

4.2 Calibration of Rotation and Inversions

To be able to successfully correlate the images and their corresponding diffraction patterns, it is necessary to know not only precisely from where the diffraction pattern originates, but also how it physically relates to the orientation of the specimen. As the electron beam is focused through the magnifying projector lenses, the image must go through a series of cross over points (Fig. 1.13). Also, as the strength of the magnetic lens is varied, the image experiences a rotation (angular shift). Therefore, to effectively use microscopy and diffraction information, the geometrical relationships between the image and the diffraction pattern must be known.

The simplest method to determine the image rotation relative to the diffraction pattern is to use a specimen of MoO_3 crystals on a carbon support grid. Such specimens can easily be obtained by heating a strip of molybdenum and catching the oxide smoke on the carbon support film. The MoO_3 crystals tend to grow as long plates with straight edges running parallel to $\langle 100 \rangle$. If the image of the MoO_3 crystal is photographed for a series of intermediate lens settings and its diffraction pattern is superimposed on each image, the rotation can easily be measured (e.g., Fig. 1.16).

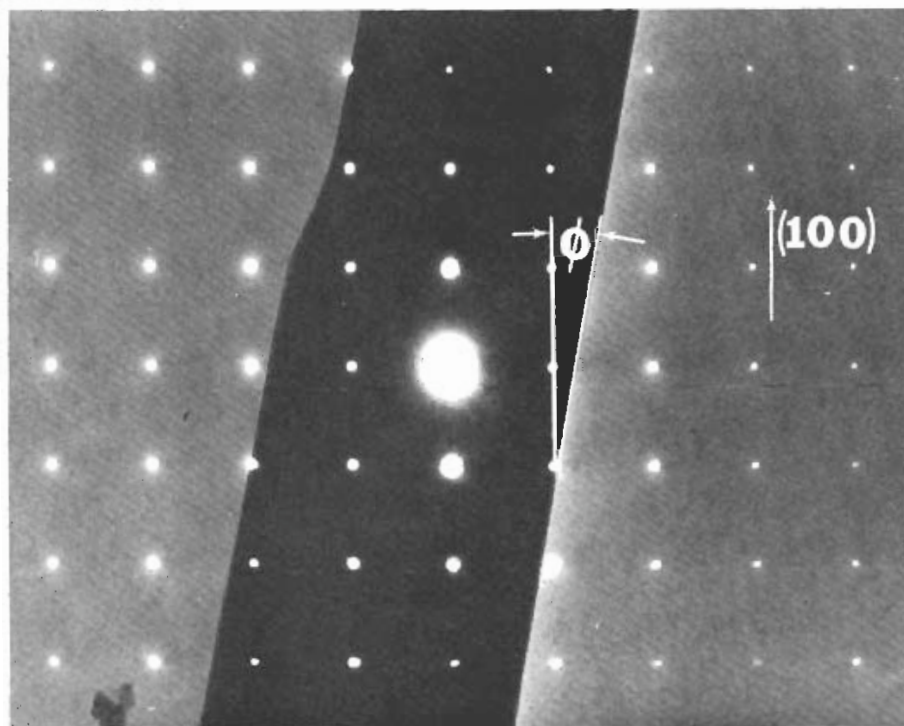


Fig. 1.16 Single crystal of MoO_3 with its selected area diffraction pattern superimposed (100 kV). The rotation ϕ ($\approx 11^\circ$) is the angle between the edge of the crystal and the $[100]$ row of spots ($\times 7750$).

