1. Introduction

In 1971, members of the Section of Hematology, Presbyterian-St. Luke's Hospital, began to collaborate with Corning Glass Works in the development of an instrument, the LARC (Leukocyte Automatic Recognition Computer), which automates the white blood cell differential examination. This collaboration included the steps of: 1) participating in the original design and development of the machine, and 2) performing field trial evaluations of various pre-production models (Bacus 1972a, 1972b, Bacus and Trobaugh 1975, Trobaugh and Bacus 1972, 1975). In April 1975, we placed two production models of the LARC in routine on-line operation (Bacus and Trobaugh 1976, Trobaugh and Bacus 1976). This article describes the instrument, defines its accuracy in classifying cells, and describes its performance in a routine hematology laboratory operation.

2. Design

The LARC™ itself consists of a minicomputer, an operator's console with a video display, and an automatic microscope. The LARC system also includes two sample preparation units; a spinner which prepares a uniform blood film on a standard microscope slide, and an automatic stainer. These components are all shown in Fig. 1.

The instrument works in the following manner. First, a film of blood is automatically spread on a glass microscope slide by the spinner. The slide is then stained by the automatic stainer, and placed on the microscope stage. The operator then pushes the “start” button and the instrument automatically acquires 100 cells and classifies them into one of six normal categories. Any abnormal cells found are classified as “other,” and at the end of the run are automatically reacquired so that the operator can review them through the microscope oculars and classify them manually.

A. Sample Preparation

The blood films for this system are prepared according to the centrifugal “spinner” technique (Ingram and Minter 1969, Bacus 1974). A standard microscope slide is placed on a platen, which is connected to a motor, and approximately 0.2 ml of blood is placed on the
The LARC spinner. This figure shows how the blood is added to the slide, which is positioned on the spinner's platen. Notice the holes in the platen which permit the beam of light to be projected through the spinning slide onto the light sensors in the lid.

The lid of the spinner is then closed, activating the spinner's motor to a speed of 5,000 rpm in 250 ms. This spins most of the blood off, leaving a monolayer of cells which is evenly distributed over the surface of the slide. A feedback mechanism controls the spinning time by monitoring the light scattered from the cells on the slide's surface. That the spin time can be effectively controlled by monitoring the spread of the cells as the slide is spun has been demonstrated (Mansfield et al. 1974). Fig. 3 shows photomicrographs of two blood specimens prepared by this technique. The blood in 3A is from a normal patient, and it is clear that the morphology of both red and white blood cells is preserved and that the cells are spread out in a non-touching fashion. The blood shown in 3B is from a patient with thalassemia minor; note that the characteristic red blood cell morphology of that disease is preserved.

Characteristically, we find fewer "smudged" cells in blood films prepared by this spin technique than in films prepared by either the "wedge" or "squash" techniques. This is particularly
true of lymphocytes. A “wedge” film of blood from a patient with chronic lymphocytic leukemia is shown in Fig. 4A; it contains many of the characteristic “smudged” cells of that disease. Fig. 4B shows a photomicrograph of a spinner slide prepared from the same sample of blood shown in Fig. 4A; note that none of the lymphocytes are “smudged.”

The LARC system also includes an automatic staining device shown on the left in Fig. 1. This device controls the quality of stain (Dean et al 1976, Gilliland et al 1974), and this control is important with regard to the measurement of features used in the pattern recognition system discussed below.

### B. Automatic Classification of Leukocytes

Fig. 5 is a block diagram of the major subsystems of the LARC automatic microscope, electronics and computer. The automatic microscope system maintains focus, finds leukocytes, and quantizes cell images into the digital computer for analysis. Conventional light microscopy objectives are used. The operator may view the images continuously through the microscope objective and binocular microscope system.

Fig. 6 indicates the process by which the cells are acquired for analysis. The microscope stage moves the slide in the direction indicated by the arrow, a scanning mirror system sweeps across the slide with $5 \times 300$ micron scans to locate all dense objects in a narrow band width of light. If a dark object is found, the stage moves over and places the cell in the center of a “digital aperture” or window. The cell is digitized at 0.42 micron resolution and classified while the stage moves on and the scanning mirror system finds the next cell. Fig. 7 shows the transmission of visible light (400 to 700 nanometers) by leukocyte nuclei and
Transmission of visible light by leukocyte nuclei and erythrocytes. The LARC’s filtering system is designed to detect objects absorbing light rays between 550 and 600 nanometers. At this color, the leukocyte nuclei absorb approximately 90% of the light and the erythrocytes only 20%.

