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Working Group V/3: Application to Non-conventional Image Acquisition Systems.

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THE APPLICATION OF PHOTOGRAMMETRY TO QUANTITATIVE MICROSCOPY

Die Anwendung der Bildmessung in der quantitativen Microscopie

L'application de la photogrammétrie dans la microscopie quantitative

ABSTRACT The design of commercially available, camera-optical microscope, non-metric image acquisition systems (primary data acquisition system), empirical calibration procedures based on length measurement, end and line standards and grids, and stereophotomicrographic techniques for the production of stereopairs of small objects, small object features or phenomena in the microscope range are discussed. The evaluation, interpretation and plotting of formlines and sections of these non-topographic stereopairs on conventional analogue photogrammetric instrumentation are described on some examples taken from materials science measurement and testing problems. The possibility of image acquisition, overlap of scales and calibration methods from the close-range field to the optical microscope and from the optical microscope to electron microscopy are mentioned. Photogrammetric output of data can be presented in graphical (analogue) or numerical (digital) forms. The methods described are applicable in the fields of physical as well as the life sciences and in their respective technologies. Links between photogrammetry, image analysis and stereology are mentioned.

1. INTRODUCTION, OBJECTIVE AND SCOPE

Studies of spatial geometry of surfaces of small objects are of interest in many contexts. From the authors' viewpoint, the objects of immediate interest are: Diamond tipped indenters (used in indentation hardness measurement and testing of metals) and microscopic asperities of facets of aggregates. Aggregate facets come into contact with the rubber tyre tread of road vehicles. The surface texture characteristics of aggregates form a significant part of current research on vehicle skid resistance of road pavements. This research is greatly assisted by an ability to portray the relevant spatial geometry of aggregates in magnitudes of the order of millimetres and micrometres.

The research objectives were to demonstrate that commercially available camera-microscope systems could be used to acquire stereograms which, when restituted in conventional analogue photogrammetric instruments, would be capable of portraying millimetre and micrometre magnitudes with some confidence. These objectives have now been realised. Only more recent research in photogrammetry as applied to quantitative microscopy is within the scope of this paper while (1, 2 and 3) report on the authors' collaboration initiated in 1976.

1.1 Quantitative Microscopy

All techniques of counting or measuring (length, area, volume, angle and duration) on microscope specimens are designated as quantitative microscopy in one, two or three dimensions. Some of the more recent techniques come under the designation of stereology and image analysis (automatic or semi-automatic). Some three-dimensional techniques are designated as multiple beam interferometry, light slit or profile microscopy and optical shadow casting. Photogrammetry can be applied to any of the techniques mentioned. However, photogrammetry not only complements the above techniques, it also enlarges the number of methods of study of quantitative parameters in microscopy in general (3, 4, 5, 6, 7 and 8).

1.2 Non-Topographic Photogrammetry - Some definitions

In this paper, microstereophotogrammetry will refer to those techniques which deal with mapping and measurement of objects where the photographic image is larger than the object. Where the image is object size or smaller, but where camera focussing is not set at infinity, the definition "close-range photogrammetry" will be used. Electron microscope photogrammetry (sometimes designated nanophotogrammetry) and close-range photogrammetry, are outside the scope of this paper.

2. RELATIVE AND ABSOLUTE SPATIAL MEASUREMENTS BY PHOTOGRAMMETRY

In topographic applications of photogrammetry, a valid aim is to portray the geometry of a large object - the surface of the earth - and to relate that portrayal to geodetic co-ordinate systems. Photogrammetrists and geodesists tend to think in terms of standard deviations of portrayed magnitudes related to these "absolute" geodetic co-ordinate systems.

Photogrammetrists were and are aware that photogrammetric methods of measurement are much stronger in recording and portraying "relative" quantities than they are in portraying "absolute" quantities. In topographic applications, the difference between relative and absolute measurement derives from "ground control". However, for some engineering applications, the measurements which are required are relative rather than absolute.

2.1 Problem Statement in Microstereophotogrammetry

When we come to portable small objects, our geometric studies need only to be portrayed in terms of relative quantities. The necessary scale of portrayal will vary with the purpose of the study. Objects which it is desired to portray may have overall dimensions of centimetres, millimetres, or fractions of millimetres. This paper deals with objects where overall dimensions of fractions of millimetres seem most pertinent. Camera-microscope systems (with inherent limitations in several matters of detail) were used to produce stereograms which were observed in conventional analogue photogrammetric restitution instruments. At a later stage, the greater versatility available in the analytical restitution instrument may be utilised.

