A DATA ACQUISITION SYSTEM FOR AUTOMATIC GRAIN COUNTING ON CHROMOSOME AUTORADIOGRAPHS

by
Victor Pollak and Dusan Badal
University of Saskatchewan
Saskatoon, Canada

Abstract: The rate of synthesis of DNA during the progress of the mitotic cycle is different for chromosomes of different types. Its determination represents, therefore, a highly useful tool for chromosome analysis.

Controlled incorporation of H_3 - labelled thymidin is commonly used to this purpose. Autoradiographs are then produced of the chromosome assembly at different stages of mitosis. The incorporated tritium shows up in the form of dark grains on these autoradiographs. The number of grains found in a particular chromosome is a measure of DNA synthesis up to this stage.

The present technique is to count these grains manually. This is a tedious and time consuming process. It was therefore decided to develop a system, which could perform this operation automatically and besides that determine certain dimensional parameters of the chromosomes to assist in the morphological classification.

The complete system can be divided into three parts: A data acquision unit digitizes the optical information stored on photographic enlargements of the chromosome autoradiographs. A data preprocessing unit removes redundant information and stores the retained part on magnetic tape. The third part is the software for processing these data on a general purpose computer. Only the hardware part of the system has been completed so far and will be described in the paper in more detail.

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The chromosomes are the memory devices of the cell, where most of the genetic information is stored in the form of molecules of DNA. There are 46 chromosomes in the normal human cell, all of which are in pairs with the exception of one group, the sex chromosomes of the male, which are unpaired. For classification purposes the chromosomes are divided into 8 groups based upon morphological criteria like overall size, symmetry, length of the arms, etc. Within the individual groups the chromosomes are labelled by index numbers. The morphological differences between the members of a certain group are, however, frequently so small, that even a highly trained experimenter may have difficulties in assigning the proper index number. Still larger difficulties are encountered with abnormal chromosomes.

When a cell divides the individual chromosomes divide also. Several phases can be distinguished in this period, which is called the period of mitosis. During mitosis the chromosomes synthesize DNA at a rate which depends upon the phase of the cycle and upon the type of the particular chromosome. Determination of the rate of DNA synthesis in dependence upon the time the chromosome has spent in mitosis is, therefore, an efficient method for their classification.

The basic building blocks of DNA are several amino-bases, which the chromosome takes in from the surrounding medium. One of theses bases is thymidin. To determine the rate of DNA synthesis the chromosome is given a supply of tritium labelled thymidin and the amount of incorporated labelling substance is measured after predetermined intervals of time. To this purpose after the desired part of the cycle has been completed further DNA synthesis is stopped and autoradiographs of the chromosome assembly are made. Care has to be taken to have the assembly sufficiently spread so that overlap of the chromosomes is avoided.

Decay electrons emitted by the labelled thymidin produce distinct dark grains on the photographic emulsion. The number of these grains within the boundaries of a particular chromosome is a measure of the amount of DNA which had been synthesized up to this instant. Manual counting of these grains is a tedious and time consuming process. It was therefore decided to develop a system for its automation. The design of the hardware part of the system is being described below.

Enlarged prints of autoradiographs of the chromosome assembly are used as the primary source of information. Before processing the areas belonging to the individual chromosomes are delimited by hand using closed contour markings in red ink. The purpose is to make machine identification easier and to avoid ambiguities. The prepared prints are loaded into a facsimile type optical scanning device, a diagrammatical sketch of which is shown in Fig. 1. The motor driven picture drum rotates with about 3-1/2 revolutions per second. It is designed to accept prints of any size up to a maximum of 11 x 13" without readjustment. Loading and unloading the paper is very simple and takes only a few seconds. A small spot on the surface of the drum is illuminated by a light source mounted on a carriage. The carriage is driven by a spindle and a gear from the drum motor; it moves along the length of the drum at such a speed, that the scanning spot covers successively the whole area of the picture.

The light reflected from the paper surface is is picked up by a magnifying lens and directed towards a beam splitter. Here the incoming light is divided into two beams of approximately equal intensity. One beam is passed through a blue filter, the other one through a neutral grey filter; they converge then upon two silicon photodetectors. The blue filter sensitizes the following detector to the presence of the red boundary marking, delimiting the area belonging to a particular chromosome. An aperture in front of the detectors limits the size of the scanning spot on the paper to about 0.25 x 0.25 mm.