Fig. 8 illustrates the acquisition and focus systems in more detail. The automatic stage with the X, Y stepping motor system is indicated clearly. The scanning mirror with the light path to the red cell acquisition photocell, #40, is shown. Signals from the two photocells positioned on either side of the acquisition photocells, #42 and #44, are constantly compared and fed back to a focus motor connected to the objective lens; this maintains focus. Note that focusing is done on the red cell monolayer and not on the white cells. The pulse shaping circuitry, #46, produces an electrical signal, #55; the high frequency components of this signal come from the red cells and the dip comes from a white cell. During the process of acquisition, when the logic circuitry, #52, centers a cell, this cell’s image is projected through the beam splitter, #32, through two color filters, a yellow and a blue, and then onto the face of the vidicon, #36.

Fig. 9 shows a typical lymphocyte, “A,” and its digitized or quantized image, “B.” This image has been quantized into 2,500 picture elements, or pixels, 50 columns by 50 rows. In “C,” the optical densities obtained along the indicated scan line in “A” are plotted; the low parts of the curve correspond to light absorption by the background and the high ones correspond to absorption of light by the nucleus. In “D,” the optical densities along the scan line have been quantized into gray level values, and in “B” each of these is displayed as an individual pixel. Thus, each number in the field of “B” represents a single pixel and the value of each number is the gray level at that point.

Figs. 10A and 10B show the results of analysis. As the slide in the arrow, a 300 x 5 micron scan, for each step along the path, relates to the polygonal scanning mirror in Fig. 8. (Copyright © 1974 by The Institute of Electrical and Electronics Engineers, Inc. Reprinted with permission from Second International Joint Conference on Pattern Recognition, Copenhagen, Denmark, Aug. 13-15, 1974.)
Fig. 8

Details of automated microscope cell finding, focusing, and color filtering apparatus. Conventional microscope optics, Nos. 18 and 20, project the slide image into three paths: 1) through the binoculars, 2) to the cell finding rotating mirror, No. 38, and 3) onto the television vidicon, No. 36, through the beam splitting and color filtering device, No. 32. Beam splitting optics, No. 32, project two identical images side by side on the television vidicon, the only difference being that one is color filtered through a yellow filter and the other is color filtered through a blue filter. The rotating mirror, No. 38, produces the scan path shown in Fig. 6 and also produces a signal, No. 55, which is constantly compared by the photocells, Nos. 42 and 44, to focus the slide automatically on the red cell monolayer. Positioning motors, Nos. 54 and 56, position white cells which are detected by the cell acquisition photocell, No. 40.

(Reproduced with permission from Adkins, 1975.)
Figure of a typical lymphocyte and its quantized image. (A) Photomicrograph of lymphocyte with scan line depicted. (C) Optical density trace of the scan line. (D) Quantized gray levels of optical density scan shown in (C). (B) Digitized image of lymphocyte shown in (A).

Digitized images of eosinophils: (A) image digitized through yellow filter; (B) image digitized through blue filter.
the color filtering illustrated in Fig. 8. Fig. 10A shows an eosinophil digitized through the yellow filter. Notice how the nucleus stands out with high gray levels and the cytoplasm effects lower gray levels. Fig. 10B shows the same eosinophil digitized through the blue filter. Notice the increased gray levels of the “red” cytoplasm. These differences in gray levels illustrate how color can be measured.

Table 1 lists typical features which may be computed from these digitized images, and which can be used to classify each cell. The exact method of feature calculation from digitized images has been described previously (Bacus and Gose 1972). Nine features similar to these are used to classify cells in the LARC system.

Fig. 11 shows the digitized image of a neutrophil. It is readily apparent that the size of the nucleus can be determined by counting the pixels with a gray level above a certain threshold, and that the perimeter of the nucleus can be measured by keeping track of the picture elements which are on the edge where there is a transition from light to dark.

These two features are effective in classifying many cells, as shown in Fig. 12, which illustrates the separation of two cell types, lymphocytes and monocytes, in the feature space of nuclear size and shape. Nuclear shape is calculated by dividing the square of the perimeter by size (P²/A); using this calculation, the shape of a perfect circle is 4π, or 1.26, and this provides a reasonable separation for these two cell types. This figure illustrates the method of feature calculation, as previously described.