Amongst the issues which needed clarification and eventual amendment, was the tendency of topographic photogrammetrists to think principally in terms of assessing the geometric characteristics of any series of photogrammetric measurements on the basis of an analysis of a series covering the major part of the format of the relevant imagery.

Camera-microscope options, used for image acquisition in this research were limited to -

(a) Parallel axes; vertical case; using a stereomicroscope.

(b) Non-parallel axes; slight convergence case; also using stereomicroscope.

(c) Convergent axis case on monocular microscope.

Past and current research received some instrumentation and other support from camera-microscope manufacturers' local representatives and from the Commonwealth Scientific and Industrial Research Organisation (see Acknowledgements).

The latest research and development produced results which are more convincing than any which had been produced previously.

2.2 Image Acquisition for Microstereophotogrammetry

The image acquisition procedure can be considered as nine interdependent operations:

These are -

1. Determination of size of object; or of object features or of phenomena in planimetry;

2. Determination of the extent of vertical displacement of items in (1);

3. Determination of the necessary magnification (objectives and oculars) in planimetry and vertical dimension on microscope only or, with camera only or, with the camera-microscope image acquisition instrumentation;

4. An assumption or determination of the allowable uncertainty in measurement of standard reference materials (SRM) or calibration artifacts with relation to the object to be measured and the measurands; (6).

5. Selection of the appropriate SRMs for planimetry e.g. grid, haemacytometer, etc.

6. Selection of appropriate SRM for the extent of vertical displacement, e.g. gauge blocks, precision steel balls or rollers, etc.;

7. Experimental work to ascertain the depth of field needed for vertical resolution using items in (6) in observation, measurement and mono or stereo photography of objects.

8. Empirical and analytical determination of parameters producing parallax for the restitution of the stereo model in the photogrammetric instrumentation the Wild Autograph A10 and the Carl Zeiss (Jena) Stereometrograph). These photogrammetric parameters are either the lateral shift for the vertical or parallel axes case; or the convergence of the camera axes for the convergent axes case; or a combination of convergence and lateral shift of image acquisition instrumentation with reference to the object; or of the object with reference to the instrumentation or, of a combination of both.

9. Ascertaining relevant data for image recording or display such as film or plate characteristics, e.g. resolution, exposure, light sensitivity, flatness, processing, stability and determination of quality of results.

In this research and development, the parallel axes and the convergent axes cases were investigated.

Standard reference materials (SRM) refer to those calibration artifacts where the critical length dimensions and other metrological properties are known to be of a higher accuracy, or of less measuring uncertainty, than is required for photogrammetric mapping and measurement. They also serve as SRM in the substitution method of measurement. (see Section 3.).

The instrumentation used and the techniques described are the results of attempts to provide mapping and measuring facilities in the micro region. With the exception of the Layton tilting stage, all equipment used was commercially available, without modification. Thus, these results may be duplicated by others.

Many workers in the close-range field have used, with notable success, a comparator approach, measuring plate co-ordinates of discrete points. As the requirements of this research included continuous plotting, a comparator approach was not possible.

2.2.1 The Image Acquisition System - parallel axes case

This image acquisition system consisted of an adjustable base with trans-illumination facility, an XY co-ordinate stage, the Zeiss (Jena) "SM XX" stereomicroscope, photographic extension tubes and a 35 mm format camera body. A tilting stage designed by E. Layton for the convergence case is an additional attachment. This system provides facilities for measuring, observing and photographing small objects in incident or transmitted light, or in a combination of illumination sources with provisions for mono-, stereo- and chronophotogrammetry.

This system was also used for the convergent axes case (as follows).

Figure 1 shows the arrangement with a hardness testing diamond indenter set up for photography of the right stereo photo.

