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Accurate Determination of Magnification in the Electron Microscope

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The magnification of the Siemens Elmiskop I at about 57,000 times can be determined with an accuracy of better than 1% for any object plane position and any region in the final image. This is achieved by taking into account magnification variations due to hysteresis effects in the lenses, changes in the accelerating voltage, changes in object plane position, and distortion produced by the projector lens.

In most instances, e.g., in biological work, the user of the electron microscope does not need to know the magnification of his micrographs with an accuracy better than that quoted by the manufacturer of his instrument. This means that an error of about 10% is accepted. Sometimes however the need for a better knowledge of magnification arises, especially in those cases where measurements of periodic structures in the electron microscope can be compared with those obtained from X-ray analysis. This is the case in studies on ordered lipid systems, which are of importance in biology and where repeating distances of the order of 50 Å are found.

A method to reduce error in the determination of magnifications up to about 10,000 times has been indicated by Heckman and Roper (*2*) for a microscope in which the magnification variation is obtained by projector lens current variation. In this method, however, not all the variables that influence magnification have been taken into consideration. The same applies to the method devised by Oster and Skillman (*3*) for magnifications up to 5000 times using the Philips E.M. 100 microscope.

Both methods suffer from the lack of precision with which the magnification is characterized once the calibrating aid is substituted by the sample to be measured.

The aim of the present study was to find the means to determine the magnification of the Siemens Elmiskop I at a magnification setting of about 40,000 times with an accuracy of better than 1% for any object plane position and any region in the final image. The method given here was developed essentially following the lines indicated by Hall (*1*).

The magnification of an electron lens varies with its focal length, which in turn is dependent on coil current, accelerating voltage, and hysteresis effects in the iron parts of the lens. The focal length of the objective lens, moreover, is determined by the position of the object along the lens axis. The magnification in the plane of the final image may vary owing to distortion produced by the projector lens.

In the Siemens Elmiskop I a final magnification of 40,000 times is obtained by the combination of three lenses: the objective, the intermediate, and the projector lens, the last with its pole piece no. 2. For each of these lenses, therefore, the above-mentioned variables must be determined, and it is obviously of importance to keep most of them at one constant value.

The objective and intermediate lens were used in the coupled condition, that is, with the same current flowing through the lens coils. This gives a greater variation of magnification with lens current, but there is only one current to determine.

Hysteresis effects were eliminated by a procedure which was found suitable by experiments. Prior to focusing for a micrograph, the objective (and intermediate) lens current was switched off and the coarse current control was set at maximum. The lens current then was switched on and, by an equal number of steps of the coarse control, diminished to the focusing value. This procedure was twice repeated while checking the focus condition. The focus condition prevailing after the second switching sequence was always found back after the third sequence, an indication that remanent magnetism of the iron circuit of the lenses was brought to a constant value.

The constancy of the accelerating voltage was checked in the following way. An object was focused at 40,000 times magnification and an accelerating voltage of 60 kV. Switching off and on the high tension had no influence on the magnification. The next step, after having noted the objective lens current, was to switch off the current to the microscope. When the microscope was switched on after a day and remanent magnetism was brought to a constant value as mentioned before, the image was found back in focus at the same objective lens current as the day before. When the magnification was checked after some months, however, it appeared that a rise of about 3% had occurred with respect to the value expected from the calibration measurements. Analysis of the circumstances led to a lowering of the accelerating voltage as the cause of this difference.

It proved, therefore, necessary to keep the accelerating voltage under control too. Its level is determined by the current through a voltage divider parallel to voltage reference tube 7 (85 A 2) of the high tension regulating circuit. This current can be checked by inserting a resistor of 180 ohm in the voltage divider and measuring the voltage developed across this resistor by means of the same compensator as that used for the lens current measurements. The step resistor W 36 in the high tension regulator serves the purpose of keeping the current through the voltage divider, and thus the accelerating voltage, at a prefixed value.

The last step, as regards the objective and intermediate lenses, was to determine magnification as a function of the position of the object plane along the microscope axis, that is as a function of objective lens current.

