IMAGE ROTATION VIS-A-VIS MAGNIFICATION - A PHENOMENON IN ELECTRON MICROGRAPHY

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ABSTRACT

Calibration of Electron Microscope (EM) Systems would reveal certain aspects heretofore unknown in the world of photogrammetry. One such phenomenon is the image rotation, which can be directly correlated to the image magnification at the microscope. Research studies on this aspect in relation to other EM features are discussed. Studies made on both, Transmission Electron Microscopes (TEM) and Scanning Electron Microscopes (SEM), are presented. The phenomenon is found to be more profound in the TEM than in the SEM.

INTRODUCTION

There seems to be an increasing demand for more accurate three-dimensional information on objects used in their studies by the users of various Electron Microscope (EM) systems. This is one aspect challenging to modern photogrammetry. In order to meet this challenge, intensive calibration of the specific EM system would be deemed necessary.

One interesting EM phenomenon is image rotation. This was studied earlier at some length by Ghosh and ElGhazali (1977). In EM systems, within the magnetic lenses, the electrons are known to travel spirally with regard to the 'principal beam'. This is behind the apparent image rotation and is caused by changes in condenser lens current, electron irradiation of regions on the specimen, condenser lens excitation, misalignment of lenses, change of projection distance during refocusing, etc. (see Agar et al, 1974).

In order to examine this phenomenon further, newer studies were made with regard to both, Scanning Electron Microscopes (SEM) and Transmission Electron Microscopes (TEM). Various aspects of these studies made at the Laval University Department of Photogrammetry are discussed below. Two represetative cases, one for each, SEM and TEM, are presented for the purpose.

IMAGE ROTATION IN SEM

Seven micrographs were used in this study. These were obtained with an SEM, model JSM-25SII, manufactured by JEOL, Japan. A SIRA calibration specimen with cross-ruled gratings of 2160 lines to the mm was used as the specimen. Details of the micrographs are in Table 1. "Letraset" crosses adhered to the four corners of the CRT screen of the SEM camera end served as fiducial marks (see Fig 1). During the production of the seven micrographs, nothing was disturbed at the SEM excepting the knobs for magnification and focusing. These are absolutely necessary for changing magnifications of micrographs.

As the basis for comparison, the same grid intersection points of 5x5 units were observed on each micrograph (see points 1,2,3 and 4 in Fig 1). The Zeiss Stereotop developped into an analytical plotter as described in

Ladouceur et al (1982) and Ghosh (1982) was used in the mono-comparator mode for reading the x,y photo-coordinates. The left stage of comparator was only used and each point was observed ten times to ensure sufficiant reliability in the observations and to avoid gross observational errors. The mean values of the observations ($\bar{x},\bar{y}$), their standard deviations (σ_x and σ_y) averages of these standard deviations ($\bar{\sigma}_x$ and $\bar{\sigma}_y$) and the resulting positional errors (σ_p) were obtained in order to appraise the data acquisition. The results are given in Table 2.

Table 1 : Arrangement of SEM micrographs

Micrograph no.	Dial value of Magnification at SEM
1	2 000 x
2	3 000 x
3	4 500 x
4	7 000 x
5	10 000 x
6	15 000 x
7	20 000 x

Note: Tilt and Rotation in all cases were kept at zero degree, while the nominal projection distance remained constant at 10 mm.

