TWO-DIMENSIONAL TRANSFORMATION OF VERTICAL TRANSMISSION ELECTRON MICROGRAPHS IN MICRO-RANGE PHOTOGRAMMETRY

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ABSTRACT

Vertical electron micrographs at different magnifications taken by a Philips EM 200 transmission electron microscope are investigated. In view of the fact that a large number of users rely on two-dimensional information extracted from highly magnified TEM micrographs, it is essential to recover the nature of transformation between image and object spaces. Calibration of the system using a replica grid with 2160 lines per mm in crossed directions produced by the Ernest E. Fullam, Inc. was performed. Since most electron microscopic users have no photogrammetric interests as a priority and due to their reluctance to apply rigorious procedure, two simple mathematical models are thus considered. Results showed that a six parameter affine transformation reduced the residuals significantly compared with a four parameter conformal transformation at a significance level $\alpha=0.05$. Relations were also established between instrumental tap settings on one hand and differential x and y magnifications, angle of rotation and affinity on the other hand.

INTRODUCTION

In the Transmission Electron Microscope, an electron beam is used for the illumination and is allowed to impinge upon the object to be photographed. The resulting microscopic photograph is known as a micrograph which is usually magnified several thousands of times. Extracting reliable information from these micrographs falls under the sub-discipline of micro-range photogrammetry (Elghazali 1984). The image formed is a measure of the scattering power of each point of the microscopic object. The magnified image is finally displayed on a fluorescent screen. The electron micrograph is obtained by inserting a sensitized film of glass plate in place of this screen. Due to their enormous magnifications, these micrographs have indeed opened great opportunities to a vast number of scientific and engineering disciplines. Three dimensional coordinates based on stereo micrographs offer complete information about the object (Boyde 1970, Ghosh 1971 and Ghosh et. al. 1978). However, information extracted from two dimensional coordinates using one micrograph is generally satisfactory for many users. This statement is substantiated by the fact that a fundamental requirement for producing TEM micrographs is that the microscopic object should be an ultra thin section, thus minimizing the significance of the third dimension.

The objective of this paper is to check and compare the adequacy of conformal and affine transformation equations in two-dimensions in describing the relation between object and image spaces of vertical micrographs. This in turn lead to studying the effect of magnification on image behaviour of TEM micrographs.

EXPERIMENTAL SET-UP

The experimental set-up is schematically represented in figure (1), where the data acquisition phase will be discussed under this heading, while data processing and results will be given next.

Object

The magnification can be calibrated by taking micrographs for an object of known dimensions. Earliest effective calibrations were performed by using polystyrene latex spheres of very uniform size (Ghosh 1971). However, these latex spheres showed some dimensional instability and thus should not be relied on to give absolute accuracies of high standards. In this research the object used is a replica grid, mounted on a 200 mesh copper grid, made from a master diffraction grating. According to the manufacturer's specifications, the grid has a number of 2160 lines per mm in crossed directions inscribed with an accuracy of 3%.