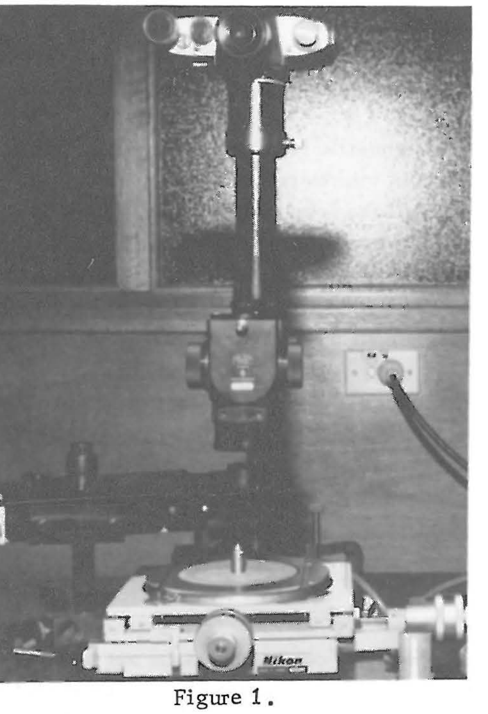


Figure 1.

Zeiss (Jena) SMXX Stereomicroscope

2.2.2 The Image Acquisition System - Convergent Axis Case

The first camera microscope system used was the Carl Zeiss (Jena) "SM XX" stereomicroscope and ancillaries (as previously described). The second system used was a Wild M7A (parallel axes) stereo zoom microscope with the photographic facility operating only through one axis of the microscope. The tilting stage was used. The objects of photography were the haemacytometer with a 1/64 inch (approx. 0.4 mm) diameter precision steel ball (the smallest ball bearing commercially produced).

More recent research and development (see also 2.1) resulted in successful convergent axes microstereograms produced on the Wild M400 Makroskop, a monocular camera-microscope system (Fig. 2). Convergent stereo photography was made in this case, by placing the objects on the tilting stage and photographing them with the stage tilted. As stage tilt increases, however, useable photographic area may decrease due to small depth-of-field unless one elects to accept longer exposure time and smaller aperture. For reasons of relative orientation, it was found necessary to set convergence tilts within a few centigrades. This was done with a Wild A10 triangulation cross level, whose bubbles had been centred on the A10 plate carrier with the elements set to kappa 100.00, omega 100.00, phi (convergence) 106.00. When this was done, many convergence problems disappeared and the affinity problem was also minimised. Further work is needed in this area. (The cross level acted as a metrological transfer standard for convergence between the Wild A10 Autograph and the tilting stage).