Individual features are combined in a 9-space representation. The space is a 9-dimensional space defined by the features listed in Table 1. Every cell is represented by a point in this space, and by calculating the probability that it belongs to one of two specific classes. This is done by calculating the probability that a cell is in a class when the probability is defined as:

\[
p(j|X) = \frac{\pi(X|j) p(j)}{\sum_{i} \pi(X|i) p(i)}
\]

where \(X\) is a feature vector, \(p(j|X)\) is the probability that class \(j\) is the correct class for cell \(X\), and \(\pi(X|j)\) and \(p(j)\) are the probability density and prior probability of class \(j\) respectively.


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<table>
<thead>
<tr>
<th>Feature</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁</td>
<td>Nuclear Size</td>
</tr>
<tr>
<td>X₂</td>
<td>Nuclear Shape</td>
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<td>X₃</td>
<td>Nuclear Density I</td>
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<td>X₄</td>
<td>Nuclear Density II</td>
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<td>X₅</td>
<td>Nuclear Density III</td>
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<td>X₇</td>
<td>Nuclear Color</td>
</tr>
<tr>
<td>X₈</td>
<td>Cytoplasmic Color</td>
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<td>X₉</td>
<td>Cytoplasmic Texture I</td>
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<td>X₁₀</td>
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<tr>
<td>X₁₁</td>
<td>Cytoplasmic Texture III</td>
</tr>
<tr>
<td>X₁₂</td>
<td>Nuclear to Cytoplasm Ratio</td>
</tr>
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</table>
MULTIVARIATE GAUSSIAN PROBABILITY COMPUTATION FOR CLASS j

\[ p(j|X) = \frac{\exp\left(-\frac{1}{2}[(X-M_j)^TQ_j^{-1}(X-M_j)]\right)}{\sum_{i=1}^{r} \exp\left(-\frac{1}{2}[(X-M_i)^TQ_i^{-1}(X-M_i)]\right)} \]

- \( p(j|X) \) probability of class j given the unknown pattern vector X
- \( M_j \) mean vector of class j
- \( Q_j \) covariance matrix of class j
- \( p(j) \) a priori probability of class j

Fig. 13
Multivariate Gaussian probability computation for class j, and equal probability contours for abnormal cell classification.

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calculation, a round object has a value of 4\(\pi\), or 12.6. The decision boundary separating these two cell types is clear. This figure provides an introduction to the mathematical model used for cell classification, shown in Fig. 13.

Individual cells are classified using a 9-space multivariate Gaussian classification. The formula shown in Fig. 13 simply states in mathematical terms what was obvious from Fig. 12, e.g., if multiple features of an object are measured and the resulting feature vector falls in a region of high sample density for a specific cell class, that object has a high probability of belonging to that cell class. If the feature vector falls outside the probability limits, that object has a low probability of being any standard, or known, cell and will be classified as an abnormal cell. In general, the clusters become more separated in a higher dimensional vector space.

Fig. 14 illustrates the flow diagram for processing at the specimen level. In the first step, an object is found, then a determination is made as to whether that object is an artifact or a cell. If decided that it is a cell, the probability of each cell class is computed with the multivariate Gaussian classifier and the cell type is decided with the maximum probability rule. If the probability is sufficiently high, the cell is classified, otherwise it falls outside the probability limits indicated in Fig. 13. In this case, the physical coordinates of the cell on the slide are stored for review by the operator. Figs. 15, 16, and 17 are exam-
Normal cells which are classified automatically by the LARC classifier.

Typical specimen artifacts, including stain debris, ruptured, and clumped cells. Such objects are detected and ignored by the logic shown in Fig. 14.

3. Performance

We have evaluated the performance of the LARC classifier since 1972.
Examples of abnormal cells which are outside the probability limits in the feature space indicated in Fig. 13. The slide coordinates of such abnormal cells are stored so that the automated microscope can return to display each cell to the operator for visual classification.

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Fig. 17

Examples of abnormal cells which are outside the probability limits in the feature space indicated in Fig. 13. The slide coordinates of such abnormal cells are stored so that the automated microscope can return to display each cell to the operator for visual classification.

3. Performance

We have evaluated the LARC's performance in four formal field trials. In 1972 we tested the first prototype model, and in 1973 a second prototype model. In 1974 we tested a preproduction model, and in March 1975 we purchased two of the manufactured models and tested them for one month. In April 1975 we incorporated them into routine operation in our clinical hematology laboratory. Our hospital has approximately 850 beds; all of the patient hematology samples are processed in the clinical hematology laboratory and approximately 110,000 differentials are performed annually. On a regular working day in a seven-day week, 300 to 500 differentials are performed.

