AUTOMATIC MEASUREMENT OF THE OPTICAL DENSITY OF MICROOBJECTS

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A digital integrating microdensitometer is suggested. A special feature of this instrument is that it transforms a light signal into a number of pulses which is proportional to the optical density of the dots in the frame. The design of the logarithmic transducer, the frequency generator, and the digital voltmeter is based on the use of the advantages of the integration principle and can be applied in high-precision cytospectrophotometers for studying cytological objects of any geometric shape.

To determine the quantity of light-absorbing substance in a cell or in subcellular structures with a nonhomogeneous distribution of optical density, various types of spectrocytophotometers are used. A digital integrating microdensitometer suitable for precise microphotometric investigations of objects of any geometrical shape has been built at the Institute of Chemical Physics, Academy of Sciences of the USSR. The electronic part of the instrument is a device which yields measurements of high accuracy and which can be used in measuring instruments of various types.

The working principle of the microdensitometer is based on measurement of the integral optical density of the frame [2]. The result of the measurement is determined as the difference between the sums of the logarithms of the transmitted light flux:

$$D_i = \Sigma \log \Phi_0 - \Sigma \log \Phi_i,$$

where D_i is the integral optical density of the object, and $\Sigma \log \Phi_0$ and $\Sigma \log \Phi_i$ are the sums of the optical densities of the dots in the frame without the object and with the object, respectively.

By contrast with the integrating microdensitometer described by Deeley [5] and manufactured in Scotland by Barr and Stroud, in the instrument now described the magnitude of the signal from the photoreceiver is transformed into a number of pulses, which then undergoes summation.

The instrument consists of the following units (Fig. 1): 1) a stabilized source of light; 2) a light microscope; 3) a scanning device; 4) a monochromator; 5) a type FÉU-27 photoelectronic multiplier with stabilized power supply; 6) a logarithmic converter; 7) a digital recorder; 8) an external generator to trigger the digital voltmeter.

The object to be investigated is placed in the light microscope. The scanning device displaces the image relative to a stationary narrow beam hole located in the plane of the microscope image. Light passing through the hole falls on the monochromator, and then on the photoreceiver, where it is converted into an electrical signal. The result of integral measurement of the optical density of the frame is recorded on the digital signal panel of the recording instrument. The final result is obtained by subtracting the readings of the instrument in accordance with the method of cytophotometry used. If the reproducibility of the

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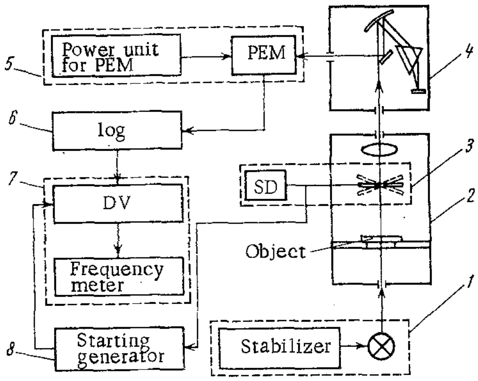

Fig. 1. Block diagram of the digital integrating microdensitometer (explanation in text).

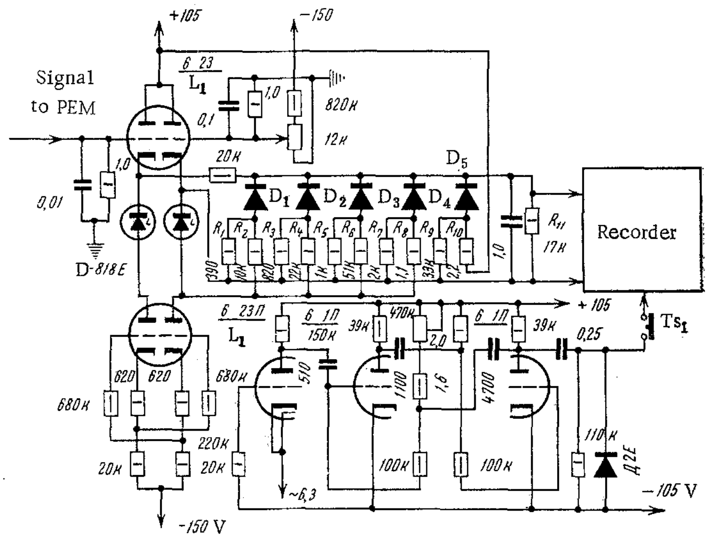

Fig. 2. Scheme of electronic part of the digital integrating microdensitometer (explanation in text). PEM) photoelectric multiplier.

measurements is high, the first measurement (determination of the optical density of the frame without the object) is carried out only rarely, mainly to check the working of the instrument.

The source of light is a type K-30 170-W incandescent lamp. A type P71-M voltage stabilizer is used to stabilize the light flux. The light flux is regulated by means of an autotransformer.

A type FÉU-27 photoelectronic multiplier was used as the photoreceiver because its sensitivity is maximal in the working region of the spectrum (about 5500 Å). The working conditions are chosen so as to make use of the most linear portion of the light characteristic curve which, in the present case, covers the region of optical densities up to 1.8 D.

The logarithmic converter (Fig. 2) consists of a cathode follower incorporating the tubes L_1 and L_2 and a functional diode transducer (FDT) incorporating semiconductor diodes D_1-D_5. The principal element of the FDT is formed by the semiconductor diodes D-223 which serve the purpose of electronic keys, commuting connecting of the resistors R_1-R_{10} to R_{11}, the load resistance of the logarithmic converter. In this way the segments approximating parts of the logarithmic function are given the assigned slope. Silicon diodes of this type were chosen because of their high back resistance and the low temperature coefficient of their parameters. The ratings of the resistors R_1-R_{10} must be chosen with an accuracy of not less than 0.1%. Further details on calculations and adjustment of the FDT are described in the literature [1, 3, 4].

In this instrument the continuous signal is fed into the input of the recorder, consisting of a type VK7-10A digital voltmeter, serving the function of transformer of the "Analog Code" with a decimal basis, and a pulse counter consisting of a type Ch3-12 frequency meter. To feed volleys of impulses proportional to the optical density of the points in the frame into the input of the frequency meter, the circuit of the digital voltmeter is slightly modified: the signal is fed directly from the output of the counting pulse generator unit to the input of the frequency meter.

In principle the combination of two instruments – the digital voltmeter and frequency meter – can be replaced by a single integrating voltmeter provided that it can perform all the functions enumerated above.

The digital voltmeter works on a discrete program from an external starting generator, incorporating a 6N1P tube as in the circuit of a multivibrator. The frequency of the generated pulses is synchronized with the frequency of the power supply. The beginning and end of operation of the digital voltmeter are set by making and breaking the terminal switch TS_1 (Fig. 2), which is connected mechanically with the scanning device.

The magnitude of the function of the input signal is thus measured by the digital voltmeter in a complete cycle of measurement of the frame with a frequency of 200 Hz, i.e., every 5 msec. The time for complete measurement of the frame is 18 sec. Under these conditions the frame is measured at 3600 points.

The suggested scheme of conversion and recording of the signal enables maximum use to be made of the advantage of the integration principle, for with an increase in the interrogation number of the frame (other conditions being equal), the accuracy of the measurements is increased. The error of reproducibility of the results of the measurements by this instrument without an object is not more than 0.05%, but this value can be reduced, for example, by increasing the interrogation time of the frame. By using a digital voltmeter, it is possible to connect a digital printing device and thus to obtain a digital picture of the frame. This is particularly important for quantitative investigations in cytology.

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