P. Waldhäusl
Technische Universität Wien
A-1040 Wien, Austria

An Approximate Solution for the Restitution of Stereo Electron Micrographs

The mathematical basis and the procedures for restituting stereo electron micrographs in photogrammetric analog plotters such as the Jena Topocart are described.

Electron microscopes are standard instrumentation of natural research centers. The demand for metrical restitution of electron micrographs is increasing. From the geometry of the scanning electron microscope (SEM) as well as of the transmission electron microscope (TEM), we know that the angle between the corner ray and the central ray of the normally 30 by 30 mm photograph is very small, e.g., for an 8 000 times enlargement it is only 1.6 centigrades. The micrograph can be regarded, therefore, as an enlarged parallel projection of the object. The greater the enlargement, the smaller are the deviations from the parallel projection. So called "goniometers" enable us to produce convergent stereopairs. From the difference of the goniometer readings, the angle, α, of convergence of the two parallel projections can be determined. The three-dimensional restitution of electron micrographs by means of photogrammetric analog stereorestitution instruments is described in this paper. For more rigorous solutions special instruments are required, but

Abstract: *For approximate restitution of stereo electron micrographs their geometry can simply be regarded as an enlarged parallel projection of the object. The formulae for this parallel perspective are derived and are compared with the pantometer formulae for aerial photogrammetry. Those plotters can be used, in which different principle distances can be introduced for the two image coordinate directions and where a pantograph can be connected easily with the left photo carrier, e.g., the Jena Topocart. Plotters with reflective illumination have a further advantage. A sample is given with complete technical data, which shows in stereo the apex of a supporting cell of mouse olfactory epithelium. Plotting has been done with 25 nm contours at a scale of 200 000:1.*

for simple metrical stereo interpretations and relatively exact restitutions of *small* sectors of the micrographs, some of the available analog plotters are usable.

In Figure 1, the subscripts o and α identify the two orientations of the object coordinate system (x, y, z). Let us accept the left photograph to be the reference picture with the photo-coordinate system (O', ξ', η') orthogonal to the direction of the parallel projection rays and parallel to the reference object coordinate system (O, x, y, z; in zero-position, Figure 1). After rotating the object through the angle α we get the second image (O'', ξ'', η'') which in fact has the identical position of the first one. The object space is shown at the scale of the micrographs. Micrograph coordinates, in terms of object coordinates, are, then

$$
\begin{aligned}
\xi' &= x \\
\eta' &= y \\
\xi'' &= x \cos \alpha - z \sin \alpha \\
\eta'' &= y
\end{aligned}
\qquad (1)
$$

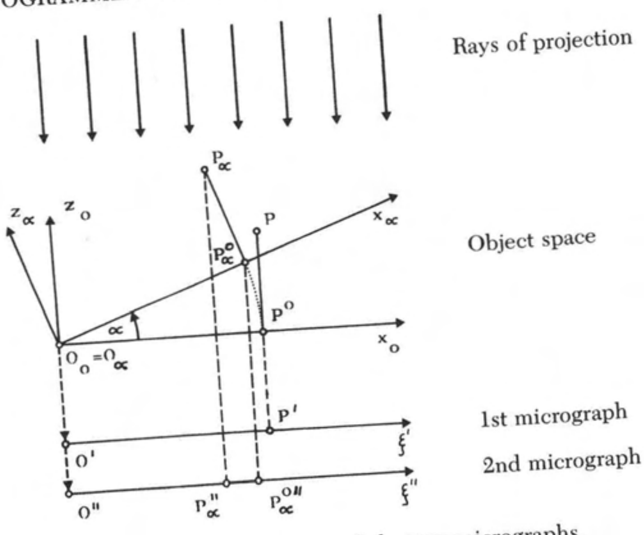

FIG. 1. The geometry of electron micrographs.

For the determination of the object coordinates (O, x, y, z) from the micrograph coordinates, Equation 1 is inverted; that is,

$$x = \xi' \tag{2a}$$
$$y = \eta' = \eta'' \tag{2b}$$
$$z = \cot \alpha(\xi' - \frac{\xi''}{\cos \alpha}) \tag{2c}$$