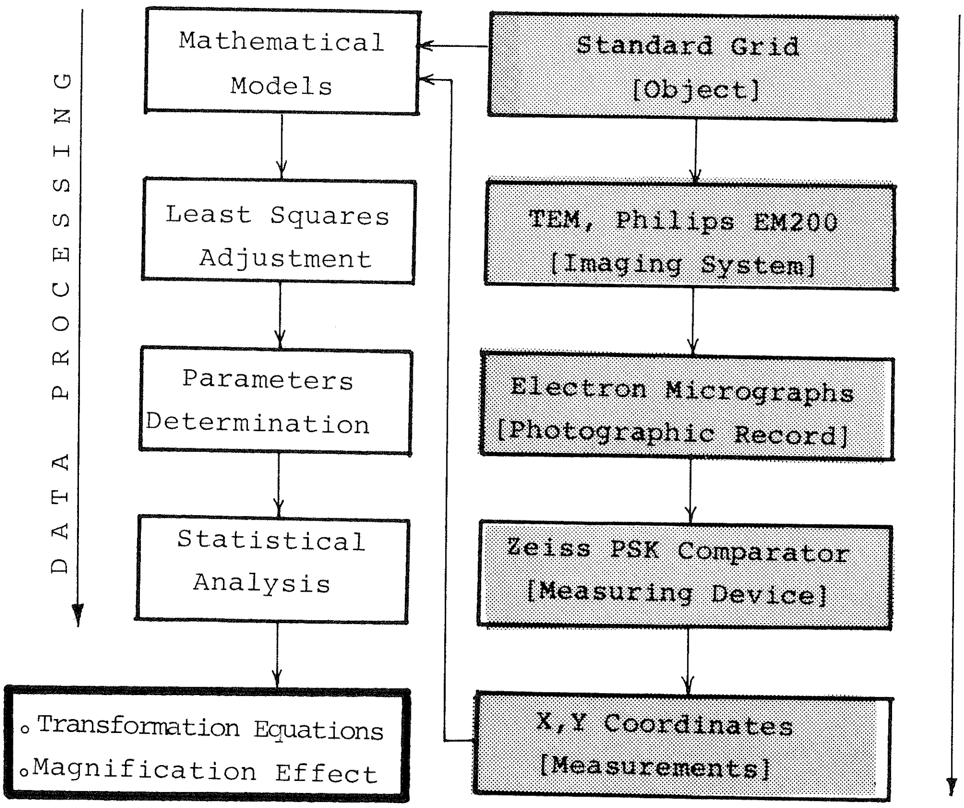

Figure (1) Schematic Diagram of Data Acquisition and Data Processing Phases

Imaging System

The transmission electron microscope used for producing the micrographs is the Philips EM 200 model. Its magnification varies in steps from approximately 500 to 220,000 times. The specimen stage on which the grid is mounted has a total tilt angle of 60°, a rotation around the vertical axis from 0° to 360° and two X & Y shifts of 2.4 mm each. A plate camera is fitted just below the TEM projection chamber. Fiducial marks were introduced in the TEM to control coordinate measurements from different micrographs.

Photographic Record

In view of studying the effect of magnification on image behaviour and recovering the nature of the transformation between object and image spaces, five vertical micrographs at different magnifications were taken under same conditions. Table (1) gives a summary of the geometrical configuration of these micrographs, their magnifications and the corresponding tap settings on the transmission electron microscope.

Table (1) Configuration of TEM Micrographs Used in the Study

Micrograph #	Tap Setting	Magnification	Tilt	Rotation
1	16	18,000	0°	0°
2	18	33,000	0°	0°
3	20	57,000	0°	0°
4	21	75,000	0°	0°
5	22	100,000	0°	0°

Measuring Device

The Zeiss PSK stereocomparator is used in a monocomparator mode to read the x & y coordinates of the grid points. The left stage of the comparator is only used in this case.

Measurements

The x & y coordinates of the grid intersections were observed ten times for each magnification. This increases the reliability of the observations by determining their standard deviations, and helps in locating and eliminating any gross error. All measurements were performed by the same operator. Table (2) gives a sample of the mean values of observed coordinates at four grid intersections forming one cell as well as their standard deviations.

Table (2) Mean Values of Coordinate Measurements and Standard Deviations at Four Grid Points

