is moving slowly in a horizontal line. To illustrate how the total counting operation is performed on image spot S_I in Figure 6(a), assume that the center of the hypocycloid cycle is moving from left to right on the horizontal line, A-A'.

Note that an arc segment of about 60° of the circles associated with the hypocycloid are drawn in full line while the rest of the circle is drawn in dotted line. This is the detection arc mentioned above and is used to denote that while the scanning beam is in what is called here the detection mode of operation, the signal from the Vidicon is gated (by the Gating and Sampling Charts in Figure 4) so that it is being transmitted only when the beam is tracing this detection arc. When the beam is traversing an area that contains no image spots, no signal is being generated. As soon as the beam trace enters an area containing image spots so that the detection arc enters points on the Vidicon face containing stored image charge, electrical signals will pass from the Vidicon into the Testing and Decision Circuits. This situation is illustrated by the circle in Figure 6(a) whose center is at C_1, the point at which signal first starts being generated.

If the electrical signal is sustained for a certain time interval (arbitrarily chosen here to be about $(r/5V_C)$, where V_C is the velocity of the circle center), the Testing and Decision Circuits switch to what is called here the test mode of operation in which the Gating and Sampling Circuits are then by-passed. Here the decision is made whether or not the detected object is to be registered as a count. This decision is to be based entirely on the following criterion: <u>In the time interval, $\tau_T = 2r/V_C$, immediately after detection, there must exist a</u>

