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Scanning microdensitometer*

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A scanning microdensitometer (Fig. 1) for biomedical purposes has been designed in Central Laboratory of Optics. It comprises a typical Biolar microscope with halogen illuminator 100 W, trinocular head, photometer scanning head, electronic processor unit and stabilized power supply. A set of planachromat objectives

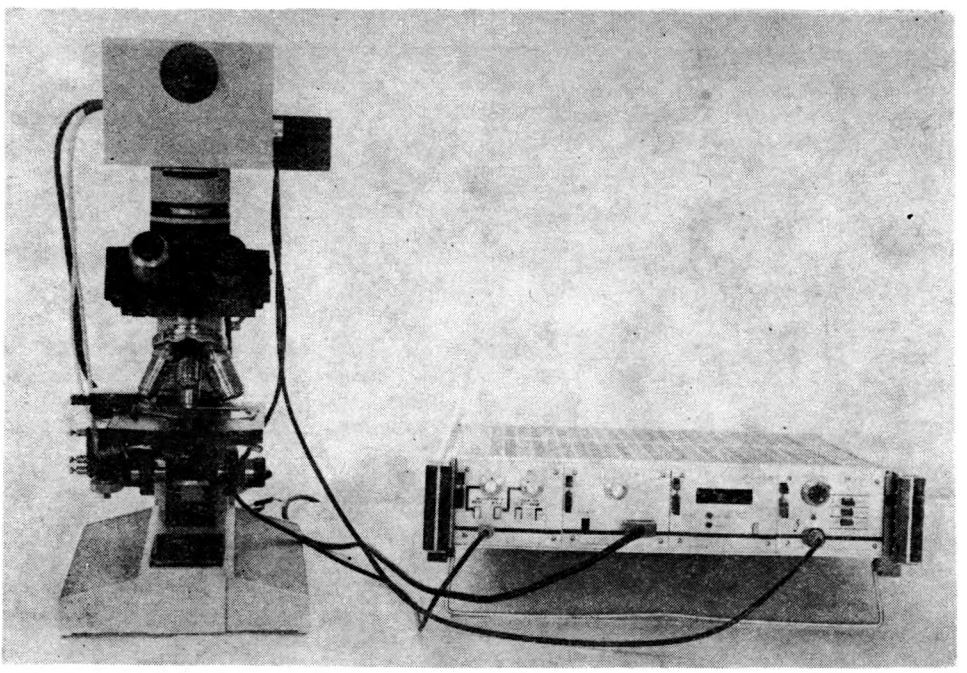

Fig. 1. Microdensitometer

is applied. Optical scheme of the arrangement is shown in Fig. 2. An object is illuminated according to Köhler principle. The primary image of the object is produced in the plane π'. In the same plane a disc with five masking diaphragms of different diameters is positioned. When the switchable beamsplitter is in the light path, the object and the mask can be observed in the viewer.

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If the beamsplitter is beyond the light path the whole beam reaches the scanning system positioned in the image plane π'' of the objective. The switchable mirror enables the observation of scanning points. Position b of the mirror switches on the lamp and allows the scanning point to be illuminated. When the beamsplitter is in the light path an objective gives

