IMPROVEMENT OF THE MORPHOMETRIC METHOD AND ITS USE IN ELECTRON-MICROSCOPY

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A method of obtaining morphometric parameters from the screen of an electron microscope is suggested and a description is given of a morphometric attachment to electron microscopes in order to put this method into operation. The prospects for the use of ultrastructural morphometry in electron-microscopic investigations is discussed.

The use of the electron microscope in modern histological and cytological investigations can often be reduced simply to a visual study and description of the visual image, giving the fullest qualitative idea of an object. However, the need to use objective quantitative criteria when assessing morphological structures makes it imperative to use morphometry. The use of the morphometric method during electron-microscopic studies of subcellular organelles enables not only quantitative characteristics such as volume, surface area, number of organelles, etc., to be obtained, but also reveals correlation between electron-microscopic, biochemical, biophysical, physicotechnical, and other parameters obtained during the study of the cell and its organelles in order to evaluate their morphological and functional states.

The use of the electron microscope for morphometric investigations raises a whole series of difficulties and limitations connected with the construction of these instruments. The absence of any special measuring systems in present-day electron microscopes compels investigators to adapt various methods developed for morphometry in the light microscope [1, 2].

For instance, it has been suggested that the fluorescent screen of the electron microscope be used for application of a measuring test system [2, 10]. Unfortunately, this method has many disadvantages, the most important of which is a change in the linear dimensions of the object accompanying a change in the angle of inclination of the viewing screen, as a result of which disparity is constantly being recorded between the dimensions of the object being studied.

Yet the use of morphometry to study mitochondria [6, 8] and also numerous investigations made by other workers have demonstrated the good prospects for its use in electron-microscopic investigations and have necessitated improvements of the technique of ultrastructural morphometry. A method of recording morphometric parameters directly from the screen of an electron microscope has been suggested for this purpose [3]. However, for this method to be realized to the full, a number of technical problems had to be solved [4, 5], and in particular, a morphometric attachment had to be developed for use with electron microscopes [7].

The method we suggest is based on the property of the projection lens of the electron microscope to have very great depth of sharpness (up to several meters). In other words, the scale test system, located at any point along the optical axis below the focal point of the projection lens can be used to obtain a sharp image of it on the screen of the electron-microscope. So that the scale system does not interfere during visual observation and photography of the specimen, we suggest placing it between the projection lens and the screen.

On the basis of analysis of versions of scale systems used in light optics and for analysis of electron micrographs, known in the literature, we chose as the prototype a quadrant grid of the most widely used ocular installations. For our purpose, we used a two-period quadrant grid, made from wires of different diameters. The small period was formed by a grid with wire with a diameter of 0.01 mm and a step (distance between the wires) of 2 mm, the large period with a diameter of 0.02 mm and a step of 1 cm, respectively, the large period containing 25 small periods. If this scheme of formation of the scale system is adopted, similar test systems can be used for electron microscopes and with a large number if periods for facilitating calculations. When

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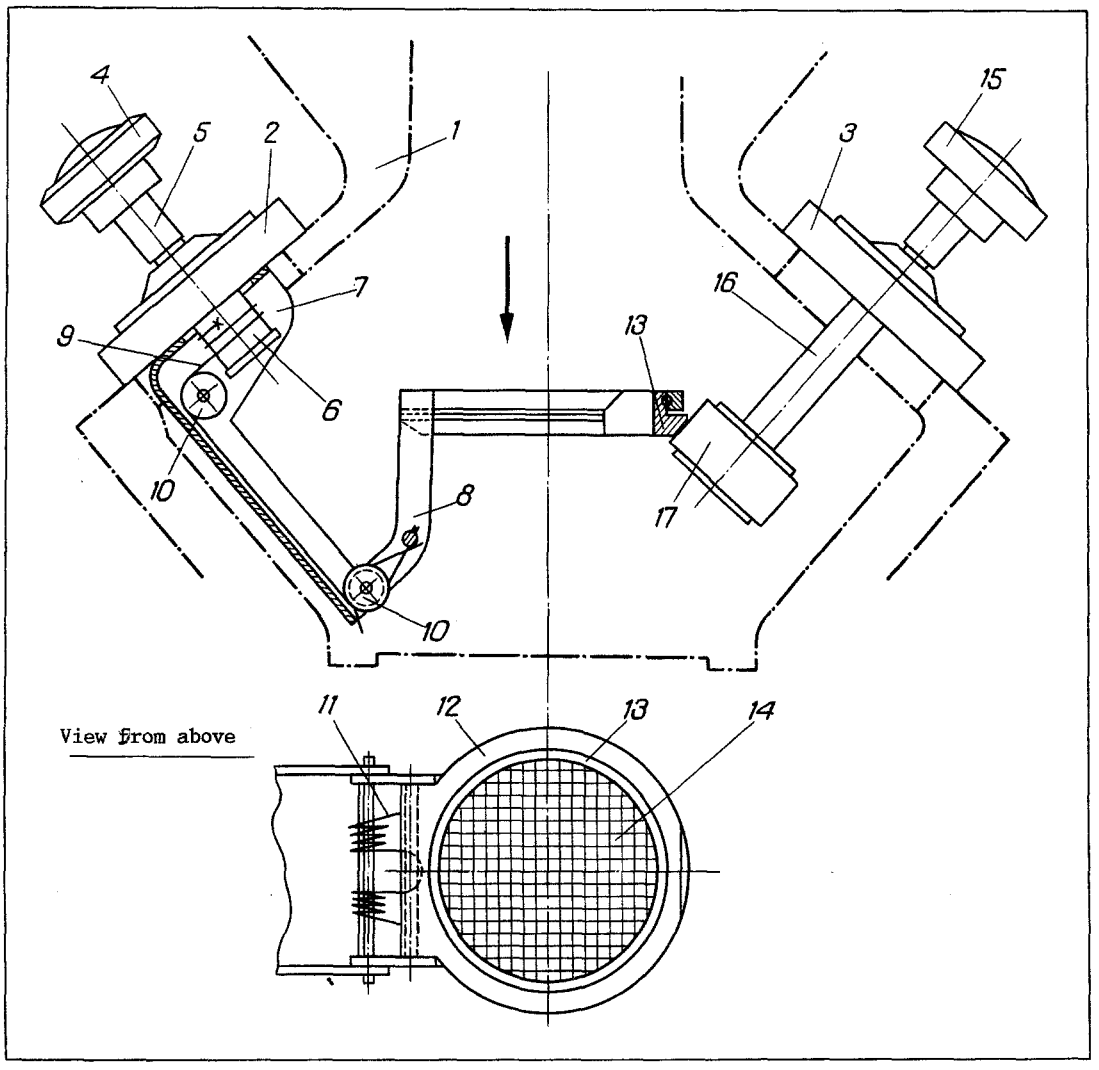