Magnification	Point	X(μm)	y(μm)	σ_x(μm)	σ_y(μm)
18,000	A	296981.8	216734.5	4.96	5.15
	B	304618.7	215954.4	4.99	4.40
	C	296160.9	208886.3	4.61	6.28
	D	303864.8	208133.5	6.12	6.26
33,000	A	294231.5	224908.0	19.14	8.69
	B	307922.0	222250.6	7.73	14.95
	C	291950.6	210910.7	5.38	8.77
	D	305623.1	208443.8	12.56	11.52
57,000	A	290788.3	233580.8	14.91	22.60
	B	313205.5	224398.2	31.00	25.71
	C	281928.4	210896.0	7.62	22.67
	D	304278.4	201415.0	19.29	16.75
75,000	A	299397.7	240413.2	42.75	35.81
	B	321995.9	219384.2	17.32	34.54
	C	277749.8	216844.6	33.82	30.67
	D	301219.2	195289.4	30.40	29.74
100,000	A	305162.0	251089.7	50.78	36.91
	B	329784.4	217509.0	31.51	46.07
	C	271514.1	225771.0	57.82	44.18
	D	296004.1	191726.9	52.44	48.31

MATHEMATICAL MODELS

It has been established from previous work that in electron microscopy, the relation between object and image spaces is based on parallel projection (Maune 1973 and Nagaraja 1974). Visual inspection of the micrographs revealed a clear relation between magnification (λ) and image rotation (θ). Considering the case of vertical micrographs as employed by the majority of users, two most commonly used 2-D transformations are employed to check their applicability to TEM micrographs. The first mathematical model is the 2-D conformal transformation expressed by equation (1).

$$\left.\begin{aligned} x_i &= a\, X_i + b\, Y_i + x_o \\ y_i &= -b\, X_i + a\, Y_i + y_o \end{aligned}\right\} \qquad \ldots\ldots\ldots\ldots\ldots (1)$$

where

(x_i, y_i) are the observed micrograph coordinates

(X_i, Y_i) are the corresponding object coordinates

(x_o, y_o) are the two shifts in x & y directions

(a, b) are transformation parameters whose values are determined according to equations (2) & (3) (Moffit and Mikhail 1980).

$$a = \lambda \cdot \cos\theta \qquad \ldots\ldots\ldots\ldots\ldots (2)$$

$$b = \lambda \cdot \sin\theta \qquad \ldots\ldots\ldots\ldots\ldots (3)$$

In view of checking the homogenity of the magnification (λ) in x and y directions and the orthogonality of the transformed coordinates, a two-dimensional affine transformation is also used to check its adequacy with TEM micrographic images (Equation 4).

$$\left.\begin{aligned} x_i &= a_1\, X_i + b_1\, Y_i + x_o \\ y_i &= a_2\, X_i + b_2\, Y_i + y_o \end{aligned}\right\} \qquad \ldots\ldots\ldots\ldots\ldots (4)$$

where

(a_1, b_1, a_2, b_2) are transformation parameters whose values are determined according to equations (5), (6), (7) & (8) (Moffit and Mikhail 1980).

$$a_1 = \lambda_x (\cos\theta - \delta \sin\theta) \qquad \ldots\ldots\ldots\ldots\ldots (5)$$

$$b_1 = -\lambda_y (\sin\theta) \qquad \ldots\ldots\ldots\ldots\ldots (6)$$

$$a_2 = \lambda_x (\sin\theta + \delta \cos\theta) \qquad \ldots\ldots\ldots\ldots\ldots (7)$$

$$b_2 = \lambda_y (\cos\theta) \qquad \ldots\ldots\ldots\ldots\ldots (8)$$

where

λ_x and λ_y are the magnifications in x and y directions.

δ is the affinity angle.

RESULTS AND ANALYSIS

To test the adequacy of the equations based on two-dimensional conformal and affine transformations, observations using micrograph #1 at 18,000 magnification were used. It was assumed that all observations are of equal weights and that no correlation exists between them. Furthermore, the variance of unit weight (σ_o^2), being a scalar was assumed to be unity. This has no effect, since the solution is invariant with respect to (σ_o^2). Table (3) gives a summary of the numerical values of least squares estimates of the parameters from the two-dimensional conformal and affine transformations. However, table (3) does not give the magnitude of the actual physical parameters of the transformations in terms of magnifications and rotations. Therefore, based on equations 2, 3, 5, 6, 7 & 8, these physical parameters are determined and given in table (4).