It was found that the magnification variation by displacing the object, e.g., bending the copper grid or changing the gridholder may amount to 10%. An accurate magnification determination once for all remains therefore of little value. From preliminary measurements it was calculated that a magnification variation of about 0.5% would be produced by an objective current variation of 1 mA. This means that a variation of 0.25% of the current has to be measured, which precludes direct current reading on a milliammeter.

Instead, for measuring current, a compensation circuit of sufficient precision (Fig. 1) was designed. In this circuit the lens current flows through a measuring resistor of 2 ohm. The voltage across this resistor is compared with a standard by means of a precision potentiometer and a galvanometer. The potentiometer has 100 scale divisions, one scale division corresponding to a lens current variation of 0.355 mA. Its range corresponds to four steps of the coarse lens current control. The resistances in the compensation circuit were calculated so as to demand a potentiometer setting at 50 for a "normal" lens current of 372,75 mA as specified by the manufacturer of the microscope. Furthermore the resistances have been chosen in accordance to the voltage of a commercially available reference battery, the maximum allowed current drain from this battery, and the available series of precision resistors.

As the magnification calibration, with this method, has become dependent on the electromotive force of the reference battery, means are provided to check this EMF from time to time with the aid of a Weston standard voltage cell and to correct the voltage drop across the measuring resistors if necessary. In order to limit the drain of current from the reference battery, this battery is switched on by means of a relay which is energized at the same time as the objective lens coil. The same relay short-circuits the galvanometer before the reference battery-circuit is broken, thus minimizing pointer deflections during lens current switching. A selenium rectifier prevents this relay from chatter caused by induction currents at lens switching. The compensation circuit is designed for lens currents at 60 kV accelerating voltage; the relay therefore is switched off at the other high tension settings by means of the high tension switch. A multipole switch offers the possibility to check the reference battery EMF irrespective of lens current.

The magnification variation in the final image on the photographic plate, due to distortion by the projector lens, was determined for projector pole piece no. 2. This

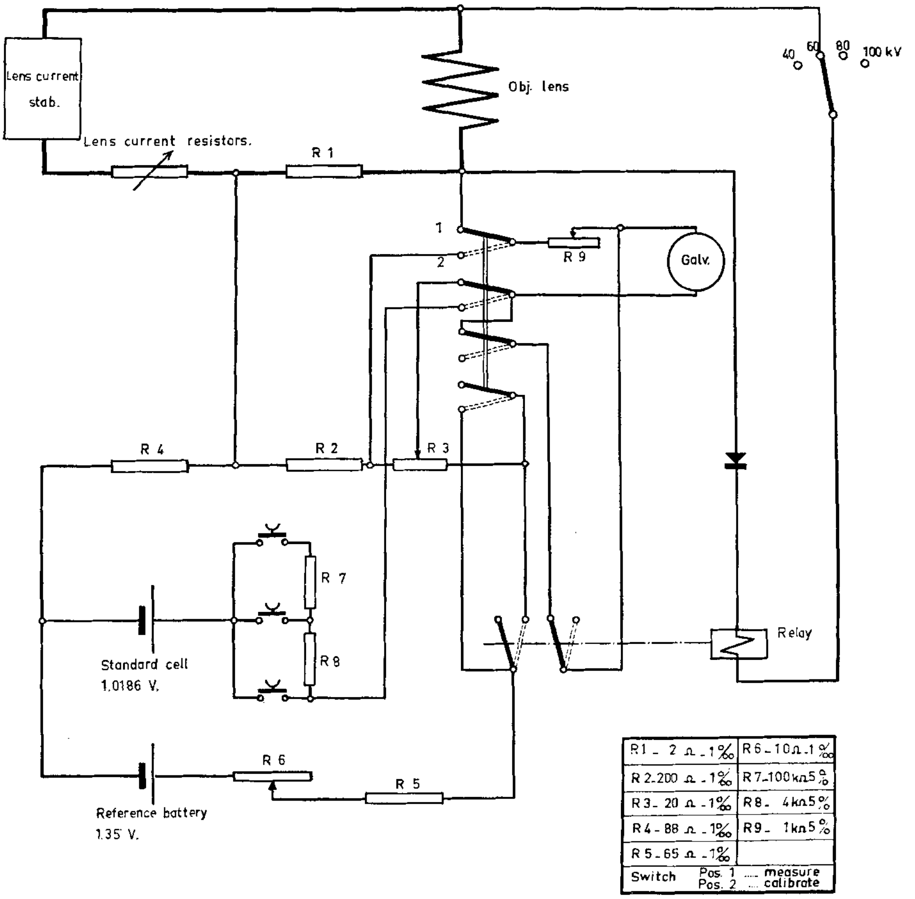

FIG. 1. Circuit diagram of the compensator for lens current measurements and high tension control.