A. Cell by Cell Comparisons

The major sources of error in performing differential blood counts are well known: 1) errors of classification, or accuracy, and 2) errors of distribution. In evaluations of this instrument, our major efforts have been exerted in assessing
the accuracy of classification. To do this, we have compared the observer’s classification versus the LARC’s classification of the same cells. For example, in such experiments, the machine was operated to do a differential in such a manner that when it found a cell it stopped, holding the cell in view. A technician then identified the cell, either by looking through the microscope or by looking at the television screen, and entered the classification on the keyboard. Simultaneously, the machine entered its classification and the data were recorded internally in a confusion matrix format (Bacus 1973). Table II illustrates the results of one such differential examination. The machine’s classifications are identified along the left side of the table and the observer’s across the top. Thus, for each single entry, the technician’s classification is recorded in a column and the LARC’s is recorded in a row. In this example, they each identified six cells as monocytes, but the technician identified two other cells as monocytes which the LARC identified as lymphocytes. Of course, if all of the elements were recorded on the diagonal, there would be no classification errors and no disagreements between the observer and the instrument.

This type of cell identification eliminates two types of errors in the analysis of comparison differential counts: 1) those of distribution, and 2) those of cancellation. In the evaluation of differential counts, errors of distribution result from performing the counts on separate samples of the same blood, in which different cells are identified and counted to estimate the two differential counts. These errors are largely governed by the standard statistics for cell counting, e.g., counting more cells reduces the error of distribution. However, errors of cancellation can only be evaluated by the matrix comparison indicated above. For example, in the results of this differential examination, the technician reported 13 band forms and the LARC reported 12. However, by looking at the classification of the individual cells, you can see that of the 13 true forms, the machine identified 6 as segmented forms. Also, of the 63 true segmented forms, it identified 5 as bands. The final cumulative results mask such errors of cancellation.

In one such study, we performed cell by cell classifications of 12,456 individual cells on 101 slides. The machine was run in the automatic mode in which it stopped on every cell, at which time the technician and the machine entered their classifications independently. The results, shown in Table III, indicate a good agreement between the average response of the technician and that of the machine. There is an 89% average per class accuracy for the five major classifications of cells, polymorphonuclear leukocytes, lymphocytes, monocytes, eosinophils, and basophils.

Table III provides a good estimate of the accuracy of classification of a large number of cells. However, it

<table>
<thead>
<tr>
<th>HUMAN OBSERVER</th>
<th>Total</th>
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<tbody>
<tr>
<td>SG 63</td>
<td>BD 13</td>
</tr>
</tbody>
</table>

| DIFFERENTIAL |
|-----------------|-------|
| SG 65 | BD 12 | LY 12 | MN 7 | ES 1 | BS 1 | UN 2 | TOTALS 100 |
| HUMAN OBSERVER | LARC |
The variance of the cell by cell comparison results from Table III. The percent of each cell type found in each differential count, the LARC versus the technician, is plotted with a symbol corresponding to the cell type. For example, the x’s for neutrophils are all plotted around the 50% level and above. Likewise, the v’s for lymphocytes are plotted around the 30% level.

B. Specimen by Specimen Comparison

To compare the results of these cell classification studies with results which could be expected in routine laboratory performance, we evaluated the effect that the installation of these machines would have on reproducibility of differential counts in our laboratory. Fig. 19 illustrates the reproducibility between technicians in our laboratory before the LARCs were installed. These data were obtained in the following manner. Each morning five blood samples were split into two samples. These were given different names and sent through the laboratory independently. This was part of an ongoing quality control program which gave us the ability to measure the reproducibility within our laboratory. In such a study, both distributional errors and classification errors contribute to the amount of variance or spread of the scattergrams. However, since we performed provides no estimate of the variance or spread of the results. Fig. 18 indicates the variance of the results of the 101 differentials reported in Table III. The data are presented in a scattergram to demonstrate the spread or variance of the classification accuracy.