Table (3) Least Squares Estimates of Transformation Parameters Using Micrograph #1, With 18,000 Magnification

Two-Dimensional Transformation			
Conformal (4-parameter)		Affine (6-parameter)	
Parameter	Least Squares estimates	Parameter	Least Squares estimates
a	16742.53	a_1	16569.43
b	1677.92	b_1	1698.55
a_o (μm)	296142.22	a_2	-1658.13
b_o (μm)	208939.72	b_2	16924.51
		a_o (μm)	296177.51
		b_o (μm)	208893.01

Table (4) Least Squares Estimates of Physical Parameters Using Micrograph #1, With 18,000 Magnification

Two-Dimensional Transformation			
Conformal (4-parameter)		Affine (6-parameter)	
Parameter	Estimates	Parameter	Estimates
Magnification (λ)	16826.86	Magnification (λ_x)	16659.10
Rotation (θ)	5° 43.2'	Magnification (λ_y)	17004.83
x-shift (μm)	296142.22	Rotation (θ)	5° 35.8'
y-shift (μm)	208939.72	Affinity (δ)	13' 40.5"
		x-shift (μm)	296177.51
		y-shift (μm)	208893.01

In order to check the correlation between the different parameters, the correlation coefficients were computed based on the variance covariance matrix of the unknown parameters (Σ_x). The correlation coefficient matrices of the transformation parameters resulting from conformal and affine transformation showed favourable correlation between different parameters. This shows that the two additional parameters of the affine transformation did not change the correlation pattern significantly. Strong correlation between two parameters suggests the possibility of eliminating one of them without affecting the solution. Having solved for the unknown parameters, the residuals were computed. Numerical values of the residuals at the four grid points of table (2) are given as an example demonstrating a significant 70 percent reduction in their magnitude when using a two-dimensional affine transformation (Table 5).

Table (5) Observational Residuals Resulting from Conformal and Affine Transformations (Units in μm)

Point	Residuals	Conformal Transformation	Affine Transformation
A	V_x	-63.0	-17.9
A	V_y	-43.0	- 5.4
B	V_x	52.1	16.9
B	V_y	-39.5	6.9
C	V_x	-18.6	16.6
C	V_y	53.1	6.7
D	V_x	29.5	-15.6
D	V_y	29.4	- 8.2

Figure (2) shows these residuals at the four grid points resulting from the two transformation equations.

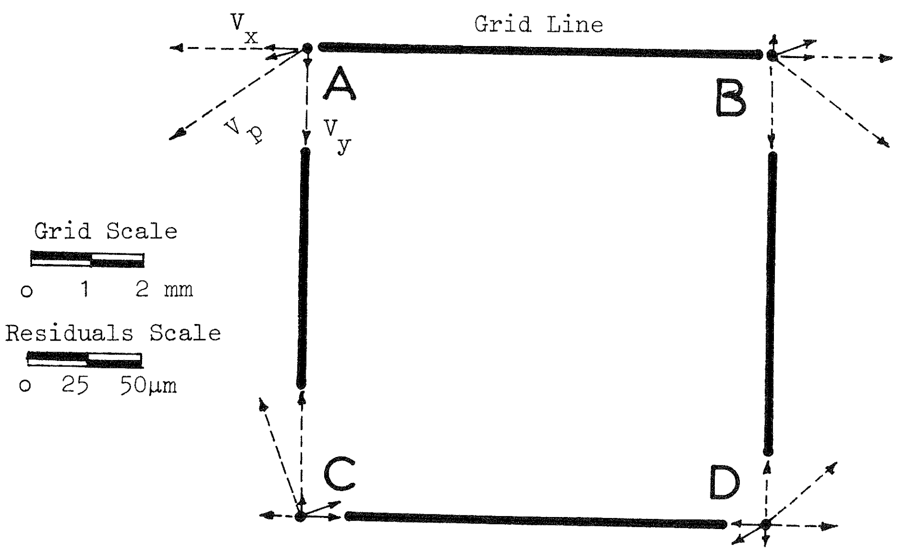

Figure (2) Observational Residuals at the Four Grid Points
---→ Using 2-D Conformal Transformation
——→ Using 2-D Affine Transformation