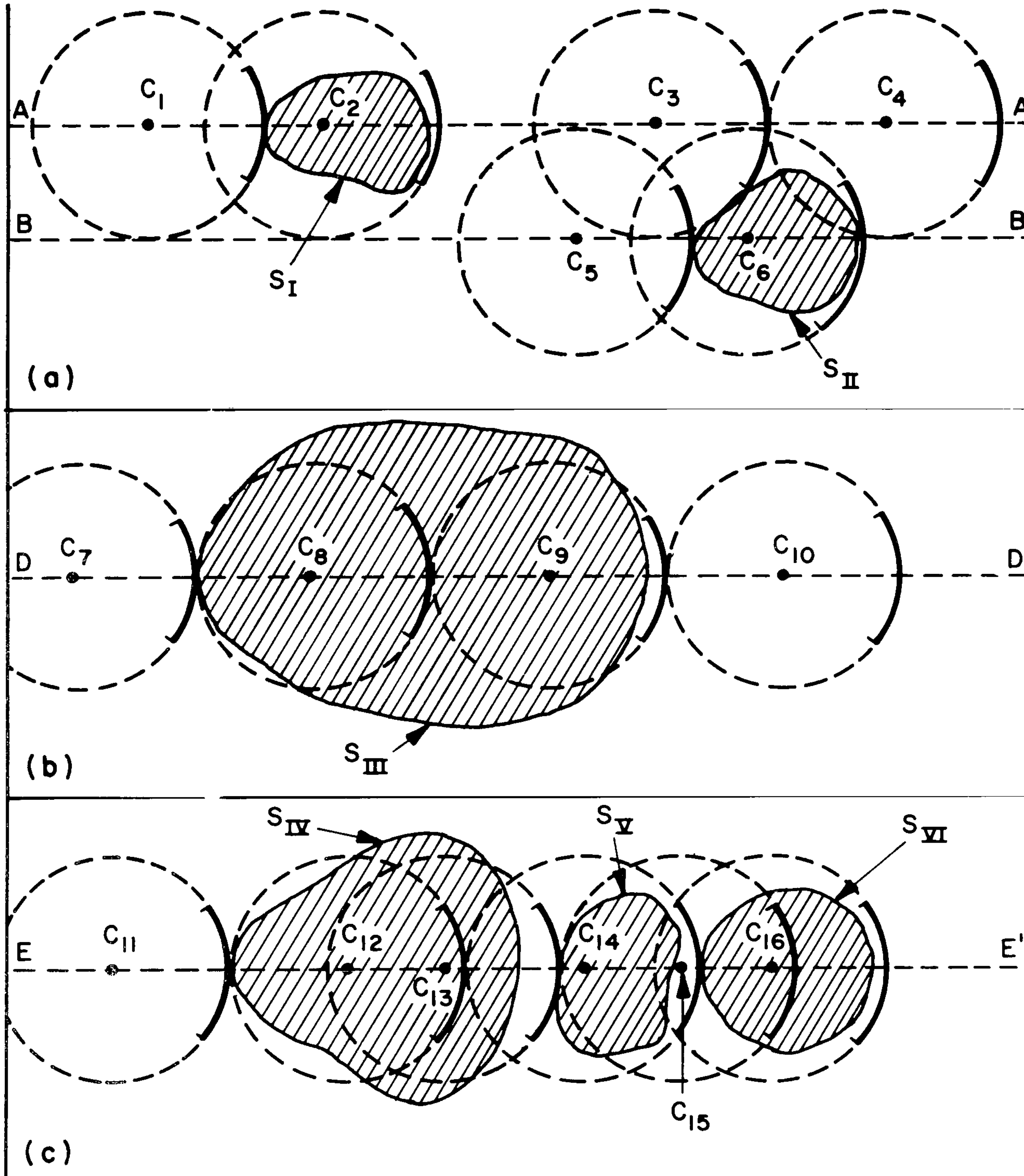

Figure 6.- Image spot detecting, testing and counting decision

sub-interval, $\tau_s \geq \frac{2\pi r}{V_o}$, over which no signal is being generated. (V_o is the velocity with which the beam trace travels around the circle.) The physical interpretation of this criterion is that in time interval, τ_s, the beam trace made at least one complete circle without running into any image charge. This situation is illustrated by the circle in Figure 6(a) whose center is at C_2. If the criterion is met, then the detected spot is registered as a count. Failure to do so results in no count.

As soon as a count is registered (or at the end of the testing interval, τ_T, if there is no count,) the operation state is switched back to the detection mode assuming there is no signal being generated. If there is a signal being generated in the interval τ_T to $(\tau_T + r/5V_c)$ after the initial detection, the operation state is not switched back but this is held off until a point is reached where no signal has been generated over a time interval of $r/5V_c$. Once back in the detection mode, the same sequence of events that is described above will occur if the detection arc of the beam trace encounters a new image spot.

It may seem that the procedures described above for the detection, testing, and decision making of the counting operation are somewhat arbitrary. It is felt that a few pertinent remarks at this time can provide some degree of qualitative insight so that some of the reasons for the above procedure may be inferred and thus be found more acceptable. In studying the detection operation, it can be seen in effect the electron beam is searching for stored image charge by making a set of short vertical lines advancing horizontally by steps of r/10. Hence, unless the maximum horizontal dimension of an image spot is less than r/10, it will certainly be found if it lies in the horizontal stripe whose width is the detection arc. Thus, the detection mode of operation seems straightforward requiring no added comments.

In the test mode, it was in effect required that the beam trace make a complete circle (actually a loop of the hypocycloid) without encountering any stored image charge. The reason for this becomes apparent when it is noted that this requirement could only be satisfied if the image spot is an area of stored charge that is isolated from any other stored charge areas and if the maximum dimension of this area in any direction is less than 2r. The procedure can therefore be seen to test if the detected charge area is an isolated spot of less than a certain critical size. This is exactly the type of test that is required for the mode of counting of interest here.

It might seem curious that the operation state was not switched back to detection mode from test mode right after a count or at the end of the test interval, τ_T, instead of adding the extra complication of requiring no signal to be present. Actually, the reason for the extra complication is that it will avoid missing counts in certain situations. One such situation is illustrated in Figure 6(c). Just how the missing count arises here in the absence of the extra switch-back criterion will be taken up in detail in the section below, along with the analysis of the counting operation for other situations.

Illustrations of the Counting Operation

In this section, some of the more common examples likely to be encountered in practical cell counting are analyzed in detail to illustrate how the counting operation described above actually performs. In the interest of brevity, only a few examples are treated. There are a number of important examples that are not considered but it is hoped that their treatment will be included in a future work.

The first example is that of the image spot, S_I, in Figure 6(a). This has already been considered above and from these remarks, it follows that S_I would register a count with detection taking place when the scanning circle is at C_1 and the count registering when it is at C_2. This illustrates proper functioning since the maximum dimension of S_I in any direction is less than 2r and therefore should register.