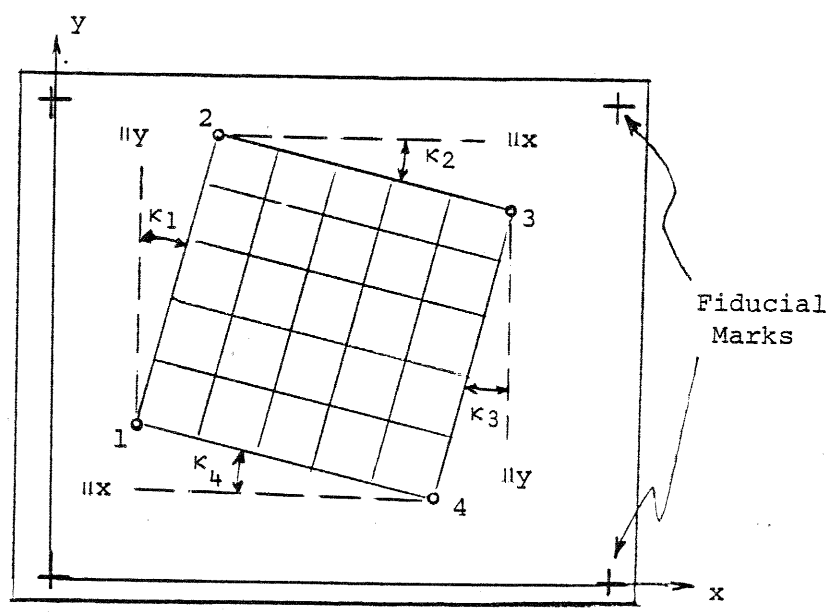


Fig 1 : Micrograph coordinate system and rotation angles at SEM

The distances of the four sides of the square connecting points 1,2,3 and 4 are considered in determining the magnifications. The average distance ($\bar{d}$) of each case is compared with the actual distance known from the manufacturer's data (grid specifications) to calculate the actual average magnification (M). These are illustrated in Table 3.

Image rotations were studied with regard to the four angles (κ_1, κ_2, κ_3 and κ_4 in Fig 1) for each magnification. The results, with regard to all the magnifications, are presented in Table 4. This would indicate closeness of values of horizontal angles in each micrograph with similar closeness in the vertical angles. The average values and their standard deviations are given.

Micrograph no.	$\bar{\sigma}_x$ µm	$\bar{\sigma}_y$ µm	σ_p µm
1	31	21	37
2	21	24	32
3	32	28	43
4	43	44	62
5	45	54	70
6	66	63	91
7	91	180	202

Table 2 :

Standard deviations of the observations [SEM]

Micrograph no.	Average distance $\bar{d}$ in µm	Magnification M	σ_M
1	2 931.7	1 266	16.0
2	4 380.8	1 892	13.8
3	6 492.2	2 803	18.6
4	10 077.0	4 351	26.8
5	14 208.7	6 135	30.2
6	21 267.8	9 183	39.3
7	28 474.7	12 295	87.2

Table 3 :

Magnifications and standard deviations [SEM]

Micrograph no.	Average rotation $\bar{\kappa}$ in degree	Standard deviation σ_κ in degree
1	19.06	0.60
2	18.90	0.34
3	18.84	0.30
4	18.76	0.33
5	18.64	0.28
6	18.94	0.21
7	18.88	0.21

Table 4 :

Rotation angles and standard deviations [SEM]

The κ angles are computed by using the following equations:

$$\kappa_1 = \tan^{-1}\frac{(x_2 - x_1)}{(y_2 - y_1)} \qquad \kappa_2 = \tan^{-1}\frac{(y_2 - y_3)}{(x_2 - x_3)}$$

$$\kappa_3 = \tan^{-1}\frac{(x_3 - x_4)}{(y_3 - y_4)} \qquad \kappa_4 = \tan^{-1}\frac{(y_1 - y_4)}{(x_1 - x_4)} \qquad \text{Eqs 1}$$

and $\bar{\kappa} = (\kappa_1 + \kappa_2 + \kappa_3 + \kappa_4)/4$

The average rotation angles against corresponding magnifications are illustrated in Figs 2 and 3. One would note in Fig 2 that there is a tendency of change from the normal trend at magnification of around 6 to 9k times. This change is conjectured to be due to change of final condenser lens

