Semi-Automated Grain and Cell Counting

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ABSTRACT

A commercially available bacterial colony counter has been adapted for the counting of radioautographic grains over individual cells in smears, and for counting cells in histologic sections. For the counting of radioautographic grains, the correlation coefficients between counts obtained visually by two observers, and between counts obtained visually and using the instrument were similar ($r = .999$ and $r = .998$ respectively). The instrument counts were obtained more rapidly than the visual counts and were associated with less observer fatigue. While performance of the instrument in counting cells in mouse bone marrow sections was less accurate than in counting radioautographic grains, a good estimation of marrow cell number was obtained ($r = .968$). Data on bone marrow cellularity was obtained far more rapidly than with semiquantitative methods.

INTRODUCTION

Recently reported improvements in radioautographic methodology may lead to considerable shortening in the processing time of radioautographs (1, 2). However, these methodological improvements do not alleviate the tedious and time consuming aspects of labelled cell identification and grain counting in studies of cell population kinetics.

We have recently adapted a commercially available electronic bacterial colony counter (Countall^{R} Artek Systems, Inc.) for semiautomated grain counting in cell smears. We have also found this instrument useful for semiautomated cell counting in histologic sections. The validation of the instrument's usefulness for these purposes is reported in this communication.

METHODS AND MATERIALS

The instrument consists of a television camera mounted on a research microscope, a television monitor and an electronic processor unit. In the studies reported here, the television camera was mounted on a Zeiss photomicroscope II^{R}, and the studies were performed with a 100X Zeiss Neofluor oil immersion objective, NA 1.3, and an Optovar^{R} setting of 2.0. The video signal from the television camera is transmitted to the electronics unit and processed to provide a single count for each object. Simultaneously, the video image is displayed on the television monitor. An electronic window is also displayed on the monitor, outlining the region within which objects are to be counted. The size and position of the window are adjustable and its shape may be circular or square. Within the window, objects whose optical density exceeds a manually set threshold are sensed and counted, and an illuminated dot or "flag" appears superimposed on each counted object on the video monitor display.

In practice, individual cells are manually brought into optimal focus on the monitor display and the circular window is adjusted to frame the cell nucleus. The detection threshold is then adjusted until a visually confirmed flag appears over each grain. The grain count is then displayed when the count button is depressed. For each cell the instrument grain count is adjusted to correct for three potential sources of deviation from the true grain count: 1) Since the circular window cannot frame each nucleus perfectly, occasional extranuclear background grains will be flagged and counted. 2) Overlapping grains (doublets, triplets, etc.) may be flagged only once. 3) Dust particles on the slide or on lens surfaces may be imaged within the counting window and flagged.

Our grain count studies were performed on smears of Sarcoma 180 cells grown in tissue culture. The cells were prepared by the method of Hungerford (3), employing hypotonic KCl to burst and flatten the cells on the slide. The cells were stained with acetic orcein and the slides were dipped in Kodak NBT2 liquid emulsion diluted 1:1 with deionized water. To insure a flat, thin, and uniformly apposed radioautographic emulsion, the slides were dipped and withdrawn at a uniform rate by means of a motor driven pulley. A more detailed description of the methods of specimen preparation is given elsewhere (4,5).

Quantitative studies on bone marrow cellularity were performed on histologic sections of mouse femur. Five micron transverse histologic sections were prepared from the midshafts of femurs from formalin perfusion-fixed mice, and stained by a modification of the Feulgen

technique (6,7). With the aid of an eyepiece reticule, a sampling band was constructed of 80 x 80 micron squares spanning the marrow at its widest point. Visual cell counts were obtained within each square. Instrument cell counts were obtained by adjusting the dimensions of the Countall square counting window such that the microscopic field included in the window corresponded to that in which visual counts were performed.