In aerial photogrammetry the central projection is employed (Figure 2) and small height differences, Δz, in object space are determined by the approximation Equation 3; that is,

$$x = \frac{(h_g - z)}{c}\xi' = s \cdot \xi' \tag{3a}$$

$$y = \frac{(h_g - z)}{c}\eta' = s \cdot \eta' \tag{3b}$$

$$p = (\xi' - \xi'') \cdot s$$
$$p_o = (\xi'_o - \xi''_o) \cdot s \tag{3c}$$
$$z = h_g - \frac{b \cdot c}{p} \qquad \Delta z = z - z_o$$
$$z_o = h_g - \frac{b \cdot c}{p_o} \qquad \Delta p = p - p_o$$
$$\Delta z = \frac{b \cdot c}{pp_o}(p - p_o) = \frac{b \cdot c \cdot \Delta p}{p_o^2\,(1 + \frac{\Delta p}{p_o})} = \frac{b \cdot c}{p_o^2}\Delta p(1 - \frac{\Delta p}{p_o})$$

$$\Delta z_o = \frac{b \cdot c}{p_o^2}\Delta p = k \cdot \Delta p \text{ as the first approximation,}$$

$$k = \text{a constant} = \frac{h_g^2}{b \cdot c}$$

$$\Delta z = \Delta z_o\,(1 - \frac{\Delta z_o}{h_g}) \text{ as the second approximation.}$$

where
s is the scale factor,
p is known as the stereoscopic or x-parallax in model space,

Δp is the parallax difference, and

h_g is the flight altitude above the reference plane, which corresponds to the parallax p_o.

We shall try now to use the aerial photogrammetric analogue instruments for the restitution of stereo micrographs.

At first c', the principal distance of the left hand camera, and the projection distance h_g are set to preselected values so as to result in a round model scale. A value for the base, b, is then introduced such that

$$\frac{h_g}{b} = \cot \alpha$$

$$b = h_g \tan \alpha \tag{4}$$

Further, we introduce at the right hand camera a shorter principle distance, *i.e.*,

$$c'' = c' \cos \alpha \tag{5a}$$

The photogrammetric plotter then interprets any ξ'' to be longer, as required by Equation 2c, i.e.,

$$\bar{\xi''} = \frac{\xi''}{\cos \alpha} \tag{5b}$$

At the same time the η'' coordinates also get enlarged. This causes vertical parallaxes, which have to be eliminated by hand for all points. This can be avoided by using only those photogrammetric plotters in which different principle distances can be introduced for the ξ and η directions, e.g., the Jena Topocart or the Kern PG 2 or PG 3.

There we only change c_x''

$$c_x'' = c_x' \cos \alpha$$

$$c_x' = c_y' = c_y'' \tag{6}$$

The η''-direction will not be influenced by the shorter c_x''; therefore, no η-parallaxes will occur. The rotation, κ'', is corrected so as to guarantee that a second point with the same η as the first reference point is free of η-parallax.

Finally, the micrographs are placed into the photo carriers so that the two pictures of one selected reference point correspond at the left as well at the right hand side with the respective measuring marks. (The camera rotations ϕ and ω are set to zero.)

If the Jena Topocart is used, positive paper prints can be restituted when the reflected illumination is switched on. Where only transmission illumination is available, diapositive micrographs are required.

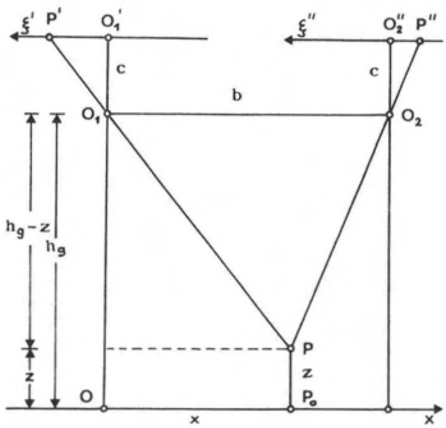

FIG. 2. The geometry of normal case photogrammetry.

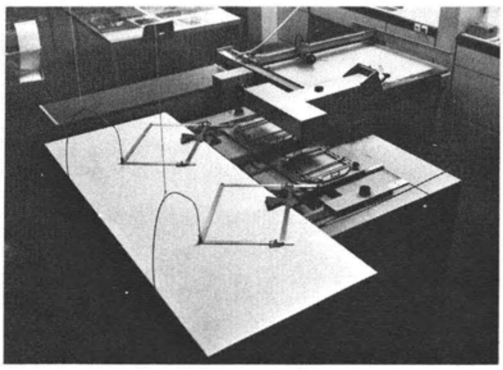

FIG. 3. The Jena Topocart B with auxiliary plotting table adjusted to the height of the photo carriers. For drawing, an OTT pantograph has been connected to the left photo carrier. The second pantograph is used for stereo photo montages (reversed photogrammetry), steared from the project on the main drawing table.