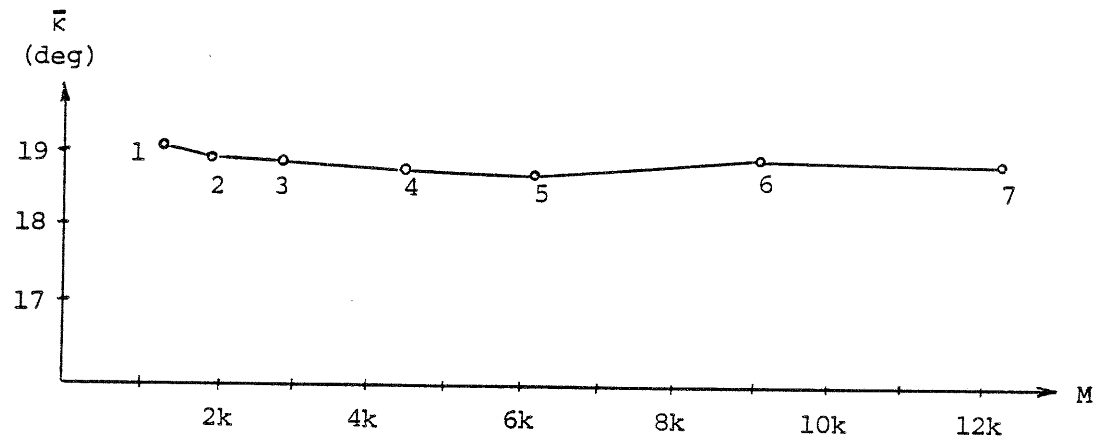
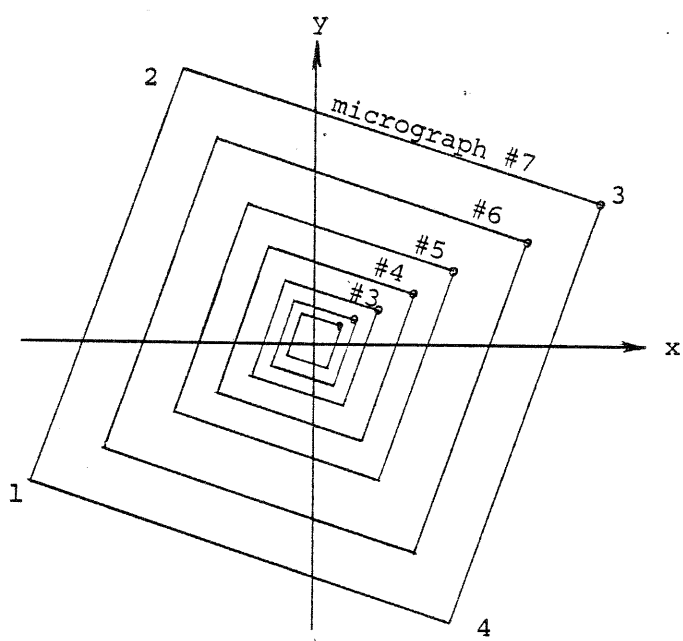


Fig 2 : Average angle of rotation versus magnification at SEM

Fig 3 :
Graphic presentation of the square at various magnifications in SEM



and/or refocusing the image at SEM. Other than this, the correlation between magnification and rotation seems to be systematic. There also seems to be some difference of systematic nature between the vertical angles (κ_1 and κ_3) and the horizontal angles (κ_2 and κ_4). These indicate differential scale variations or 'affinity' between the x and y directions on the micrographs. Further details in this regard are discussed in Ghosh (1982). These variations in the rotation and scale-affinity are, however, not very significant in the SEM systems and may be neglected for most applications.

IMAGE ROTATION IN TEM

Nine micrographs were used in this case. These were obtained with a Philips TEM model 300 by using the same replica grid of 2160 lines per mm and operating at 80 kV. Table 5 gives the details and Fig 4 gives the schematic of the micrograph format in which certain available fixed (but easily identifiable) points were used as fiducial/reference marks on the micrographs.