It is evident that whether or not an image spot can register a count will depend not only on its having the correct size but also on its vertical position. A case where an image spot will not register a count even though it is of the correct size and is detected is illustrated in Figure 6(a) by the spot, S_{II}. Here, it can be seen that when the scanning circle center is moving on the horizontal line, AA', detection of S_{II} takes place when the circle center is at C_3. As the center moves from C_3 to C_4 (which is its path over the testing interval, τ_T), at no time will there be no signal over the trace of a complete scanning circle. Thus no count will be registered. In the case of the spot, S_{II}, there will be a count registered eventually when the circle center moves on its next horizontal line, BB'. As can be seen detection would take place when the center is at C_5 and a count would be registered when it is at C_6.

It turns out, however, that this need not always be true for spots of the correct size. It can be shown that, in general, such an image spot would always register a count only if its maximum vertical dimension, $\hat{y}$, is such that $\hat{y} \leq r$. If the maximum vertical dimension is such that $r < \hat{y} < 2r$, then depending on its vertical position there is a possibility that it will miss being counted even though it is detected. The a priori probability of a missing count can be shown to depend on the quantity, $(\hat{y}/r)$, with the greatest probability being when $\hat{y} = 2r$. On the other hand, if $o < \hat{y} \leq r$, there is always a possibility (again depending on its vertical position) of there being a double count*. This is not so for the case, $r < \hat{y} \leq 2r$. A summary giving the exact a priori probabilities for missing counts and double counts for various image spots is given in the following section. The details of the counting behavior of image spots of various sizes can thus be obtained by referring there.

As already mentioned, an image spot having a maximum dimension in any direction exceeding 2r, should not be able to register a count. To demonstrate that this is so, an example illustrated in Figure 6(b) is now considered. It is seen here that the scanning circle center moves along the horizontal line DD' and that detection of the spot, S_{III}, takes place when the circle center is at C_7. The test mode will then last until the center reaches C_9 and during this time there can be no count registry since all the scanning circles will intercept signal areas. Because of the extra switch-back criterion mentioned earlier in Components and Operational Description, switch-back to the detection mode will not take place until the circle center reaches C_9.** It is seen that as the circle center moves to the right from the point C_9, the detection arc will not intercept any signal areas unless it reaches a new image spot. During the whole sequence of events in the encounter of the scanning circles with the spot C_{III}, note that no count was ever registered, as was to be expected for proper functioning.

The next example to be considered is one that illustrates how the counting system under investigation can malfunction. This case is illustrated in Figure 6(c) where there are three image spots, S_{IV}, S_V, and S_{VI}. Here it can be seen that counts should be registered for S_V and S_{VI} but not for S_{IV}. (It it too large). Actually, the counting system will count S_{VI} but miss counting S_V. This can be seen by studying Figure 6(c). S_{IV} is detected when the scanning circle center is at C_{11}. There will be switch-back to the detector mode just before the center reaches C_{13} and S_V is detected when it is at C_{13}. During the interval of the test mode when the center is moving from C_{13} to C_{15}, it can be seen that the scanning circle is always intercepting signal areas either from S_{IV} or from S_{VI}. Thus no count can be registered for S_V and this is due to the fact that the two neighboring spots, S_{IV} and S_{VI} are both too close. If only one were too close it can be shown that the malfunctioning would not occur. Further study shows that there is no problem with the count of S_{VI} with

* This term means that the spot registers a count on both horizontal sweeps that detect it.

**Otherwise it would have switched back at C_8.

detection taking place when the scanning circle center is at C_{14} and the count is registered when it is at C_{16}.

Another case where the counting system would malfunction by missing a count is where the spot has a long narrow shape where its maximum dimension exceeds 2r and its minimum dimension is less than $2\delta_s$. In this case the spot would be detected but not counted in the present action cycle. It would disappear in the next action cycle and therefore the spot would never be counted.

It was mentioned earlier that the reason for introducing the extra criterion for switch-back to the detection mode from the test mode is to prevent missing counts in certain cases. An illustration of such a case is now considered as the final example in this section. This case can be seen by again referring to Figure 6(c) where it is now assumed that the image spot S_{VI} does not exist so that there is only the spots S_{IV} and S_V. With switch-back conditions as presently postulated it can be shown that the counting system would function properly with spot S_{IV} not being counted and the spot S_V registering a count. In verifying this statement, it is seen that S_{IV} is detected when the scanning circle center is at C_{11}. There would be no count for S_{IV} during the test mode with switch-back occurring just before the center reaches C_{13} just as in the previous paragraphs. S_V is detected when the center is at C_{13}, but this time a count would be registered when the center was somewhere between C_{14} and C_{15} since now spot S_{VI} would not be present to intercept the scanning circle.