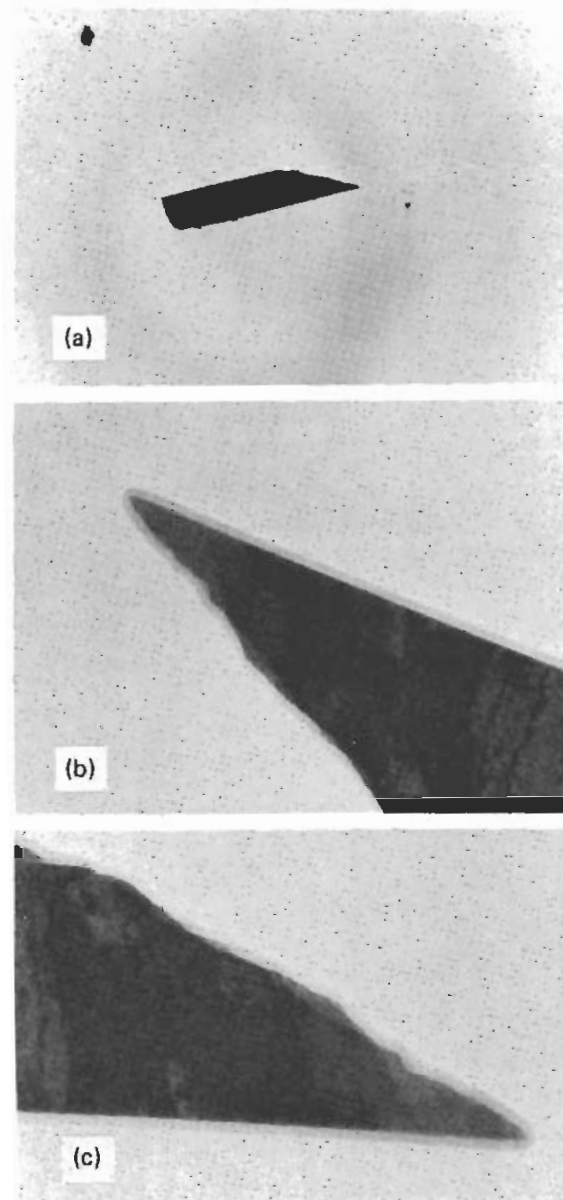


Fig. 1.17 The image inversions of a MoO₃ single crystal, taken with a Philips EM 301 equipped with a high resolution stage: (a) low magnification ($\times 1300$ – $\times 6000$), (b) intermediate magnification ($\times 7250$ – $\times 3000$) and (c) high magnification ($\times 36,000$ – $\times 360,000$).

It can be seen from the ray diagrams of Fig. 1.13 that there is an inversion between the image and the diffraction pattern. This effect is present in all microscopes; however, microscopes with more advanced imaging systems may present a more complicated situation. Figure 1.17 shows the image inversion of a MoO₃ specimen taken with a Philips EM 301 for three different ranges of magnification. Figure 1.18 shows an example of a magnification-rotation calibration for a Philips EM 301 microscope, illustrating the inversion change with magnification. Since the Philips is equipped with a diffraction lens, care must be

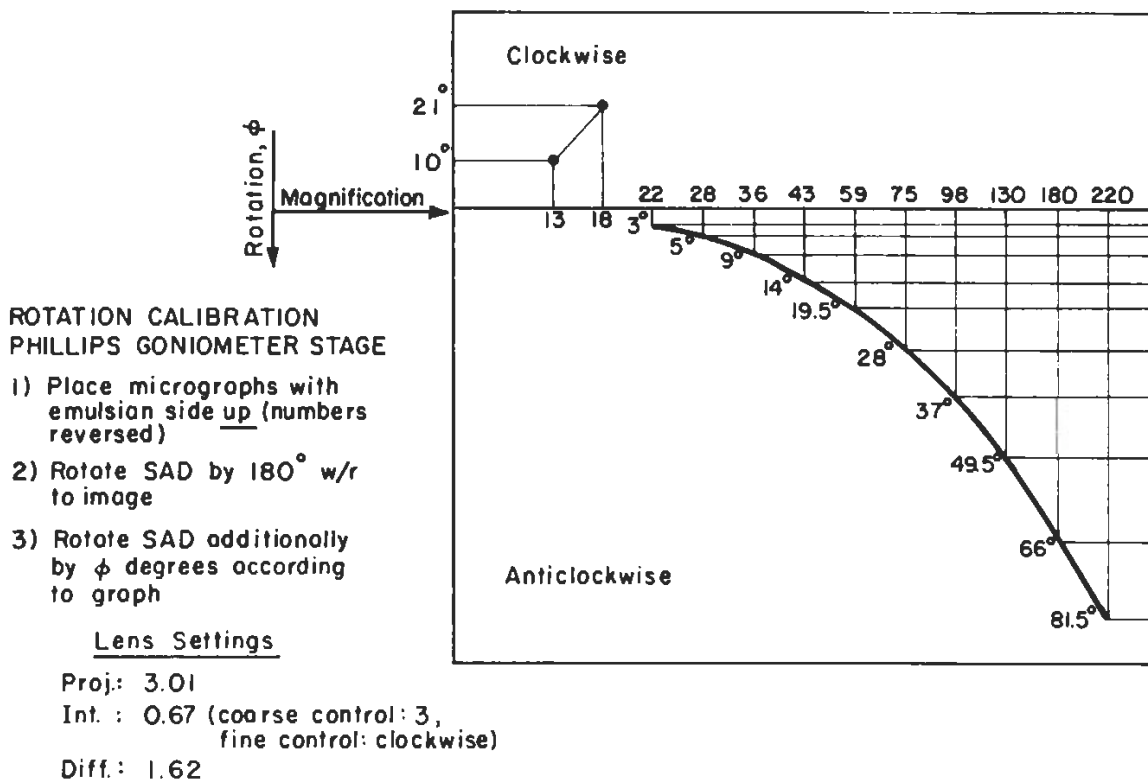


Fig. 1.18 The rotation calibration of a Philips EM 301 electron microscope equipped with a goniometer stage.

