A SCANNING MICROPHOTOMETER FOR THE ANALYSIS OF CURVED MICROSCOPIC OBJECTS

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UDC 615.478.6:087.981.6

A microphotometer intended for recording the curve of distribution of optical density along highly curved microstructures is described. Measurements are made on photographic negatives. The negative is moved along by means of friction rollers connected to a differential mechanism. By means of this device it is easy to correct the direction of scanning in the course of measurement. The instrument was used to study segmentation of metaphase chromosomes.

In medical and biological research it is often necessary to determine the distribution of a substance along lengthy microstructures such as chromosomes, fibrils, the transverse sections of membranes, and so on. Existing methods and instruments used in cytometric and fluorometric analysis are adequate for undertaking such tasks if the object to be studied is in the shape of a straight line. However, in practice objects for investigation are frequently curved to a varied degree. Only one description of a microphotometer by which the distribution of fluorescence of a fluorochrome along chromosomes which are slightly curved is known [2]. However, as the writers of this description point out, the instrument cannot be used for strictly quantitative analysis, nor is it suitable for photometry of highly curved chromosomes.

This paper describes the details of construction and the principle of action of a scanning microphotometer developed by the writer to record the distribution curve of density of a substance along microscopic objects with practically any degree of curvature. To carry out the measurements the object is first photographed with a magnification of several hundred times and photometry is carried out on the negative. Since the instrument is intended mainly for cytogenetic investigations, in which photomicrography is an essential stage in other forms of analysis also, the method of photometry described below involves only a negligible amount of extra work. At the same time, photography has the following advantages: because of the preliminary magnification, the degree of precision required in the manufacture of the parts of the scanning mechanism is considerably reduced, the contrast of the object can be varied considerably during the photographic process, the range of measurements can be widened, and in some cases the sensitivity of the method can be increased; the photometer thereby becomes a universal instrument capable of quantitative analyses of many different kinds (absorption, fluorometric, etc.).

In the instrument to be described a light projection system of a mass-produced microdensitometer (of the MF-2 or MF-4 type) is used but the photoelectric cell is replaced by a photomultiplier (FEU). In the course of measurement, longitudinal scanning by movement of the photographic negative, and transverse scanning by means of a moving diaphragm, located in the plane of the image of the object, are carried out simultaneously.

The relevant part of the instrument is shown schematically in Fig. 1. The device creating the moving diaphragm consists of a stationary rectangular slit of a light receiver and a rotating opaque disk, with a cut-out slit in the form of an Archimedes' spiral placed behind it. The negative is moved by means of a pair of driving rollers, each of which is connected through equal reducing gears with one of the half-axles of a

Laboratory of General Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 10, pp. 123-125, October, 1972. Original article submitted November 22, 1971.

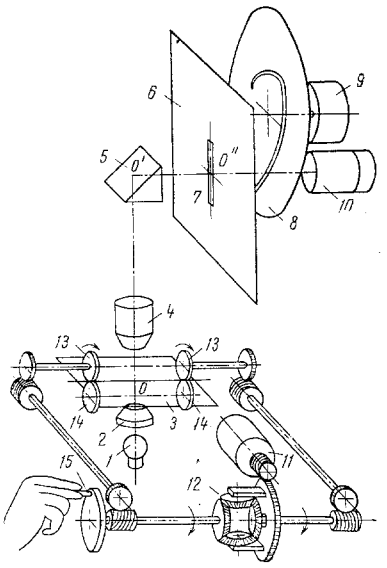

Fig. 1. Scheme of microphotometer: 1) Source of light; 2) condenser; 3) photographic negative for measurement; 4) objective; 5) prism; 6) screen for visual observation; 7) Slit of light receiver; 8) disk with cut-out slit; 9) synchronized motor; 10) light receiver; 11) synchronized motor; 12) differential; 13) driving rollers; 14) compression rollers; 15) handles for changing direction of scanning; points 0.0', 0" – optical axis of light projector.

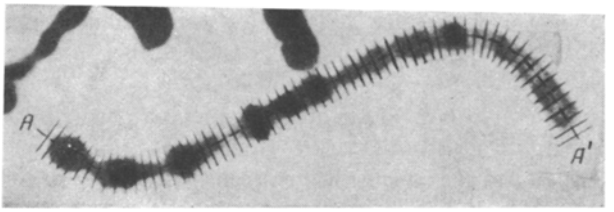

Fig. 2. Short lines represent trajectories of movement of limiting diaphragm during individual cycles of transverse scanning; line AA' represents trajectory of movement of light receiver slit center during longitudinal scanning (further explanation, see text).

differential mechanism. The main shaft of the differential is driven by a constant-speed motor. The driving rollers have rubber covers which exert friction on the surface of the photographic negative. The rollers are arranged symmetrically relative to the optical axis passing through the center of the slit in the light receiver, and they rotate around the same straight line. The line joining the points where the rollers touch the photographic negative is projected on the inlet slit of the light receiver. One of the half-axles of the differential is connected to a handle by means of which the direction of longitudinal scanning can be varied. The operator observes the position of the image of the object relative to the center of the slit of the light receiver and varies the rate of rotation of the handle so that the center of the slit coincides during measurement with the axial line of the object. Because of the properties of the differential mechanism, the quantity of movement communicated to one of its is half-axes is transmitted to the other with opposite sign, as a result of which the trajectory of movement of the photographic negative is curved. Meanwhile, with the driving rollers in the specified position, the rate and direction of movement of the object and of its image at the point of intersection with the optical axis remain unchanged. The center of the line of transverse scanning is thus displaced at uniform velocity along the axis of the object, and at any point the perpendicular orientation is automatically maintained between the line of transverse scanning and the axial line of the object. A scheme of the trajectory of movement of the limiting diaphragm during scanning of a curved object (the chromosome of a Chinese hamster after treatment with bromodeoxyuridine) is shown in Fig. 2.

If quantitative measurements are required the signal of the photodetector is led into an electronic unit where it undergoes logarithmic conversion, followed by integration during each cycle of transverse scanning, and then displayed on the axis of coordinates of an automatic recorder. The total quantity of the substance measured is also determined by summation of the ordinates and the result is displayed by means of a pointer on a dial.

Besides work under the conditions specified above, in some cases a simpler alterative form can be used in which the transverse scanning is dispensed with and the object is scanned over the whole area of the slit of the light receiver. The amplified detector signal is led without transformation to an automatic writer. During work by this method the ordinates of the resultant curve are not strictly proportional to the quantity of substance tested photometrically, but the graph objectively reflects the distribution of areas with different light transmission along the length of the object. In this way, in particular, the writer has analyzed the localization of secondary constriction bands in condensed segments of human chromosomes after treatment of cells with bromodeoxyuridine [1].

The instrument described has the following characteristics: size of negative used 24 × 36 mm (magnification usually used for photography approximately 600 ×); enlargment given by light projector varies

from six to 40 times (usually 12 times); speed of movement of negative 0.15 mm/sec, which, if converted to movement of the object is equivalent to about 0.2 μ/sec; period of repetition of transverse scanning lines 0.5 sec; dimensions of scanning diaphragm 0.8 × 0.8 mm; length of transverse scanning line controllable within the range 0-25 mm, equivalent to approximately 0.1 × 0.1 and 0-3μ respectively for the object with typical magnifications. In the case of photometry of objects lying close to the edge of the frame, the negative is placed in a light cassette, consisting of a strip of photographic film measuring about 80 mm across, with a 15-mm circular hole cut out of the middle, folded in half. The object to be measured is placed inside this hole and the cassette is introduced between the driving and compression rollers.

LITERATURE CITED

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