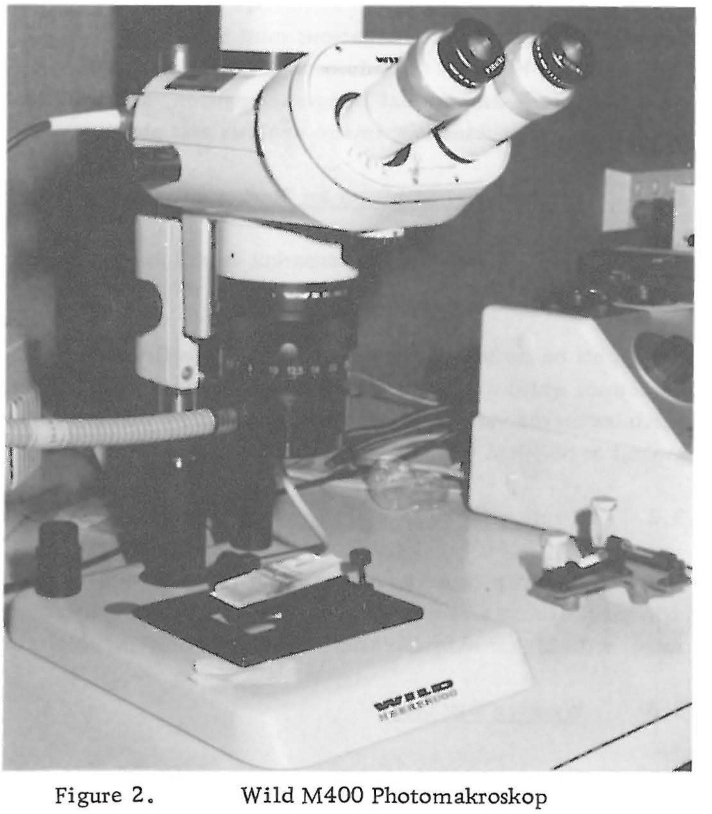


Figure 2. Wild M400 Photomakroskop

The imagery from this effort still contains evidence of limitations and problems deserving deeper study. It has been established that an appropriate method of obtaining stereograms capable of successful observation, with imaging systems in which the angular field is very narrow, is to achieve stereoscopic parallax through the convergence of the relevant photo axes. Thus the tilting stage has been a vital component of the equipment used in this work. Arising from these depth-of-field resolution limitations, limited areas of individual photographs, comprising stereograms, were in sharp focus. The areas where sharp focus in one image coincided with sharpness in the conjugate image of the stereogram were even more limited. It was not possible, therefore, to take observations which would be meaningful over large areas of the format of stereograms obtained. It was possible to record meaningful observations covering small areas of the imagery of the SRM, which contained sufficient gridpoints from which to compute standard deviations of some statistical significance.

3. STEREOPHOTOGRAPHY - NON METRIC SYSTEMS

With non-metric (uncalibrated) camera-microscope systems in microstereophotogrammetry, one exchanges the security of the fully-calibrated aerial survey camera for an uncalibrated image acquisition system without fiducials, whose principal distance is not known and which varies with changes of magnification.

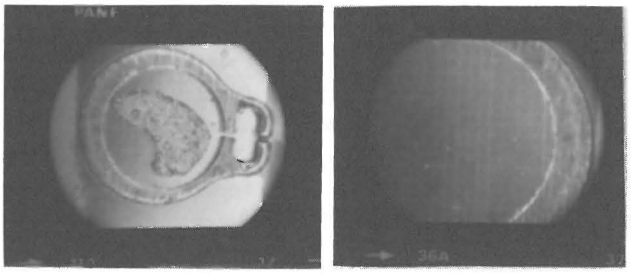


Figure 3.

Sample Microphotography

By analogy with photogrammetric ground control, SRM for planimetry and elevation should be located in or near the object of measurement. This "control" should also give an indication of model distortions, as well as some check on affinity of models (see Sections 3.5).

If the object (e.g. indenter) is magnified to fill nearly the whole microscope field of view, simultaneous photography of control points is not possible; resort must be had to the "substitution" method (a standard metrological technique) whereby the object is photographed, and removed. The SRM are then substituted and photographed under EXACTLY the SAME conditions of convergency, magnification, object distance and so on. The plotting system is calibrated using the "control" stereogram; the "object" stereogram is then introduced into the calibrated system and plotted.

The objective-ocular combinations must be chosen so that there is sufficient working distance (analogous to flying height) between the microscope objective and the object stage, sufficient depth-of-field for the specified three-dimensional image acquisition, and sufficient resolution of detail within the visual acuity of the observer for subsequent image analysis and plotting.

3.1 Preparation of Objects for Photography

Many objects will be shiny, transparent or reflective, in some attitudes and illuminations. Obviously this disturbs observation, and the need exists to render such object surfaces visible and non-reflective.

Highlights on surfaces of objects, caused by reflections or refractions of illumination, can be minimised by an aerosol white dulling spray often used in commercial photography. Polished objects should have some dust particles on the surface to aid photogrammetric observations. This also applies to machining marks on finely ground or polished metal objects (1 and 9).

3.2 Depth-of-Field Resolution Limitations

The aerial survey camera is a priori set to infinity focus and does not exhibit depth-of-field resolution difficulties in normal usage. The camera-microscope system has a very limited depth-of-field resolution zone; with higher magnifications the depth-of-field resolution zone shrinks rapidly to practically a plane.

3.3 Working Distance

With stereo-microscopes, the object or working distance as adjusted for each magnification, varies only slightly. This applies to both zoom and magnification variator types. With single tube microscopes, the working distance varies considerably, being longer for small magnifications and shorter for high magnification.

In both cases the principal distance is much larger than the working distance and has to be established empirically.

3.4 Use of the Tilting Stage

The tilting stage (10) serves to achieve suitable convergent photography. The tilt axis should be parallel to the Y axis of the resultant photography (checked using microscope graticule). The stage has a precisely calibrated tilt applied and is then firmly locked. The tilt should be the maximum phi that the observing Stereoplotter can accommodate. Most analogue stereoplotters will accept 6 grades of phi; if an analytical plotter is to be used, a convergence of 30 grades may be more appropriate. The upper limit in this case may be dictated by comfort of observation rather than anything else. (See Fig.4).