RESULTS

Grain counting: In order to establish an independent estimate of the reproducibility of the "true" grain count, two observers performed visual grain counts on each of 500 cells. Agreement between the two observers was good over the entire range of grain counts (0-250 grains/cell). The data are shown in the form of a scatter diagram in figure 1A. The correlation coefficient was 0.999. Among lightly labelled cells (0-10 grains/cell) visual counts were identical 88% of the time, and agreed within 1 grain/cell 94% of the time. Among heavily labelled cells (>50 grains/cell) the visual cell grain counts of both observers were within three grains of one another 95% of the time. Visual and instrument counts were then obtained and compared for each of 500 cells. Agreement between visual and instrument counts was good over the entire range of counts, as shown in figure 1B. The correlation coefficient was 0.998. Among lightly labelled cells (0-10 grains/cell, visual reference standard), visual and instrument counts were identical 68% of the time, and were within one grain/cell 91% of the time. When visual grain counts exceeded 50 grains/cell, visual and instrument counts were within three grains of one another 84% of the time.

Cells with intermediate (20 grains/cell) and heavy (50 grains/cell) labelling could be counted more rapidly using the instrument, and operator fatigue was reduced considerably.

Cell counting: Cell borders and cell nuclear boundaries are much less well defined optically than are radioautographic grains. Consequently, even with optimal nuclear staining conditions and optimally adjusted density threshold settings some cell nucleii may be sensed as separate objects more than once, while others may not be counted at all. In practice, we have found in Feulgen stained sections of mouse bone marrow that these two effects tend to counterbalance one another, providing instrument cell counts that are generally within 10-20 percent of visual cell counts over the same microscopic fields. A scatter diagram comparing instrument cell counts with visual cell counts over one hundred ten 80 x 80 micron fields is shown in figure 2. The correlation coefficient is .966. The correspondence between instrument counts and visual counts was found to hold for microscopic fields of low cell density as well as for fields of high cell density. Instrument cell counts could be obtained rapidly, and with minimal operator fatigue.

DISCUSSION

The Countall is a relatively inexpensive and compact television based system that is suitable for counting microsopic objects in appropriatel prepared samples. For radioautographic grain counting the best results are obtained with flattened cells in smears coated uniformly with liquid emulsion and stained with a nuclear stain of relatively low optical density. Instrument counts, corrected visually for background grains, doublets, and artifacts, are accurate and reproducible, and can be obtained rapidly and with minimal operator fatigue.

Semiautomated cell counting is not as accurate or precise as grain counting. The validity of instrument counts should be evaluated for each new tissue and for each new stain. Once validated for a given set of conditions, semiautomated cell counting can be performed rapidly and with minimal operator fatigue. Although instrument cell counts are not as accurate as visual cell counts, the semiautomated method is superior to semiquantitative estimates of marrow cellularity based on visual estimates of cell density (8,9). Semiautomated cell counting may find useful application in such areas as the rapid assessment of tissue cellularity in response to cytotoxic drugs or radiation.

REFERENCES

1. Durie, B G M & Salmon, S E, Science 190 (1975) 1093.
2. Braunschweiger, P G, Poulakos, L & Schiffer, L M, Cancer res 36 (1976) 1748.
3. Hungerford, D A, Stain technol 40 (1965) 333.
4. Shackney, S E, Ford, S S & Wittig, B A, Cancer res 33 (1973) 2726.
5. Shackney, S E & Ford, S S, Cancer res 34 (1974) 1401.
6. Shackney, S E, Ford, S S & Wittig, A B, Cell tissue kinet 8 (1975) 503.
7. Shackney, S E, Bunn, P A & Ford, S S, Cell tissue kinet 9 (1976) 363.
8. Rosenoff, S H, Bostick, F & Young, R C, Blood 45 (1975) 465.
9. Smith, W W, Alderman, I M, Schneider, C A & Cornfield, J, Radiation res 19 (1963) 621.

FIGURE LEGENDS

Figure 1. (1A) Correlation of visual radioautographic grain counts on each of 500 cells obtained by two different observers. (1B) Instrument grain counts and visual grain count on each of 500 cells. For discussion, see text.

Figure 2. Correlation between instrument cell count and visual cell count in each of one hundred ten 80 x 80 micron square microscopic fields. Diagonal lines labelled +10% and -10% bound an area within which visual and instrument counts agree within 10 percent. Diagonal lines labelled +20% and -20% bound an area within which visual and instrument counts agree within 20 percent. For further discussion, see text.