If the extra switch-back criterion were not present it can be shown that the spot S_V would miss being counted. This can be seen by following through the counting events. After detection with the center at C_{11}, switch-back would occur when it reached C_{12}. Detection would occur almost immediately and the counting system would again be thrown into the test mode. This would last until the center reached C_{14}, at which point switch-back would occur again. This time, however, the counting system would stay in the detection mode since the detection arc would not be intercepting any more signal areas as the circle moved to the right. Note that as the center moved from C_{13} to C_{14}, no count was registered. Thus image spot S_V missed being counted.

Counter Behavior Characteristics

This section summarizes the results of an analysis of how the proposed counter system would respond to image spots of various sizes and shapes when they are placed at various points in the optical field. The actual analysis itself will be omitted here, again in the interest of brevity, but it can readily be supplied if a large enough demand arises. An exact analysis for image spots of arbitrary shapes and sizes would be a rather extensive undertaking. Therefore, as a compromise between rigor and accuracy of results and between ease of implementation, certain simplifying assumptions have been made about the image spots to be counted. The essence of these assumptions are that out of the entire class of possible image spot shapes, only a certain sub-class is to be treated here. This sub-class still allows a great deal of variation in shapes of the image spots. Even though this means that the results obtained here are strictly speaking, not applicable to image spot shapes outside of the chosen sub-class, it is nevertheless felt that these results are valuable in characterizing the general behavior of the counter and also in providing a rough estimate of the counter efficiency. Moreover, in many cases it should be possible to infer in a rough qualitative way what the behavior of the counter would be for image spot shapes outside of the chosen sub-class. The main assumptions about spot shapes to be adopted here are: (1) the maximum dimension of an image spot which passes through its centroid has a direction that is either nearly vertical or nearly horizontal, (2) the ratio of the maximum dimension to the minimum dimension of an image spot should not be too large (less than 5), and (3) there is no a priori knowledge of the vertical position of an image spot on the optical field (i.e., any vertical position is equally probable). Assumption (2), incidentally, has the effect of eliminating long, narrow image spots which are particularly difficult to count.

For a counting operation over just one action cycle the characteristics of the counter behavior are presented in Figure 7. Here it can be seen that only one parameter (the maximum vertical dimension, $\hat{y}$) relating to spot size has been considered. This can be shown to be the most crucial parameter for the chosen sub-class of shapes. In fact as long as the maximum horizontal dimension, $\hat{X}$, and the shape are such that the image spot can be enclosed within the boundaries of a certain given area, the other parameters are of no importance.

Figure 7(a) is a plot of the a priori probability, P_D, that the counter will register a double count for a given image spot as a function of $\hat{y}$, its maximum vertical dimension. Figure 7(b) is a plot of the a priori probability, P_M, that the counter will miss counting a given image spot a function of its $\hat{y}$. As stated earlier, Figure 7 shows that an image spot will always register a count when $0 < \hat{y} \leq r$, and there can be no double counting when $r < \hat{y} \leq 2r$. In summary, Figure 7 provides a measure of the probabilities of the two ways that the counter can malfunction for a given image spot.

It is to be noted again that the results here are of the counting operation over just one action cycle. If the entire operation is considered, the important fact that emerges is that