Usually, adding more terms to the mathematical model will improve the agreement with the observations. However, we must weigh the utility of the transformation equations against the increased accuracy obtained with a larger number of terms by proper statistical testing of the added terms at a certain significance level (α). Accordingly, we can test the hypothesis.

H_o : Effect of differential magnification and affinity = 0,

by computing the variance ratio (Eqn. 9),

$$\text{Variance Ratio} = (DF)\frac{(V^TPV)_{conformal} - (V^TPV)_{affine}}{(V^TPV)_{affine}}; \qquad (9)$$

and comparing it with the student F distribution value, $F_{1,DF,\alpha}$, where DF is the degrees of freedom of the system. If the variance ratio is insignificant, we may conclude that the effect of differential magnification and affinity is also insignificant (Hamilton 1964). Accordingly, substituting for the terms of equation (9), we have,

Variance Ratio = 23.757

Meanwhile, using statistical F tables at significance level α=0.05, we have,

$$F_{1,DF,0.05}=7.709$$

Since Variance Ratio > $F_{1,DF,0.05}$, therefore we reject the H_o hypothesis. This simply means that the two added parameters of the affine transformation proved to be significant at significance level α=0.05.

Referring to table (4), it is noticeable that the magnification estimates using the two mathematical models are less than the given magnification by approximately 6.5%. Also, the rotation angle is considerably large for no obvious reason despite the fact that the two coordinate systems were almost parallel to each other. Accordingly, with a view of studying the effect of magnification on image behaviour, coordinates of grid intersections on micrographs #2, 3, 4 & 5 (table 1) were transformed using affine transformation equations (equation 4). Figure (3) represents the relation between the instrumental tap settings and the given and calibrated magnifications. The calibrated magnifications were consistently less than the given instrumental magnification. The reduction in magnification ranged from 6.5% at the 18,000 magnified micrograph to a maximum value of 9.2% at the 100,000 magnified micrograph. It is also noted that the magnification in y direction is consistently higher than the magnification in x direction by approximately 2%. Similar results were obtained from measurements of the grid intersections after rotating the grid through a 90° angle of rotation thus reversing x & y directions. This was necessary to check whether or not the grid itself was the reason for these differential magnifications. Figure (4) represents the relation between the instrumental tap settings, the angle of rotation (θ), and the affinity (δ). By increasing the tap setting, or in other words the magnification, both the angles (θ) and (δ) were increased. The increase in the rotation angle was non-linear while the affinity increased linearly with respect to the magnification. The large increase in the rotation angle can be only attributed to the fact that the increase in magnification causes the rotation of the electron beam emmitted from the electron gun of the microscope and accordingly the rotation of the resulting image. The effect of the rotation angle is important if only absolute positions have to be determined. Otherwise for relative positions its effect is minimal.

CONCLUSION

Affine transformation proved to be more suitable than conformal transformation in relating object to image spaces of TEM vertical micrographs. The calibrated magnifications were consistently less than the given magnification, and more over the horizontal magnification was less than the vertical magnification. The affinity effect is another parameter that affects the geometry of the resulting image and consequently shapes of depicted objects. Moreover, the rotation angle increased rapidly due to the increase in magnification only caused by rotation of electron beam. Statistical testing at significance level of α=0.05 showed that the additional two parameters of the affine transformation as compared to the four parameters conformal transformation are significant. Therefore, in view of the above results it is essential to calibrate the TEM imaging system against a higher standard grid under actual operating conditions.