An incremental optical shaft encoder is attached to the shaft of the picture drum. One increment of the encoder corresponds to a circumferen-

tial step of 0.25 mm on the paper. The output pulses of the encoder are counted by a binary counter. A master pulse is generated by the shaft encoder once at the beginning of each scanning line and sent to another binary counter - the state of which corresponds to the axial position of the carriage. The output of the circumferential counter and the line counter together determine the co-ordinates of any scanned spot on the paper.

The output signals of the two photodetectors are amplified and fed to a group of adjustable threshold detectors. The output signals of these are combined to produce a two digit binary signal, which serves as a tag to identify the character of the scanned spot. The four possibilities are; a blank, a red boundary marking, a grey spot being part of the general area of a chromosome and finally the deep black of a tracer grain.

The amount of information contained in the scanned picture area even with only four levels per element is immense and much too large for straight computer processing. Some preprocessing is therefore necessary to remove nonrelevant and redundant data. This is done here by recording only changes in the character of the scanned picture area. It is estimated that this reduces the requirements for storage capacity by about an order of magnitude.

When the first spot of a new line is scanned, the reading of the line counter containing the sequential number of that particular line is transferred to the main memory. All following data, until the beginning of the next line are hereby identified as belonging to that line. At the same time the character of the scanned spot is sensed and the identifying tag produced by the threshold detector assembly is stored in a small auxiliary memory. All operations involved are controlled by pulses from the shaft encoder, which serves also as a master clock for the input to the preprocessing logic. The advantage of this arrangement is, that the clock rate is firmly tied to the mechanical movement of the scanner.

When now the next line element is scanned, the identifier tag generated is compared with that from the previous pulse stored in the memory. If they are identical, the new one is skipped and nothing else occurs. If they are, however, different, the new tag is transferred to the intermediate storage register, where it replaces the previous one. Simultaneously the position of the circumferential counter is read and together with the identifying tag transferred to the main storage register with a capacity of about 100 words with 12 bits each. This register acts as a buffer making it possible to transfer information to the associated magnetic tape unit at a speed close to the average rate of acquisition of information, the peak rate of which may be many times higher. Two such equalizing memories are provided. When one is filled with data the input data stream is switched to the second one, and the content of the first is transferred to the magnetic tape unit.

A commerical incremental 9 track IBM compatible unit with a maximum stepping rate of 1000 steps per second is used. Gapping, parity check bit inserting and other operations required to make the written data computer compatible are carried out by the control logic of the tape unit. The command information required is delivered by the data acquisition unit. The tape with the stored pictorial information may then be transferred to the University's central computer for the final processing.

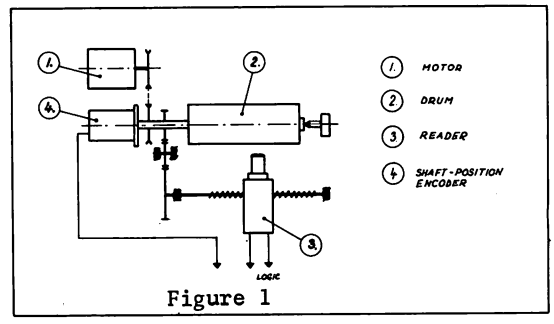

Figure 1

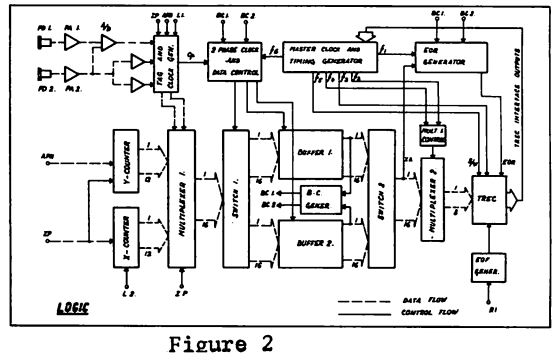

Figure 2

Figure 2 Legend:

PD - photodetectors
PA - preamplifiers
APH - incremental pulses from shaft encoder
ZP - zero pulse from shaft encoder
TREC - incremental magnetic tape recorder

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