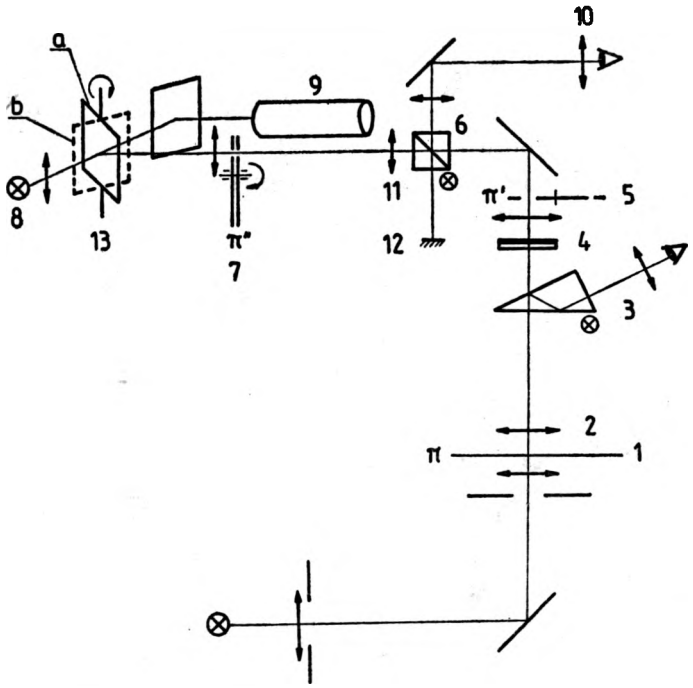

Fig. 2. Optical scheme of microdensitometer. 1 – object, 2 – objective, 3 – trinocular head, 4 – interference filter, 5 – masking diaphragm disc, 6 – beamsplitter, 7 – scanner, 8 – lamp, 9 – photomultiplier, 10 – viewer, 11 – objective, 12 – mirror, 13 – switchable mirror

the image of the scanning point in the plane of the mirror. In this manner the scanning point superimposed upon the object and the masking diaphragm can be observed. Position a of the mirror sends the photometer beam to the photodetector, placed in the exit pupil of the microdensitometer.

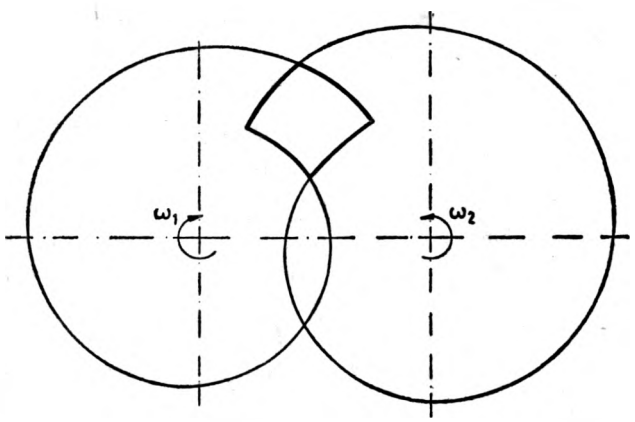

Fig. 3. Scanning principle (ω_1 and ω_2 denote angular speeds of discs)

Scanning realized in the second image plane π'', is based on the principle shown in Fig. 3. Two opaque discs with transparent Archimedes-type spiral slits rotate with different speeds. Speed ratio is 1 : 50. Slit cross forms the scanning point. X-Y displacement of this point gives the scan pattern. Slit width is 0.1 mm and spiral pitch is 5 mm.

Light beam which passes through the scanning point reaches photodetector M10FS29 photomultiplier made in GDR is used as a photodetector. The signals leaving photodetector are processed in the electronic unit the block diagram of which is shown in Fig. 4. These signals are proportional to the trans-

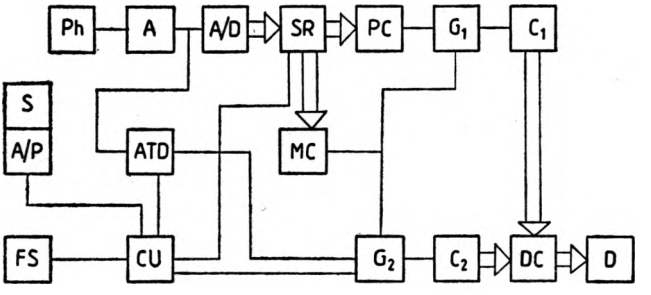

Fig. 4. Block diagram of electronic system. Ph – photomultiplier, A – linear and logarithmic amplifiers, A/D – analogue-digital converter, SR – shift register, PC – pulse converter, G_1, G_2 – gates, C_1, C_2 – counters, DC – display control, D – display, S – scanner, A/P – angular-pulse converter, CU – control unit, FS – function selector, ATD – area threshold discriminator, MC – mask control

