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## METHOD OF FOCUSING ELECTRON MICROSCOPES

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5 Claims. (Cl. 250—49.5)

The present invention relates to a method of improving or facilitating the focusing of electron microscopes.

In the operation of electron microscopes, especially for photographic reproduction, it is very important that the image of a substance to be examined in the microscope is focussed sharply on the fluorescent screen. However, there are numerous substances, for example, viri which have very little contrast. When adjusting the electron microscope, it is therefore very difficult to show the image of such substances in focus on the fluorescent screen and to determine whether the image is sharply adjusted or out of focus. This difficulty is enhanced by the fact that the intensity of the electron beam decreases in proportion to the increase of the magnification factor, so that with high magnifications the fluorescence of the screen is only very low and weak contrasts in the image are hardly perceptible.

It is the principal object of the present invention to overcome the above mentioned disadvantages according to the invention, the substances to be examined are provided with means contrasting therewith, so that the difference in contrast between the added means and the actual image of the substances can be easily determined on the fluorescent screen. The strong contrasts thus appearing in the magnified image of the conglomerate permit an easy and sharp focusing of the image.

In practicing the invention, the substances to be examined in the electron microscope, for example viri, are provided with materials having a specific density considerably higher than the density of the substances. Only small particles of these materials are added to the substances to be examined, and the size of these particles must correspond substantially to the size of the substances. The added materials must be of a type suitable for obtaining in the image on the fluorescent screen of the electron microscope the strong contrasts necessary for adjusting the image to be in sharp focus. It is advisable to use only such materials which do not react in any way with the substance itself, and it is easily possible, for example by tests, to determine the type of materials best suitable for a particular purpose. For examining viri, colloids of various metals, for example gold or silver colloids, have been found suitable, although colloidal metallic oxides may also be used.

The following is a more specific example of the method above described. The substance, in particular virus, to be examined is dissolved and gold colloid is added to the solution. The concentration of viri, for example, may be in the amount of $10^{-4}$ to $10^{-5}$ grams of virous protein for each cubic centimeter of solvent. The concentration in gold to be added thereto may then, for example, amount to $10^{-6}$ to $10^{-7}$ grams per cubic centimeter. The solvent to be used should be a volatile substance, for example, water or xylene without any contents in nonvolatile substances or salts. The gold-containing solution is applied to a supporting foil as customary in electron microscopes for supporting the object to be studied. After volatilization of the solvent, the dried residue left on the foil is a conglomerate of the substance to be studied and gold particles. The foil is then placed into the electron microscope and the focusing is effected in the customary manner, except that the strong contrasts now appearing in the magnified image render the focusing very easy and accurate.

I claim:

1. The method of electron-microscopically examining organic substances of weak electron-optical contrasts, which comprises adding to the organic substance to be examined colloidal particles of an inorganic material chemically inert with respect to said organic substance and of greater density than said organic substance so as to form a conglomerate of strong electron-optical contrasts, subjecting the conglomerate thus obtained to electron-microscopical magnification, and adjusting the magnification by focusing the magnified image on the contrasts of the conglomerate.

2. The method of electron-microscopically examining substances of weak electron-optical contrasts, which comprises adding to the substance to be examined a colloid non-reactive as regards said substance and of greater specific weight than said substance so as to obtain a conglomerate with strong electron-optical contrasts, subjecting said conglomerate to electron-microscopical magnification, and adjusting the magnification by focusing the magnified image on the contrasts of the conglomerate.

3. The method of electron-microscopically examining organic substance containing virus or other organisms of weak electron-optical contrasts, which comprises admixing to said organic substance an inorganic material non-reactive as regards said substance and of considerably greater density than said substance, said material being finely subdivided into particles of a size in the order of magnitude of the size of said

organisms, whereby a conglomerate of strong electron-optical contrasts is obtained, subjecting said conglomerate to electron-microscopical magnification, and adjusting the magnification by focusing the magnified image on the contrasts of the conglomerate.

4. The method of electron-microscopically examining substances of weak electron-optical contrasts, which comprises adding to the substance to be examined a colloid of noble metal so as to obtain a conglomerate of strong electron-optical contrasts containing said substance and colloidal gold particles in juxtaposition and non-reactive as regards each other, subjecting said conglomerate to electron-microscopical magnification, and adjusting the magnification by focusing the magnified image on the contrast of the conglomerate.

5. The method of electron-microscopically examining substances of weak electron-optical contrasts, which comprises adding to the substance to be examined a colloidal metallic oxide non-reactive as regards said substance so as to obtain a conglomerate of strong electron-optical contrasts containing in juxtaposition particles of said substance and colloidal oxide particles, subjecting said conglomerate to electron-microscopical magnification, and adjusting the magnification by focusing the magnified image on the contrasts of the conglomerate.

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