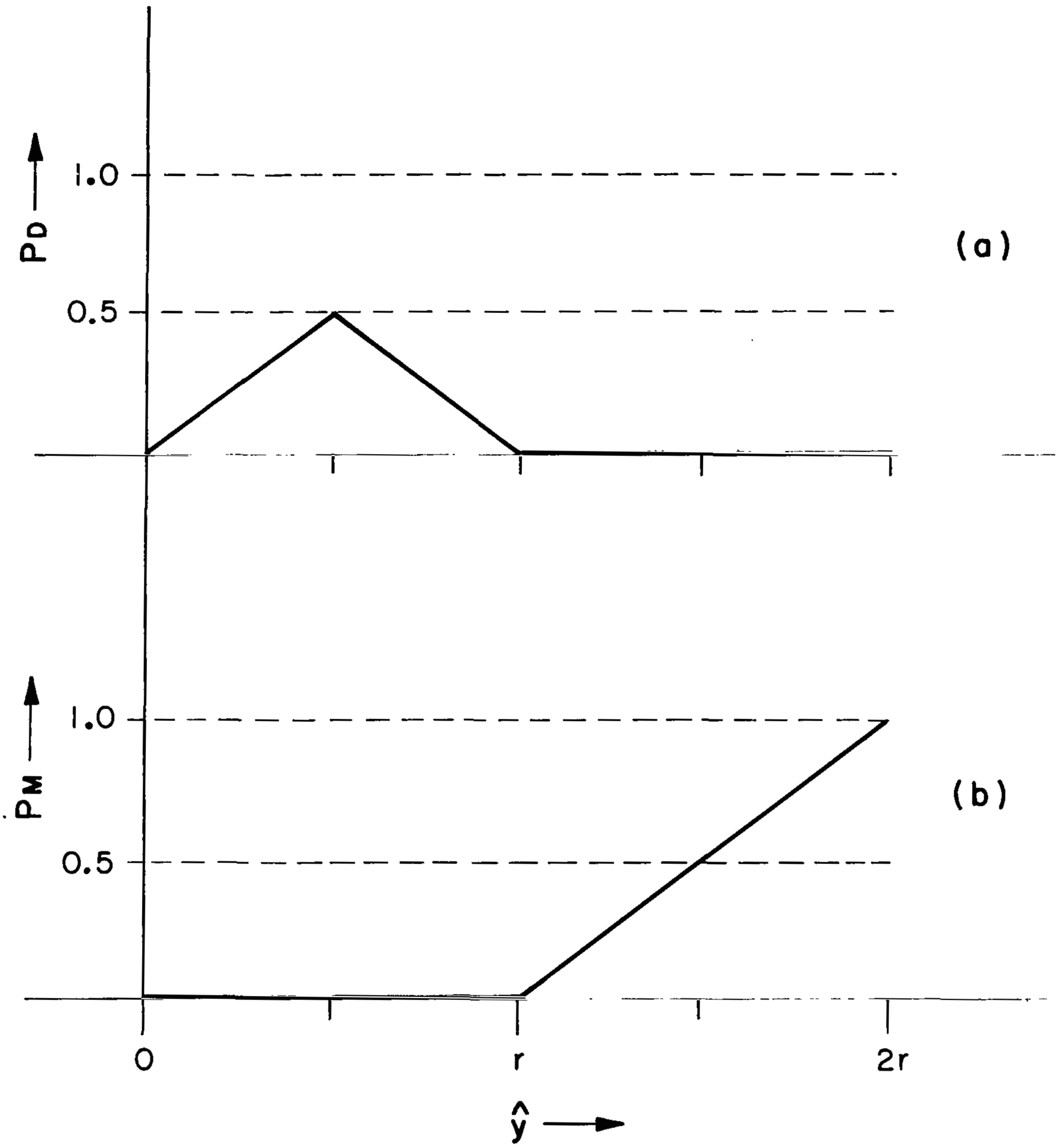

Figure 7.- A priori probability

the counts for the image spots with a dimension in the range $r < \hat{y} < 2r$ which have been missed in the action cycle of Figure 7 are not all lost. It is possible that counts for these image spots can be registered in the next action cycle where their size will have been reduced so that it can fall in the size range of $0 < \hat{y} < r$. It is even possible that such an image spot can register a double count, just as the image spots in the size range $0 < \hat{y} < r$ in Figure 7(a).

At this point a rough quantitative estimate of the counting efficiency of the whole system is to be made using the following simplifying assumptions: (a) It is assumed that the counting characteristics of Figure 7 are valid over any one action cycle. (b) To be able to estimate the counting interplay between two consecutive action cycles, the somewhat naive assumption is made that the shrinkage parameter, δ_s, can be chosen so that all image spots with a $\hat{y}$ in the range, $0 < \hat{y} < \xi r$, are removed in the next action cycle (where $1 \leq \xi \leq 2$) and that all the image spots with a $\hat{y}$ in the range $\hat{y} > \xi r$, remain (with a new $\hat{y} = \hat{y} - \xi r$).