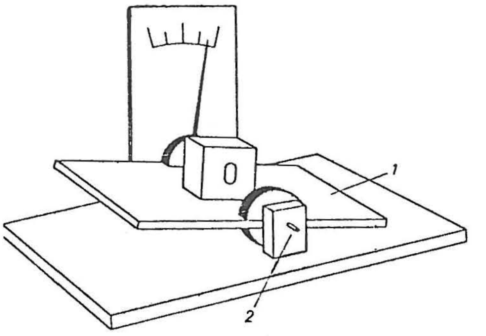


Figure 4. Diagram of Tilting Stage

1. Adjustable Tilting Surface
2. Axis of Tilt

The objects to be photographed are assembled, fixed in place and photographed. The assemblage is then rotated through 200 grades on the tilted stage (previous high end is now low) and rephotographed. The stage is not moved or adjusted between photographs (so that phi 1 equals phi 2). Focus and centring must be checked. Minor errors in the 200 grade rotation, and in the coincidence of principal points, can be tolerated; they will be cleared during relative orientation.

The stage of the microscope, either stereo or monocular, is constructed at right angles to the optical axis. The tilting stage, with appropriate tilt set, rests on the microscope stage.

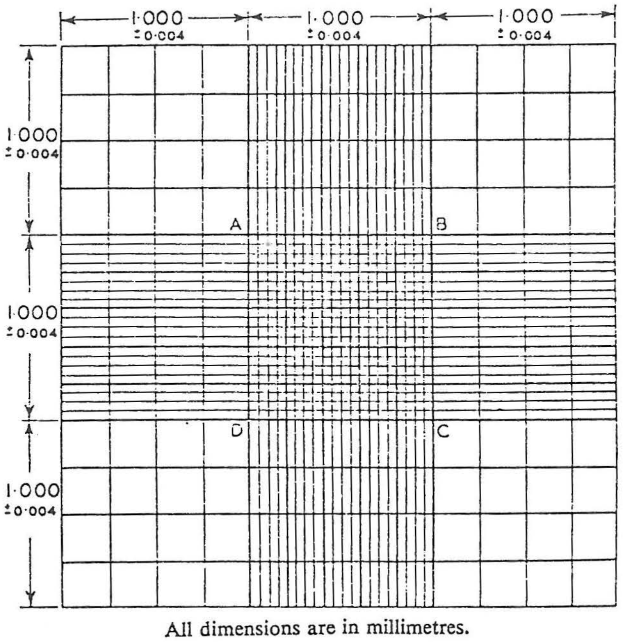
3.5 Standard Reference Materials and the Ground Control Analogy

In microstereophotogrammetry, surveyed ground control is exchanged for accurate and precisely calibrated Standard Reference Material (SRM). The SRM used define horizontal scale, vertical scale and a plane surface (which need not be level).

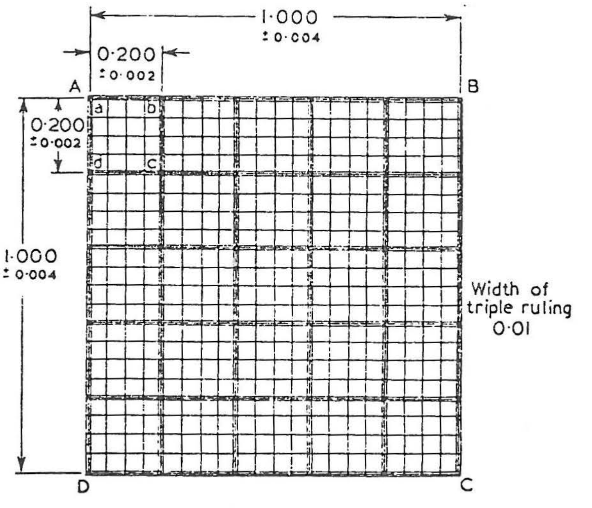
The most useful SRM was a Spencer Haemacytometer (11), a microscope glass slide of about 80 mm x 30 mm, ground optically flat in two small areas. On each of these areas is engraved a precise grid of very fine lines (Fig. 5). It serves as a line standard and as a calibrated grid plate, although its usual function has to do with blood cell counts in pathology.