A
VISUAL COUNT NO. 2
VISUAL COUNT NO.1
B
AVERAGE INSTRUMENT COUNT
VISUAL COUNT

Figure 1

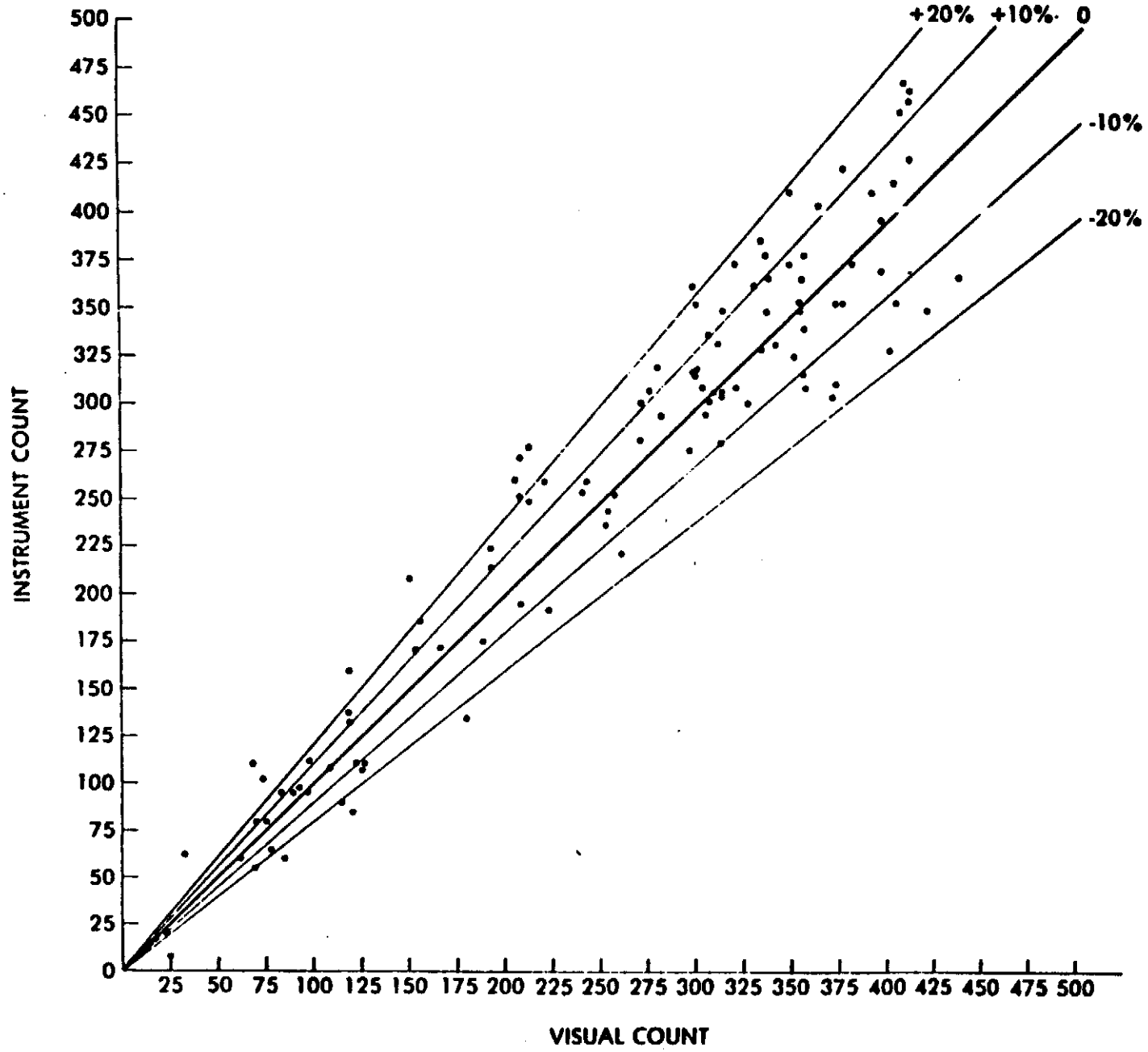
+20%
+10%
0
-10%
-20%
INSTRUMENT COUNT
VISUAL COUNT
0
25
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
450
475
500

Figure 2