Table III
AVERAGE CONFUSION MATRIX COMPARING LARC’S AND TECHNOLOGISTS’ CLASSIFICATIONS OF 12,456 CELLS FROM 101 ROUTINE BLOOD SPECIMENS

<table>
<thead>
<tr>
<th>TECHNOLOGIST</th>
<th>SG</th>
<th>BD</th>
<th>LY</th>
<th>MN</th>
<th>ES</th>
<th>BS</th>
<th>UN</th>
<th>AR</th>
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<tbody>
<tr>
<td>LARC MN</td>
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<td>.6</td>
<td>1.7</td>
<td>3.4</td>
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<td>.5</td>
<td>.9</td>
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<tr>
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<td></td>
<td></td>
<td>9.6</td>
</tr>
</tbody>
</table>

Comparisons made on the same cells

Fig. 18

Comparisons made on split samples

Fig. 19

Scattergram of replicate differential counts by technicians, plotted in the same format as in Fig. 18. (Copyright © 1975 by The Institute of Electrical and Electronics Engineers, Inc. Reprinted with permission from IEEE Electronics and Aerospace Systems Convention, Washington, D.C., Sept. 29-Oct. 1, 1975.)

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only 100 cell differentials, most of the spread was due to distributional error.

Fig. 20 shows the results of the same quality control program after the LARCs were installed. Again, each morning five samples of blood were split into duplicate samples and the samples were sent through the laboratory under different names. 100 cell differentials were performed on each sample. There is still considerable scatter, attributable primarily to distributional error, but the spread is no more than that between the technologists without using the LARCs, and perhaps less. These two scattergrams should be compared to Fig. 18, which illustrates the small variance of classification error found between a technician and the LARC. The data in Fig. 18 were obtained from the confusion matrix analysis in which the technician and the LARC looked at and classified the same cells. In the reproducibility studies, each of the observers were looking at 100 different cells from the same sample of blood.

As mentioned above, there are two sources of error in performing a differential count, errors of distribution and errors of classification. We have shown that, by using the truth table technique, distributional error can be separated from classification error. Another way to overcome the distributional error is to count more cells. Fig. 21 shows the results of another experiment displayed in the same format as Figs. 18 through 20. In this experiment, 20 slides were prepared from each of 16 different bloods. Technicians performed 100 cell differentials on ten of the slides and, on the other ten, 100 cell differentials were performed by the LARC. Thus, 1,000 cell differentials were performed on each specimen by both the LARC and by the technicians. The differential counts were then plotted against each other in a scattergram. As in Fig. 18, the spread from the 45° line is much narrower than in Figs. 19 and 20, which were prepared from 100 cell differentials, most of the spread was due to distributional error.

In addition, a critical test for the reproducibility of any classification type of task is the truth table. A summary of the work in our lab is that from 16 bloods, each slide was marked with a non-identifying second number. The technician looks at the slide and makes a quick classification of the leukocytes. This takes from the 45° line is much narrower than in Figs. 19 and 20, which were prepared from 100 cell differentials, most of the spread was due to distributional error.

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from 100 cell differentials. The scatter in these data approaches the width of the truth table scatter shown in Fig. 18, in which the spread is due entirely to classification error.

C. Efficiency of the Machine in the Laboratory

In addition to accuracy and reproducibility, throughput time is another critical measure of performance for this type of automated equipment. Fig. 22 summarizes the LARC throughput times in our laboratory. These are typical data from 16 routine slides. The first part of each line shows the time it takes for the LARC to perform its part of the differential; the average time is 56 seconds, with a range of 50 to 74 seconds. The second part of each line is the time it takes for the technician to classify the unknown cells and to add comments from the LARC keyboard. The average time for this action is 22 seconds, with a range of 7 to 40 seconds. The third portion of the line indicates the time required for the technician to verify each report and add any additional comments into our laboratory information system.

4. Summary

The LARC system controls the sample preparation and then performs an automated differential count of the leukocytes. Its major features are:

1. Its cell acquisition system operates at low resolution and in parallel with its cell identification system, which operates at high resolution.
2. It utilizes color information in the identification of cells.
3. It permits continuous viewing of cells as they are being classified.
4. It classifies normal leukocytes automatically and recalls for operator review each of the cells which it has identified as abnormal.

In our evaluation of the instrument, we have estimated accuracy of cell classification, reproducibility in routine operation, and throughput times. Accuracy of cell classification was determined by simultaneous identification of individual cells by the machine and the human observer; the overall cell classification accuracy was 89%. In routine operations, reproducibility was essentially that of distributional error and was not influenced by the much smaller classification error. The average time for the period of automated analysis was 56 seconds.

5. References


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