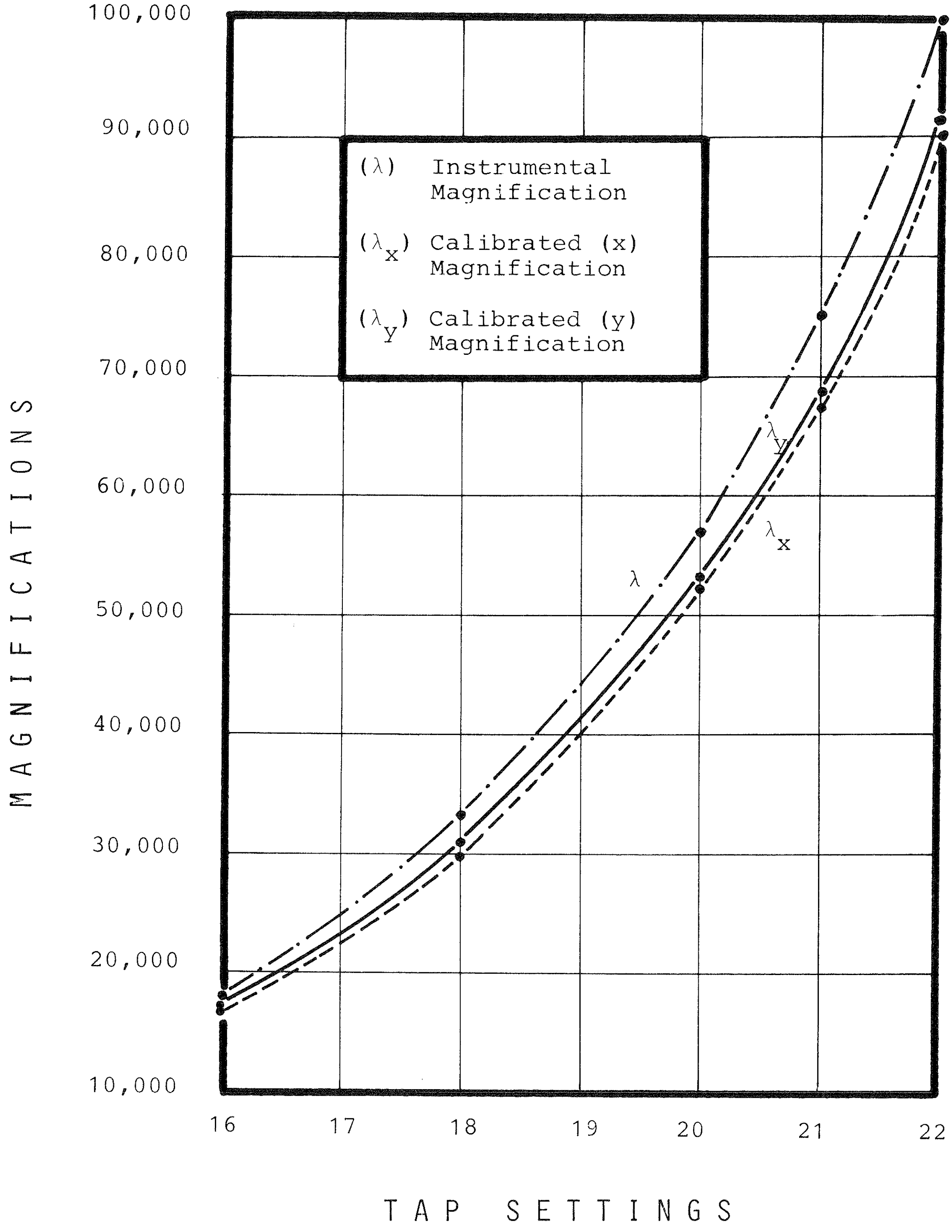

Figure (3) Relation Between Instrumental Tap Settings and Magnifications Resulting from Affine Transformation

