VARIANT OF THE METHOD OF INTEGRAL MICROPHOTOMETRIC MEASUREMENTS OF OBJECTS

Yu. E. Morozov, A. P. Chernov, and V. V. Rogozhin

UDC 612.014.481-087

A method of integral microspectrophotometric measurements is suggested for the objective and rapid obtaining of information concerning the amount of absorbing substance in an object directly on the instrument panel without further procedures. In practice the method is suitable for most biological objects and quantitative histochemical reactions.

* * *

The main difficulties in the way of microphotometric determination of the mass of absorbing substance in biological objects are due to its nonhomogeneous distribution and high local concentration, and also to the irregular shape of the microscopic object.

The need thus arises for a multiwave (two-wave) scheme of measurement, i.e., to measure the object successively at different wavelengths. A planimetric representation of the object must also be used, and this involves considerable effort and time. In the simplest variant, suggested by Sherudilo [4], the need for planimetry is dispensed with, but all the difficulties and limitations connected with the use of a multiwave system of investigation remain. Casperson uses a method of scanning microphotometry in which errors connected with nonhomogeneity of distribution of the absorbing substance are eliminated by the use of a sufficiently small scanning probe. It is assumed that under these conditions the distribution of absorbing substance within the area of the probe is uniform. The system of scanning and integration described in the literature is complex and has not yet achieved wide popularity.

In the suggested variant of the method the scheme of scanning is simplified. Scanning is combined with simultaneous summation of optical densities over an assigned frame area.

A thin beam of light repeatedly crosses a frame of area S, strictly determined for each particular series of measurements. The mass of absorbing substance (m), assuming homogeneous distribution within a stipulated volume, is described by the equation $m = C \cdot V = CSd$, where C is the concentration of the substance, V the specified volume, and D the thickness of the object. According to the laws of Bouguer, Lambert, and Beer, the optical density D is proportional to the concentration and thickness of the layer: $D = kCd$, where k is the coefficient of absorption of the given substance at the wavelength used (hence, $C = D/kd$). By substitution in the expression for man, the equation $m = D_S/k$ is obtained, in which D_S represents the optical density in an area of frame bounded by the measurements of the probe. This formula is valid for objects with a homogeneous distribution of the substance. For a nonhomogeneous distribution of the substance, the value D_{mean} must be found, and for a constant frame this is given by the formula $D_{mean} = \Sigma D_S/S$, where ΣD_S represents the sum of the optical densities of the frame.

For a nonhomogeneous distribution the mass of absorbing substance is expressed with an adequate degree of accuracy by the formula $m = \Sigma D_S/k$. Since the coefficient k for a given substance is constant, in the case of relative measurements the value of m can be expressed as ΣD_S. In turn, the latter is measured as the sum of a number of discrete values or impulses, the frequency of which is proportional to the amplitude of the electrical signal. Since in a particular series of measurements the frame is constant and the signal logarithmic, the total number of impulses recorded on the counter will be given by ΣD_S. The area of the frame, which in cytophotometry is always greater than the object, is fixed by the duration of counting

Laboratory of Histochemistry, Institute of Human Morphology, Academy of Medical Sciences of the USSR (Presented by Active Member of the Academy of Medical Sciences of the USSR A. P. Avtsyn). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 1, pp. 97-99, January, 1969. Original article submitted January 19, 1968.

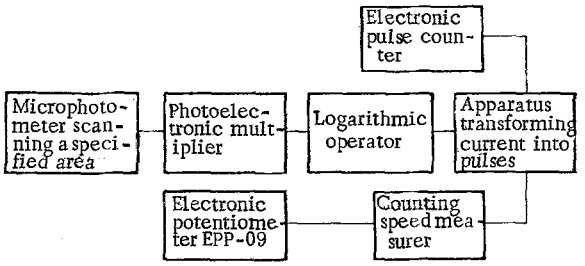

Fig. 1. Theoretical block diagram of the apparatus for microphotometric measurements.

and of delivery of the object, provided that the delivery speed of the object and the amplitude of oscillations of the probe are constant. The number of impulses recorded by the counter is directly proportional to assigned values expressing zero and infinite density or zero and total absorption, the integral of the electrical signal, and the duration of recording of the impulses.

Since the values determining the zero points are stipulated and coordinated with the indicator mechanism of the intensimeter and are constant for a given apparatus, while the duration of recording is determined by the area of the frame, the number of impulses on the instrument panel will be proportional to the integral of the electrical signal over a definite period of time. Consequently, the number of impulses recorded by the counter will show the sum of optical densities ΣD_S of the object in the given frame.

Error due to a nonhomogeneous distribution of the substance is excluded by the use of a probe not exceeding 2 μ in diameter at the image level, thus enabling measurements to be made at one wavelength.

The use of a single-wave method does away with the limitations connected with the shape of the spectral curve absorbing the components and it enables the results of many histochemical reactions to be assessed quantitatively.

The mass of absorbing substance is integrated over the area of the frame, and in cytophotometry this dispenses with the need for planimetry. When necessary, a diaphragm can be introduced in the plane of the field to isolate a particular detail of the object from the surrounding background.

In the experimental system (Fig. 1) the principal optical unit consists of a type MF-4 microphotometer, in which the optical system is replaced (apochromat 90, I, 30 objective and compensating ocular 12.5 are sustituted). An oscillating plane-parallel disk, set in motion by a Warren motor giving 60 rpm, is used for scanning. The slit of the microphotometer is used as probe. Horizontal delivery of the object combined with vertical oscillations of the disk gives the representation of the object at the slit of the microphotometer a sinusoidal movement. For conveniences of observation and to reduce the effect of scattered light, a monochromator is placed behind the microphotometer slit. The FÉU-19m photoelectric multiplier receives the monochromatic light which has passed through the monochromator. The indicator apparatus is an intensimeter, from which the zero points of measurement are deduced. The counter and self-writing EPP-09m apparatus are coordinated with it.

Choice of the optical and electronic systems of the counter and of the indicator instrument is not based on theoretical consideration. In practice, any scanning microphotometer and any system transforming an electrical signal into pulses can be used together with any counter. In our opinion the commercially available units of the BIP-1 current integrator, with counters designed to operate with it, are the most suitable.

Measurement of a standard optical density showed that the characteristic of the instrument is sufficiently linear. When the same cells were measured the relative error did not exceed 2%. Maximal deviation from the mean value usually did not exceed 5% and was mainly due to defective horizontal delivery of the MF-4 table.

LITERATURE CITED

1. V. Ya. Brodskii, Uspekhi Sovr. Biol., 42, No. 1 (4), 87 (1956).
2. N. V. Korolev and L. S. Agroskin, Biofizika, No. 4, 513 (1957).
3. A. I. Sherudilo and V. Ya. Brodskii, Optika i Spektroskopiya, 2, No. 2, 266 (1961).
4. A. I. Sherudilo, Cytophotometry (Physical and Biological Aspects of the Study of Cell Nuclei) Author's abstract of candidate dissertation, [in Russian] Novosibirsk (1965).