taken to focus it correctly since, as shown by Fig. 1.19, the image will invert when the diffraction lens is either under- or overfocused. If the direction of the foil normal, with respect to the electron beam, is to be known, any inversions must be accounted for. One of the most frequent exercises in electron microscopy involves relating the direction of the diffraction vector to structural features, such as defects, in the electron microscope image. Calibrations such as the one shown in Fig. 1.20 are thus essential. The recommended steps are given in the following list, with reference to Fig. 1.20:

1. Place image and diffraction pattern negatives emulsion side up (Fig. 1.20a) on a viewing light box or table.
2. Rotate the diffraction pattern with respect to the image, allowing for rotation-inversion calibrations (Figs. 1.20b and c).
3. When properly oriented, mark the direction of the diffraction vector (or other crystallographic data) on the nonemulsion side of the image (Fig. 1.20c).
4. Make a contact print so that a permanent record can be kept of the crystallographic relations. Afterward the marks can be erased.

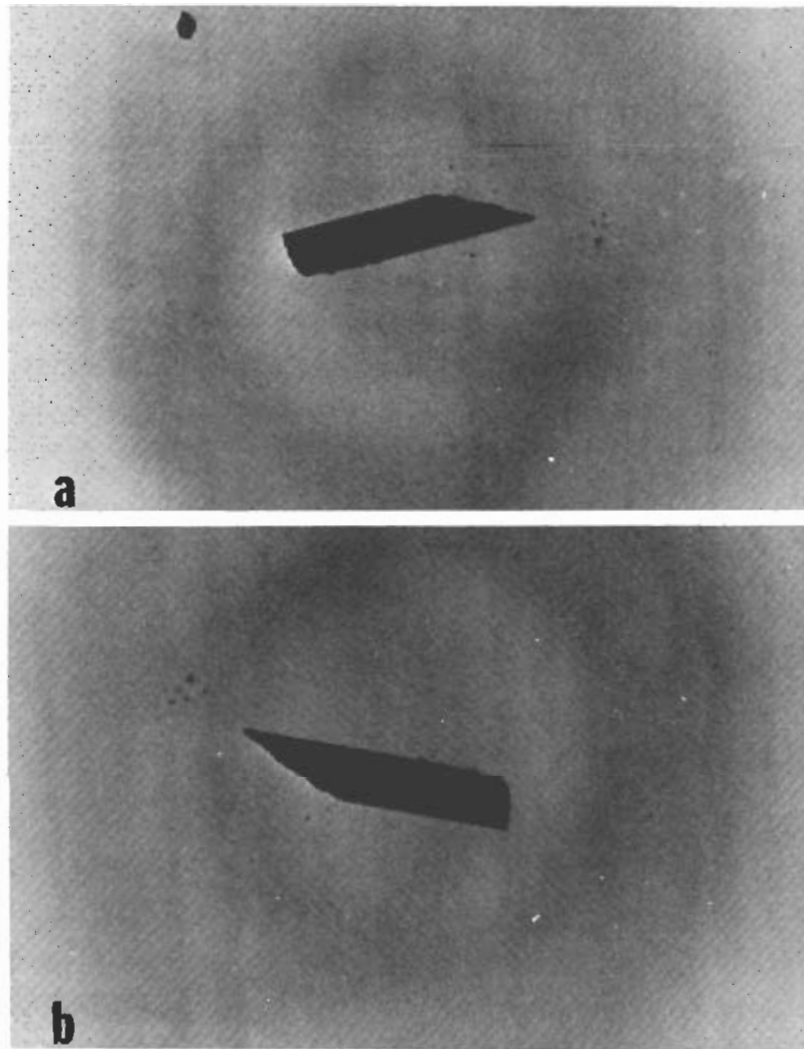


Fig. 1.19 Illustrating image inversion in a four-lens imaging system with (a) underfocus and (b) overfocus of the “diffraction” lens. Specimen is a MoO_3 single crystal.

5. Plot the data obtained on a stereographic projection, thus facilitating such analyses as true orientation and trace analysis.

4.3 Magnification Calibrations

Accurate magnification calibrations are required if quantitative measurements are to be made from electron micrographs. A wide variety of methods are available for image magnification calibrations;¹⁹ however, the methods most commonly used are diffraction grating replicas for low and intermediate magnification and direct lattice imaging of known crystal lattice plane spacings for high magnifications. Permanent calibrations are accurate to within $\pm 5\%$ and can be improved to $\pm 2\%$ for an *in situ* calibration.