was first done at the projector current setting which gives a final image of standard diameter (90 mm) on the screen. The magnification variation was determined from the size of a small tungsten oxide crystal, photographed at different sites in the final image. The magnification, measured in radial direction at the edge of the photographic plate proved to be 6% higher than at the center. Measured in tangential direction the difference was 2.5%. In this condition of the projector lens a magnification variation of less than 1% was to be found only within a circle of 3 cm diameter at the center of the plate. In order to have a larger part of the photographic plate available for accurate measurements it was decided to use the projector lens at its maximum magnification. According to van Ments and Le Poole (*4*) the lens is then free from isotropic

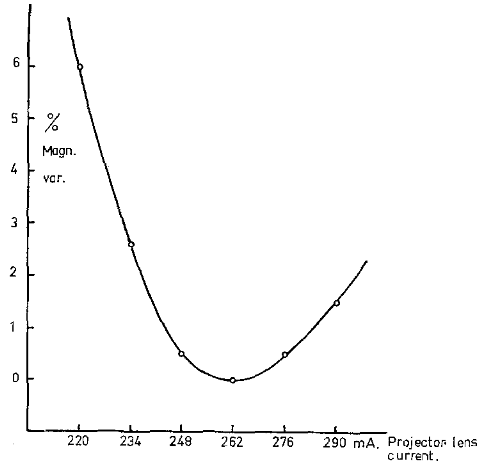

FIG. 2. The percentage of variation of magnification with the projector lens current.

distortion, and also from chromatic aberration. This was found at a lens current of 262 mA, which is given by a coarse current control setting of four steps from the maximum current end (Fig. 2). At three or five steps the magnification variation was 0.5%, so the projector current setting for maximum magnification is not very critical. For this reason hysteresis effects in the projector lens are neglected too.

The influence of the reference voltage in the lens current stabilizer was also checked. A 3 volt variation produced a lens current variation of 14 mA, corresponding to one step of the coarse control. As the reference voltages are checked regularly and kept within a 1.5 volt difference from the nominal value, magnification variations due to this source will be less than 0.25%. The projector lens magnification, therefore, is sufficiently determined, for our purpose, by the setting of the coarse and fine current controls.

At maximum magnification of the projector lens the variation in radial direction due to distortion proved to be less than 1% within a circle of 8 cm diameter on the plate. This variation is less than 0.5% within a circle of 6 cm diameter. The variation in tangential direction was not measurable. At maximum projector magnification image contrast was found to be somewhat deteriorated by electrons scattered from the projector tube walls. A suitable diaphragm therefore was placed underneath the projector lens in order to confine the final image to a diameter of 95 mm on the screen.

Determination of the magnification variation due to displacement of the object along the optical axis of the microscope

Object displacement was obtained by screwing up and down the grid holder. This holder was ground down for about 0.2 mm, allowing object positions to be taken at

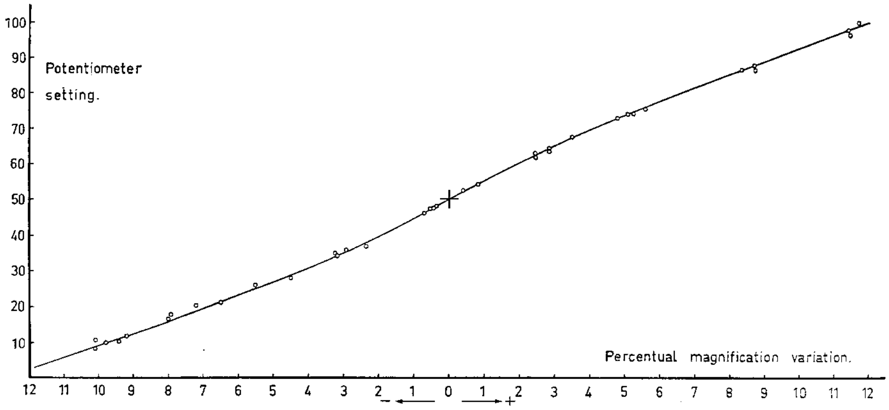

FIG. 3. The percentage of variation of magnification by the objective and intermediate lens as a function of object plane position expressed as the potentiometer setting of the compensator with focused objective lens.