Fig. 1. Diagram of morphometric system for electron microscopes: 1) tube of electron microscope; 2, 3) plates; 4) control handle; 5) axis; 6) drum; 7) bracket; 8) lever; 9) cable; 10) pulley; 11) spring; 12) fixed ring; 13) movable ring; 14) scale system; 15) control handle; 16) axis; 17) roller.

scale systems made in the form of a grid containing at least two systems of orthogonal meshes are prepared, it must be recalled that each system was made with an equal period and was placed within the mesh of the mixed system with a larger period, and that for each adjacent system the diameters (d) of the forming wires were in the relationship: $d_{i-1} = (0.01 - 0.5)d$, where i - 2,3... denotes the number of periods.

Such a scale system, fixed in a supporting element made in the shape of a ring, connected with a rod so that the scale system can be inserted into the electron beam, can be used as a very simple morphometric device for attachment to electron microscopes [4, 5].

When working on improving the convenience of use of the morphometric device and reducing the time required to analyze an object, we undertook design work on the morphometric attachment for electron microscopes [7]. The morphometric attachment consisted of four components: a counting system, a moving mechanism, a turning device, and a switching mechanism. All these units were combined into a single kinematic chain (Fig. 1).

The diagram shows the morphometric system made in the form of an attachment for electron microscopes, in which a plate (2), on which are mounted the moving mechanism, the switching mechanism, and the counting system, to the tube of the electron microscope (1) at the site of the left viewing window, and at the site of the right viewing window is fixed a plate (3)

with the turning system. The moving mechanism incorporates a control handle (4) with axle (5) and drum (6). A bracket (7) with hinged lever (a) is securely fixed to plate (2). A cable (9) is fixed at one end to the drum (6) and at the other end through a system of pulleys (10) to the spring-loaded lever (8) by the spring (11).

The second ring (12), relative to which the first ring (13) with the scale system fixed to it (14), and made for example in the form of a grid can revolve freely, is securely fixed with the lever (8). The mechanism of rotation is based on a plate (3) and incorporates a control handle (15) with axle (16), to which the guiding element (17) interacting with the first ring (13) when the scale system (14) is in the working position, is fixed. The switching mechanism, which is an auxiliary unit and serves to fix the spring-loaded lever in the required position, is not shown in Fig. 1.

OPERATION OF THE SYSTEM

In the working position on the scale system (14) is introduced into the electron beam and is arranged perpendicularly to the electron-optical axis. After recording of the image of the object, against the background of the periodic structure, the scale system is oriented relative to the object so that the characteristic directions of the contour of the object coincide with the directions of the scale system. This orientation is effected by turning the handle (15) and transmitting a rotary movement to the movable ring (13) which, together with the scale system (14), is turned relative to the fixed ring (12). To remove the scale system from the working position the handle (4) is turned. Under these circumstances, by pulling on the drum (6), the cable (9) draws away the lever (8) from the fixed and movable rings into the region of the tube free from the electron beam.

Thus, our suggested device for morphometric measurements of histological and cytological objects yields objective quantitative information. While noting the importance of developments in transmission electron microscopes with image analyzers based on the use of computers, it has to be recognized that their use will be very limited in the immediate future. Mechanization is suitable only when the manual method significantly retards further investigation [9]. To solve many modern problems in experimental, diagnostic, and descriptive morphology, in which electron microscopy is widely used, it is evidently quite sufficient at present to use the simplest statistical methods and specially developed programs for personal computers. In this connection it is more economically advantageous and expedient to equip modern microscopes (and those already in operation) with a simpler and cheaper morphometric attachment. This is demonstrated by the considerable interest displayed by experimental research workers in the present development at a number of All-Union conferences and meetings.

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