mission of the scanned sample. They are amplified by linear and logarithmic amplifiers A. The logarithmic values are proportional to the absorption of the measured sample. Both the transmission and the absorption can be read out on the display D. During such a measurement the scanning system is stopped. In this case the selection of points to be measured is possible. During the measurement of the integrated density and the area, the scanning system is working. The analogue signals leaving amplifier A are proportional to the point densities. They are converted into the digital form by the analogue-digital converter A/D. The point density values are delayed in the shift register SR in which the five following measurements are stored. If one or more of these measurements is higher than the fixed mask threshold, the point to be measured is not taken into account. The successively measured values are converted by pulse converter PC into the pulse series which do or do not pass through the gate G_1 and reach or do not reach the counter C_1. In this counter the pulses are integrated; the integrated density is displayed when the scanning cycle is completed. The mentioned delay system allows the elimination of the points which are partially superimposed upon the mask.

At the same time the area of the investigated object is measured. The angular-pulse converter A/P, connected with the scanner, produces the pulses which

are used as the time base of the arrangement. Each pulse corresponds to one measured point. The analogue values of measurement are compared with preselected density level in the discriminator. When the point density is higher than the area threshold and lower than the mask threshold the corresponding pulse leaving the A/P converter is counted in the counter C_2. This pulse is delayed to assure the time accordance between the discriminator and mask control signals reaching the gate G_2. The values of area or integrated density can be displayed alternatively. The measurement principle is typical of one-beam photometers. Two measurements: first for the object and the second for the background should be made. The substraction is realized automatically. The basic parameters are shown in the Table.

Basic parameters of the microdensitometer

Scan area	for 100 × objective	25 μm × 25 μm
	for 40 × objective	62 μm × 62 μm
Scanning point size	for 100 × objective	0.5 μm × 0.5 μm
	for 40 × objective	1.25 μm × 1.25 μm
Number of scanning points (total)		2500
Interference filters		490 nm, 560 nm, 620 nm, others for request
Density range		0–1.2
Measurement time		9 s

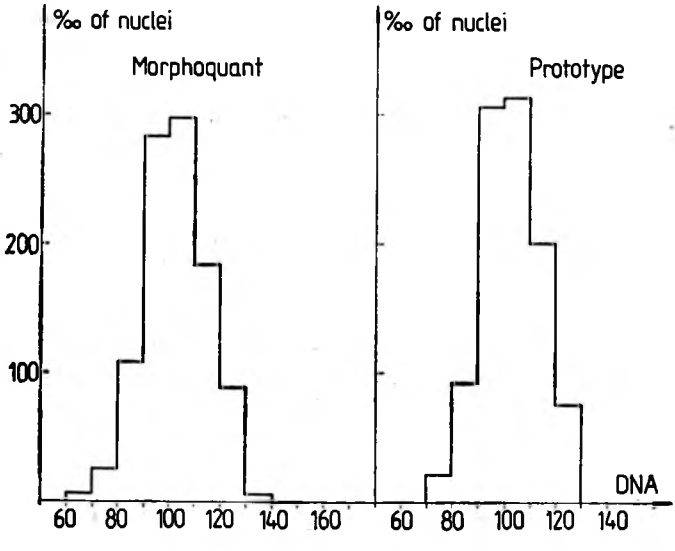

Fig.5. Histograms of DNA obtained by means of Morphoquant and the prototype built in Central Laboratory of Optics, Warsaw

The instrument was tested in Medical Academy of Szczecin, where the DNA amounts in cell nuclei were investigated. The results were compared with those obtained from the same specimen by means of Carl Zeiss, Jena, Morphoquant computer analyser. The histograms based on these results are shown in Fig. 5. It seems that a good agreement was achieved. This arrangement, though based on simple scanning principle, enables easy evaluation of basic cell parameters with good accuracy.

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