The same grid intersection points as in the case of SEM (i.e., the same 5x5

Table 5 : Arrangement of TEM micrographs

Micrograph no.	Instrument dial setting	Nominal magnification at instrument
1	7	10 260x
2	8	12 540x
3	9	15 960x
4	10	20 520x
5	11	25 080x
6	12	31 920x
7	13	39 900x
8	14	51 300x
9	15	60 420x

Note: Tilt and Rotation in all cases were kept at zero degree while the nominal projection distance stayed constant.

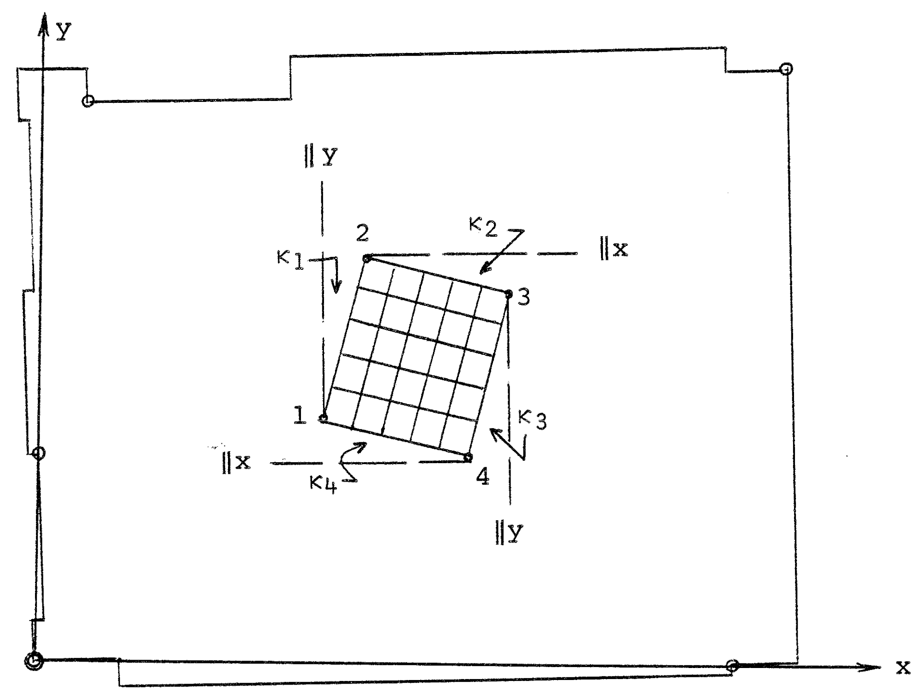


Fig 4 : Micrograph coordinate system and rotation angles at TEM

units square) were used as the basis for comparison. Certain exceptions, however, had to be made for the cases of extreme magnifications, where the grid lines appeared unusually wide and the format was not able to contain the large square. Thus, 3x3, 2x2 and 1x1 unit squares were used at magnification settings 11 & 12, 13 & 14 and 15, respectively. The micrograph coordinates (x and y) were each measured ten times at the same instrument (Zeiss Stereotop converted into an analytical plotter). From the mean values of these observations were calculated their standard deviations (σ_x, σ_y), averages of the standard deviations ($\bar{\sigma}_x$, $\bar{\sigma}_y$) and the resulting positional errors (σ_p), see Table 6.

As in the case of SEM, the distances (d's) of the four sides of grid as well as their averages ($\bar{d}$'s) are calculated. The average distance is then compared with the actual distance to compute the average magnification (M). The standard deviations of the magnifications are computed next (see Table 7).

Micrograph no.	$\bar{\sigma}_x$ µm	$\bar{\sigma}_y$ µm	σ_p µm
1	43	32	54
2	50	56	75
3	45	64	78
4	39	87	95
5*	172	45	178
6*	104	113	154
7*	37	54	65
8*	22	34	40
9*	20	24	31

* These values are with regard to measurements on intersections of lines drawn on micrographs, the imaged lines being unusually thick due to high magnification.

