

## METHODS

### MECHANICAL DEVICE FOR MORPHOMETRIC AND ORIENTATIONAL ANALYSIS OF BIOLOGICAL STRUCTURES

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A simple mechanical device for various types of morphometric analysis is suggested. It consists of a binocular microscope, luminescence illuminator, a mechanical stage with disk-chuck and nest for holding the test systems and light- or electron-microscopic negatives. Analysis is carried out by visual scanning of negatives by counting the number of times the structures are crossed by points of the test system and by recording the measurements with a key-actuated counter. The microscope is fitted with a goniometer and cross-wire ocular micrometer.

KEY WORDS: morphometric analysis; mechanical device; negatives.

Attempts to automatize morphometric analysis of biological structures are constantly being made at the present time [2-4]. However, the problem of automatic recognition of organoids and intracellular inclusions has by no means been solved. That is why, during stereometric analysis, the function of recognition of intracellular structures is performed as a rule by a human operator. Several investigators have carried out stereometric analysis of objects on photographic prints, by superposing transparent test stencils with a system of points or lines on them. Other methods also have been suggested — projection of negatives and slides, and also superposition of a system of points or lines directly on the fluorescent screen of the electron microscope [7]. However, stereometric analysis of negative material directly is most economical.

We have made a mechanical device for visual scanning of negatives by means of an optical system enabling planimetric, stereometric, and orientational analysis of negatives to be undertaken at tissue and cellular (ultrastructural) levels.

The device is of the bench-top type (Fig. 1). On its base 1 are mounted an MBS-1 binocular microscope 2, a mechanical stage 3, and illuminator with on-off control system 4. The binocular microscope 2, giving magnification of between 3.6 times and 119 times, is used for visual scanning of the negative to be analyzed. The microscope is equipped with a specially made goniometer 5 to measure angles in the field of vision. Its scale is a protractor with divisions of 1°. The mechanical stage 3 is intended for placing any part of a negative measuring 6 × 9 or 9 × 12 cm in the field of vision by means of longitudinal (6) and transverse (7) screw-operated mechanisms. On the top of the stage is mounted a disk-chuck 8 with nest 9 for holding the negative and the transparent test stencil with a system of points or lines. The position of the latter is fixed by clamps 10. The negative is rotated by turning the disk-chuck between 0 and 360°. The angle of rotation is measured on a dial graduated in degrees. The illuminating system 4 consists of two LB-8 luminescent lamps, and on-off control system 11, an on-off button 12, and plug 13. To reduce pulsation of the luminous flux a single-phase circuit with two lamps was used.

For stereometric analysis of negatives the coordinate stage is moved steadily in one direction, while at the same time the structures to be analyzed are examined by means of the binocular microscope with cross-wire ocular. The negative is moved from one edge to the other along the lines of the control system. By means of a key-actuated counter the points of the test system or the number of intersections of the control lines with the outlines of the structures to be studied are counted. If the relative volume of the component is between 1 and 5%, stereometric analysis of the negatives is carried out by the field method [1, 6], by in-

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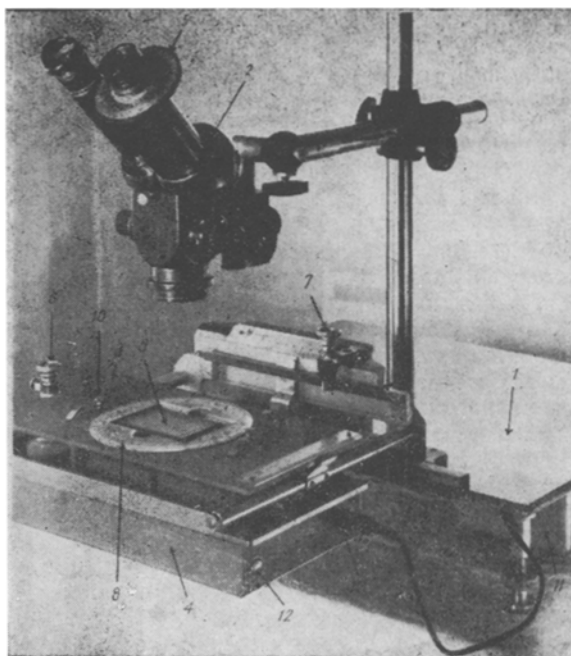


Fig. 1. Device for visual scanning of negatives. Explanation in text.

roducing a grid containing 225 squares into the ocular. Orientational analysis of structures that are not equiaxial in shape is carried out by means of the goniometer 5 and the cross-wire ocular of the binocular microscope 2. The test object is brought into the center of the field of vision by operating the displacement levers of the coordinate stage. By rotating the ocular with the goniometer the angle between the vector of orientation of the object ( $\vec{K}$ ) and the direction of the  $Ox$  or  $Oy$  axis of the corresponding system of coordinates is determined.

The suggested device is simple to make and use and has been successfully tested by the writers for stereometric analysis of electron micrographs of cardiomyocytes [5]. Its use does away with necessity for preparing series of photographic prints or slides. The wide range of magnifications of the binocular microscope, coupled with the use of different test systems and attachments to the ocular micrometer, enables practically all types of morphometric analysis to be undertaken.

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