The parameter, ξ, is now chosen to satisfy the following condition:

$$\left[\frac{1}{2r}\int_{o}^{\xi r} P_D dy + \frac{1}{2r}\int_{\xi r}^{2r} P_D \overline{P}_M dy\right] + \left[\frac{1}{2r}\int_{\xi r}^{2r} P_M \overline{P}_D dy\right]$$

$$+ \left[\frac{1}{r}\int_{\xi r}^{2r} \left(1-P_M-P_D\right) \overline{P}_D dy + \frac{1}{r}\int_{\xi r}^{2r} P_D \left(1-\overline{P}_M-\overline{P}_D\right) dy\right]$$

$$+ \left[\frac{3}{2r}\int_{\xi r}^{2r} P_D \overline{P}_D dy\right] = \frac{1}{2r}\int_{r}^{\xi r} P_M dy$$

where; $P_D \equiv P_D(y)$; $P_M \equiv P_M(y)$; $\overline{P}_D \equiv P_D(y-\xi r)$; + $\overline{P}_M \equiv P_M(y-\xi r)$

(1)

Eq. (1) is a relation equating the probability mean of the extra counts to the mean of missing counts over two consecutive action cycles. The first term in the large brackets on the l.h.s. represents the double counts in the first action cycle, the second term the double counts in the second action cycle, the third term represents the triple counts, and the last term the quadruple counts. The term on the r.h.s. represents the total missing counts. When the integrations of Eq. (1) are carried out using the P_D and P_M of Figure 7, the following algebraic equation results:

$$\frac{1}{8} + \frac{(2-\xi)^2}{4} + \frac{(2-\xi)^3}{12} = \frac{(\xi-1)^2}{4} \qquad (2)$$

Eq. (2) can be solved for ξ, giving the result that $\xi \doteqdot 1.75$. This value of ξ, is now used evaluating the term of the r.h.s. of Eq. (1) [or of Eq. (2)] which represents the total probability of missing counts averaged over all the image spot sizes. This probability, P_B, turns out to have the value, 0.14. From Eq. (1) it is seen that P_B is both the total probability of missing count and of additional counts. It is proposed here that this parameter be used as an index of malfunctioning since it can be interpreted as sort of mean counting error (with missing counts being negative errors and the additional counts being positive errors).

DISCUSSION

The foregoing section has provided the main details of the detection, testing, and decision procedure in the operation of the Counting Section of the Automatic Cell Counter. It was seen that the expected overall counting error was around 15 percent. This is about the error expected from human operators performing cell counting. The time required for the instrument to perform a counting task would be only a small fraction of that required of a human operator. A typical single counting operation should take around 5 seconds and very few series of operations should exceed 2 minutes. This, however, does not include the time required to produce the microscope photograph. If a TV camera tube and monitor were used to record the microscope image and project it into the Automatic Counter, the photograph would not be necessary and there would be a further saving of time and effort.

In review, it is apparent that the design for the Processing Section is at present on firmer ground than that of the Counting Section since the principles underlying its operation have been experimentally tested. Similar preliminary testing must be performed on the Counting Section before full scale implementation of the instrument can be justified. Moreover, more testing is required on the Processing Section to see how valid assumption (b) in Counter Behavior Characteristics can be.

The details of the electronic circuits of Figures 2 and 4 and their operating principles has not been dealt with here. It need only be mentioned that the functions required of these circuits as mentioned in PROCESSING and COUNTING Sections can readily be provided by the more or less standard circuits known at the present time. That is to say, an extensive electronic circuit development program will not be required. The actual detail of these electronic circuits must be left to a future report.

As already indicated, a cell count is taken for each action cycle. It can be seen that each of these counts is only of those cells whose sizes roughly fall within a certain rather narrow range of values (depending largely on the parameter, δ_s). Thus, the arranging of the count into subgroups associated with a size category which was mentioned in the INTRODUCTION is done automatically and is one of the advantages that the instrument has over a human operator. The total count, of course, can be obtained by summing the counts from all of the action cycles.

Among other advantages that the instrument has compared to a human operator is that since all the operations are automatic with no human skill required there would be an overall saving in operating cost. Also there would be no fatigue factor to contribute to erratic results and inefficiency such as in the case with human operators.

The one major disadvantage of the instrument in this comparison is that it would probably suffer a loss of efficiency for certain types of cell shapes (the long narrow shapes, for example). However, it is possible that a different kind of scanning method can be devised to be able to deal with these special shapes.

In summary, it would appear that a review of the foregoing sections would warrant the conclusion that the implementation of the automatic cell counter treated here is not only feasible but is actually attractive from a performance per cost viewpoint.

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