Table 6 :

Standard deviations of the observations [TEM]

Micrograph no.	Ave. distance $\bar{d}$ in µm	Magnification M	σ_M
1	21 663	9 354	23
2	26 949	11 637	32
3	33 861	14 621	34
4	42 346	18 285	41
5*	34 485	24 818	128
6*	43 386	31 223	111
7*	36 236	39 116	70
8*	46 112	49 778	37
9*	28 561	61 663	67

* see note below Table 6.

Table 7 :

Magnifications and standard deviations [TEM]

In order to study image rotations, the four angles (κ's in Fig 4) were calculated by using the same equations (Eqs 1). The results are given in Table 8. Figures 5 and 6 illustrate the relationships between image rotations with regard to the respective magnifications.

One would notice (Figs 5 and 6) a sharp change at magnifications around 18k to 24k. This change is conjectured to be due to change of final condenser lens in the TEM. The smooth portions of the curve would indicate systematic correlation between image rotation and magnification. On comparing the horizontal (κ_2 and κ_4) and vertical (κ_1 and κ_3) angles in Table 8, the affinity effect in scale at the TEM would be apparent.

Having realized that such image rotations exist in the EM systems, one is naturally concerned firstly to determine the magnitude in each case and secondly of eliminating or minimizing the mensural error (if any) caused by such rotations. Preliminary research studies at the Laval University Department of Photogrammetry indicate that such rotations can be visualized as almost similar in pattern as the 'spiral' distortion described in Ghosh and Nagaraja (1976).