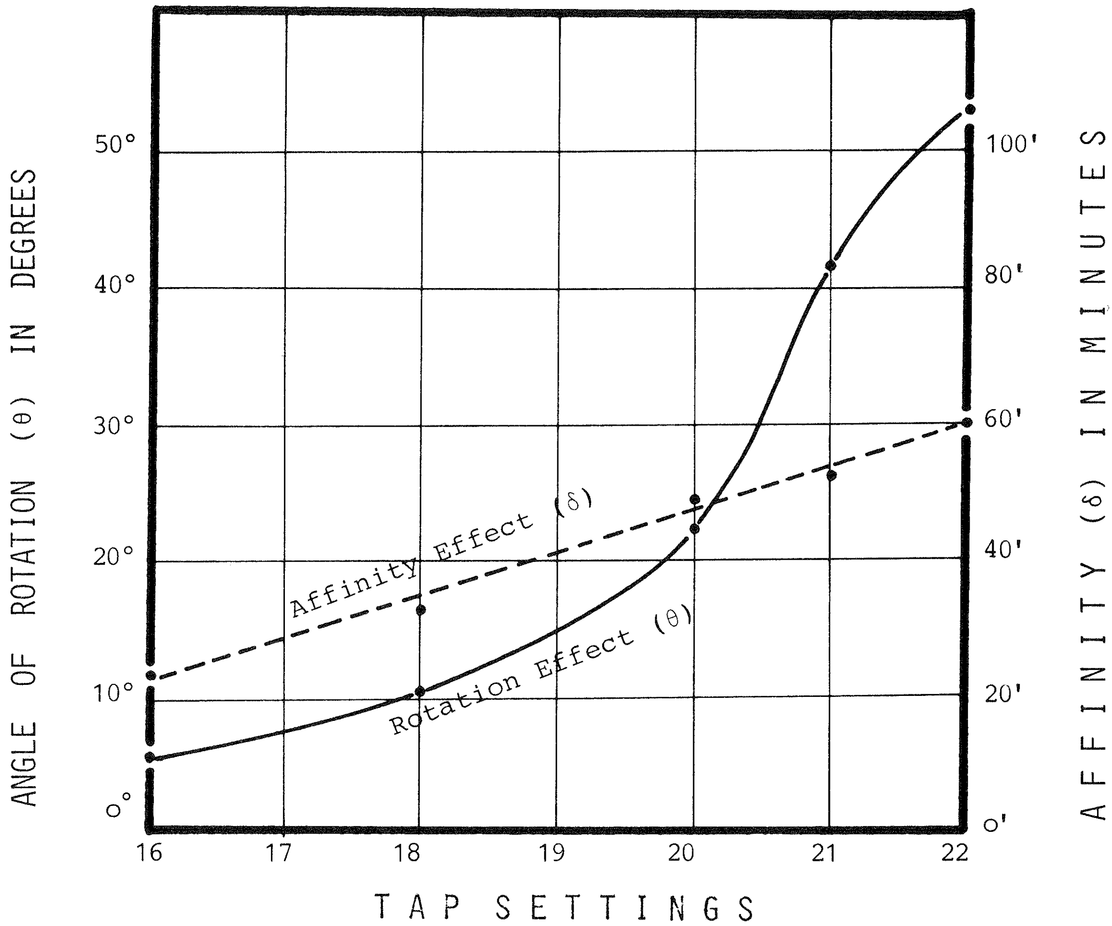

Figure (4) Relation Between Instrumental Tap Settings, Angle of Rotation and Affinity, Resulting from Affine Transformation.

The results of such calibration procedure can be used to transform TEM image coordinates to recover the actual planimetric coordinates of the imaged object. This would certainly enhance the reliability of planimetric information, shapes, areal parameters and associated characteristics extracted from single TEM vertical micrographs. The gain in accuracy against the simplicity of the mathematical model and accordingly the computational effort makes it attractive from the users point of view.

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