both sides of the standard position. The object was a carbon replica of a diffraction grating containing 28,800 lines to the inch (supplied by Fullam, Schenectady, New York). This was photographed at a magnification of 2500 times by using the coupled objective and intermediate lens only. The distance of 20 lines on the plate was measured with an accuracy of 0.2% using a photographic enlarger and an accurate ruler. By plotting magnification variation against potentiometer setting of the compensator, the curve of Fig. 3 was obtained. On the abcissa percentages of magnification deviation with respect to the magnification at potentiometer setting 50 are noted. Thirty-six measurements were made, and the curve shows a good fit to the points, especially in the middle region.

Determination of the magnification provided by the combination of objective, intermediate, and projector lens with its polepiece no. 2.

The three lenses were used under the above-mentioned conditions. The object again was a carbon replica of a diffraction grating. The mean line distance of this replica was checked in the following way.

The large central grid square was photographed in the electron microscope at a magnification of 210 times with the objective lens only. The lines proved to run nearly perpendicular to a diagonal of the grid square. The number of lines between two opposite corners of the square was counted. After correction for nonperpendicularity, a number of 221.5 lines was found for the distance between the two corners. Subsequently the distance between these two corners was measured with the aid of a light microscope equipped with an ocular micrometer. This micrometer was calibrated as follows. A razor blade of about the same thickness as the grid square diagonal was broken, and a splinter was selected which showed a sharply defined fracture perpendicular to the blade plane. The thickness of this splinter was measured near the edge by means of a compensation method. The combined thickness of two gauge blocks, 1060 ± 0.2 μ and 1200 ± 0.2 μ, respectively, was compared with the thickness of a gauge block of 2000 ± 0.2 μ combined with the razor blade splinter. Comparison was done by means of a high quality dial micrometer gauge with scale divisions of 0.2 μ. The thickness of the splinter proved to be 260 μ with an estimated inaccuracy of 0.3 μ. Next the razor blade splinter was mounted on the light microscope, with the fracture plane perpendicular to the optical axis, and the ocular micrometer was calibrated.

The value obtained from these measurements for the distance between the two grid square corners was 195.4 ± 0.3 μ. The corner distance divided by the number of lines gives a mean line repeat distance of 0.882 μ, which corresponds to 28,793 lines to the inch for this particular grid square.

Now the replica was photographed in the electron microscope with the three lenses mentioned. Forty-six photographs were made and the repeat distance of two lines was measured using a photographic enlarger with 5 times magnification. This magnification was accurately calibrated by measuring the image of a sharply defined metal plate 20.00 mm in width, which was placed at the position of the negative in the enlarger. From the 46 measurements thus obtained, a mean line repeat distance of 255.93 mm was calculated. The standard error of this mean has a value of 0.22. With 99.7% probability the line distance after magnification, which corresponds to the aforementioned calibrated mean line distance, will lie within the range 255.93 ± 0.66 mm. The percentage of error in this part of the magnification determination therefore is ± 0.25. The mean line repeat distance on the photographic plate is obtained by dividing the above mean by the enlarger magnification. This distance, 51.19 mm, was found at a mean potentiometer setting of 55.6 scale divisions.

Using the curve of Fig. 3, the magnification by the electron microscope at potentiometer setting 50 was found to be 57,400 times. For practical purposes a table of magnifications for 100 scale divisions of the potentiometer has been calculated. With the projector controls in the right position and remanent magnetism at a constant value the actual magnification on each photographic plate is now defined by the setting of the compensating potentiometer when the object is in focus.

A consideration of the magnitude of the errors, involved in the different parts of the magnification determination described here, warrants the conclusion that the resultant error in a statement on the magnification in any particular electron micrograph will lie well within the limit of 1% with this method.

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