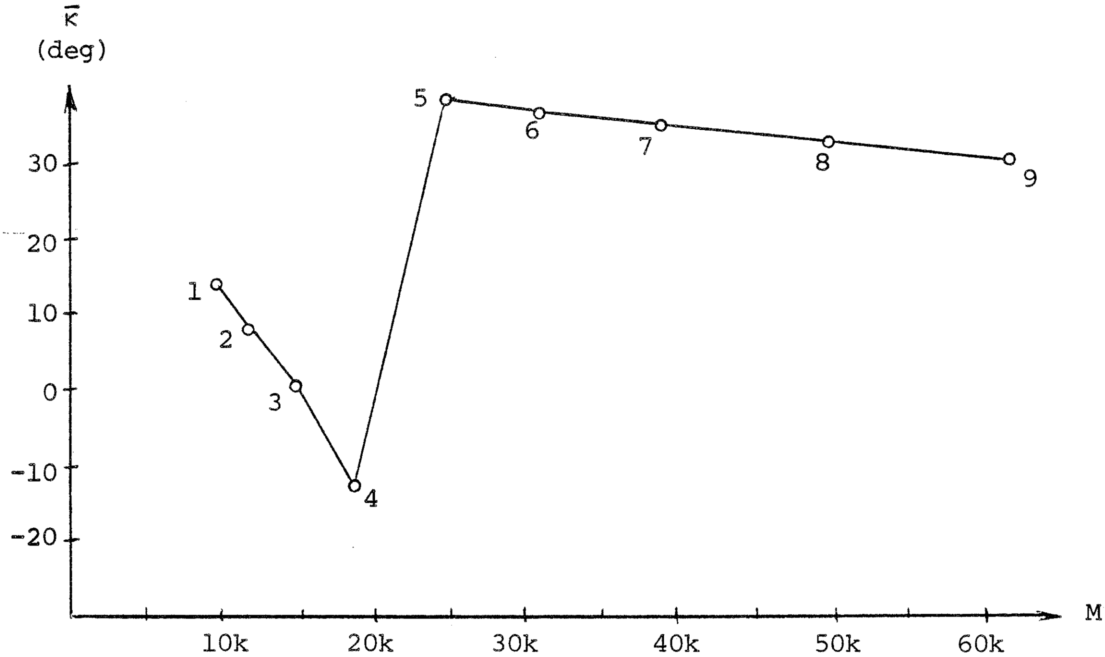


Fig 5 : Average rotation angle versus magnification at TEM

Micrograph no.	κ_i in degrees Angle order:1,2,3,4				$\bar{\kappa}$ deg.	σ_κ deg.
1	12.9	13.8	13.0	13.6	13.3	0.10
2	7.6	8.2	7.9	8.2	8.0	0.11
3	0.3	0.9	0.6	1.0	0.7	0.09
4	-13.1	-13.2	-12.9	-13.1	-13.1	0.09
5*	36.5	37.0	36.5	37.6	36.9	0.18
6*	34.5	35.1	34.6	35.7	35.0	0.14
7*	32.8	34.1	33.5	33.7	33.5	0.07
8*	30.5	31.1	30.9	31.9	31.1	0.04
9*	27.5	28.6	27.0	28.9	28.0	0.04

Table 8 : Computation of rotation angles [TEM]

* see note below Table 6.

The magnitude and specific pattern of such distortions in a particular TEM for a specific magnification can be determined through a calibration process by including appropriate mathematical models in the procedure. A pair of simple yet effective equations describing distortions of the spiral type presented in Ghosh and Nagaraja (1976) are :

$$\left.\begin{aligned}\Delta x &= S_x\,(x^2y + y^3)\\ \Delta y &= S_y\,(x^3 + xy^2)\end{aligned}\right\} \qquad \text{Eqs 2}$$

where Δx and Δy are the x and y components, respectively, of the spiral distortion;

S_x and S_y are the spiral distortion coefficients;

and x and y are the uncorrected photo-coordinates.