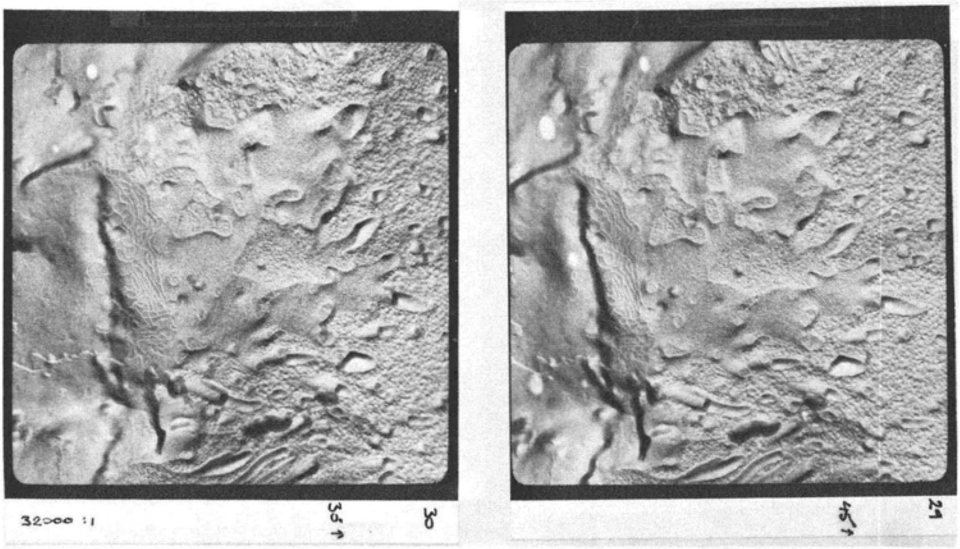

FIG. 4. TEM stereo-micrographs, with α 15°, of a freeze etch replica showing the apex of a supporting cell of mouse olfactory epithelium.

Heights are now easily determined just by reading off the model-z (which are the Δz_o) and correcting those readings due to the second approximation formula (Equation 3c) if required; i.e., the factor $(1 - (\Delta z_o/h_g))$ has to be applied as soon as the percentage $(\Delta z_o/h_g)$ becomes significant for the application at hand.

The planimetry must be plotted from the left photograph, enlarged only by a preselected scale factor, according to Equations 2a and 2b. The author uses an OTT-pantograph connected with the left photo carrier of the Jena Topocart B as shown in Figure 3.

Figure 4 shows an example of TEM stereo micrographs. The micrographs were taken with the Philips EM 301 G of the Institute of food technology of the Vienna University of Agriculture and the specimen was prepared with a Leybold EPA 100 (freeze etch device) by H. Hörandner, Institute of Micromorphology, Vienna University.

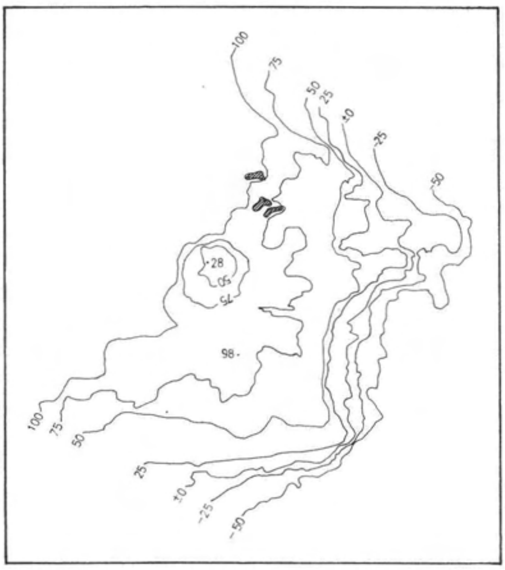

FIG. 5. Reduced scale (approx. 125 000:1) reproduction of the 25 nm contour plot of the left photo in Figure 4.

The TEM stereopair (Figure 4) has been restituted by employing the Jena Topocart B of the Institute for Photogrammetry, Technical University Vienna. A sample contour plot from that stereopair is shown at Figure 5. The technical data are listed in Table 1.

TABLE 1

Angle of convergence α	= 15.00°
$c_{\xi'} = c_{\eta'} = c_{\eta''}$	= 100.00 mm
$c_{\xi''} = c_{\xi'} \cdot \cos\alpha$	= 96.59 mm
Micrograph scale, M_{TEM}	= 32 000 : 1
Model scale, M_M	= 100 000 : 1
$h_g = c_{\xi'} \cdot \frac{M_{TEM}}{M_M}$	= 312.50 mm
$b = h_g \tan\alpha$	= 83.73 mm
Plotting scale	= 100 000 : 1

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