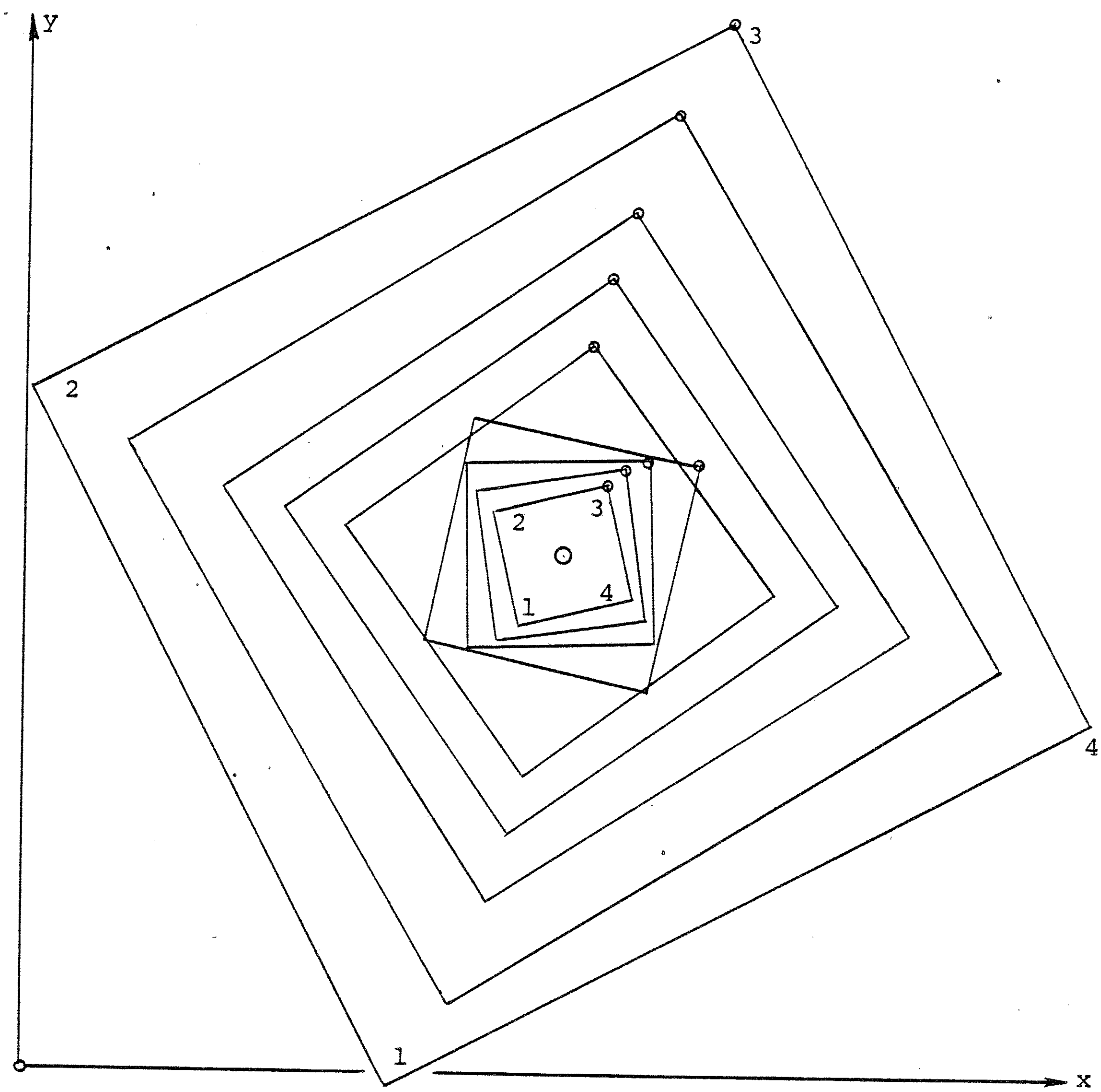


Fig 6 : Graphic presentation of square at various magnifications in TEM

These two equations also would adequately satisfy the requirements caused by the scale affinity effect in any EM system. This has been established beyond doubt through research at the Laval University (see Adiguzel, 1984).

CONCLUSIONS

Image rotation effects at an SEM system, it seems, may be negligible for almost all applications. It may be noted that the maximum variation reported here (Table 4) is hardly one half of a degree. Image rotation effect at a TEM system, however, can not be ignored in all applications. The maximum variation reported here (Table 8) is of the order of 50 degrees and deserves consideration for most microscope systems.

The effect of refocussing in the EM systems in general with regard to change of magnification can be practically nothing. In case, however, a change in the tilt of the micrograph (with respect to the specimen) is also involved, this effect may not be ignored. This is because it would necessitate a change in the projection distance, thereby it would effect scale and other geometric variations. Our studies support the observations of Boyde and Ross (1975) with regard to this aspect.

The maximum and minimum variations between the horizontal and vertical angles at the SEM are 2.3 degrees (at around 12k magnification) and 0.8 degree (at around 3k magnification), respectively. Similar variations at the TEM are, 1.5 degrees (at around 62k magnification) and 0.1 degree (at around 18k magnification). These indicate that the affinity effect is undoubtedly larger at the SEM than at the TEM. Furthermore, for the best stereoscopic measurements in view of scale affinity and other distortion effects, one would recommend a 3k magnification at the SEM and a 18k magnification at the TEM. These would indicate the optimums under the presnt state-of-the-art. This opinion is based on our research studies on various EM systems to which we had access.

RECOMMENDATIONS

The mensural errors in an EM system due to image rotation, focussing and scale affinity may be determined through intensive self-calibration processes. These can subsequently be remedied during the 'orientation' procedure by using an analytical plotter with the support of appropriate soft-ware. In particular, the following are recommended:

- The spiral nature of the electron beams can be mathematically modelled and added to the self-calibration math model.
- The effect of focussing a TEM in setting a specific magnification may change the projection distance which, in turn, would affect the value of the magnification. This can be alleviated by adding the magnification and the projection distance, both, as variable parameters in the self-calibration math model. Alternatively, one can do it somehow operationally, by changing the projection distance (for each focussing) in case the instrument permits this and by using one parameter in the solution program.
- The scale affinity effect can be determined by analysing the rotation angles or by adding magnification parameters (or scale factors) for the x- and y-directions in the self-calibration math model. For high precision work, due consideration of the scale-affinity effect is highly recommended.

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