Other SRM are metrological gauge blocks which are small metal blocks made to very precise thicknesses. They define heights and height differences. A small (0.4 mm dia.) steel ball has also been used. It has the advantage that the one object combines XY (scale) and Z (height difference) control. It can be placed on the haemacytometer or on any level surface on which the vertical diameter may be read. Horizontal and vertical diameters may be compared as a check on affinity. A small steel washer of measured thickness has been used as a height standard. If its internal diameter is known, it can also serve as an XY scale standard if no haemacytometer is available. More practically it will serve to contain the material being mapped.

Microscope specimens and SRM need to be firmly attached to whatever base is used (usually the haemacytometer). This stops any relative movement between object and SRM when the assemblage is rotated on the tilting stage. The glue used should be slow in setting (to allow final small adjustments in positioning specimens and SRM) and non-corrosive. It should also be easily dissolved (for cleaning) after photography. Finger nail polish serves well, having all the above attributes, and washing off in acetone without affecting the haemacytometer.



All dimensions are in millimetres.
Improved Neubauer ruling



All dimensions are in millimetres.
Central portion of Improved Neubauer ruling

Figure 5. Haemacytometer Ruling

4. ORIENTATION PROCEDURES

As mentioned previously, no fiducial marks appear on the microphotographs. The principal point was assumed to lie at the intersection of the diagonals of the format and this point was marked on the negatives to be used in centring them on the diapositive carrier of the Wild A10.

A purely arbitrary principal distance of perhaps 200 mm may be set; it will be changed later as part of the relative orientation. Experience with camera-microscope may lead to other first approximations.

4.1 A Relative Orientation for Convergent Photography

Careful setting-up and use of the tilting stage simplifies relative orientation.

The stage tilt axis should be parallel to both Y axis of microscope graticule and Y axis of haemacytometer. The haemacytometer Y axis (as photographed) will therefore be parallel to phi tilt axis of the plotter only when the haemacytometer Y axis parallels the Y axis of the stereoplotter.

The above conditions ensure that the calibrated convergence applied to the tilting stage appears as a tilt in a defined direction in the microphotograph. Thus, there are no significant omega components in the relative orientation; the convergence applied appears as phi only and is preset on the plotter as part of orientation.

When all the above are carried out, the relative orientation reduces to the following operations:

- i. preset phi 1 and phi 2 to the convergence used;
- ii. set both omegas to 100.00 and bz to zero;
- iii. monocularly left and right, make Y axis of Haemacytometer parallel to Y axis of plotter;
- iv. at coincident principal points (or between them if close) remove Y parallax with by;
- v. at "9 o'clock", clear half with each kappa;
- vi. at "3 o'clock", check; clear with either kappa;
- vii. iv, v and vi may need repeating.

Parallax should then be clear at "12 o'clock" and "6 o'clock" also.

Initially, convergence (phi) had not been set so accurately and an empirical solution for it had been attempted. The solution, however, was not satisfactory because of the extreme narrow-angle geometry of the model. At the same time that it was decided to calibrate convergence very accurately, observations were made which revealed the connection between correct convergence and correct vertical model scale (12, 13 and 14).

Hence, use of correct convergence seems to ensure correct vertical model scale; the model will be non-affine. Incorrect principal distance does not seem to have the influence on vertical model scale here that it has in normal near-vertical work. However, it has other influences as will be shown.

4.3 "Calibration" of Principal Distance of Photography

Because phi 1 and phi 2 (convergence) have been calibrated and preset, there can be no phi cylinder deformation. Any cylinder deformation found, seems to be caused by use of an incorrect principal distance. Such a deformation is now defined as "Principal Distance (Cylinder) Deformation". If it is present, there may also be y parallax in the "corners" of the model.

If the cylinder curves downwards in the middle, the principal distance needs to be increased until the effect is removed (and vice versa). The effect was only noticed after deciding to use convergent photography, but it offers promise in "calibration" of principal distance.

Some photographs had a principal distance greater than 308 mm (WILD A10 principal distance maximum). Observations of the latest such pair (at principal distances of 210 mm and 308 mm) showed that the larger setting did not remove the principal distance cylinder distortion, but significantly reduced it. One surmises that a larger principal distance, if mechanically possible, would remove it. This comparison was done without changing convergence.

4.4 Absolute Orientation

Most work done did not require an absolute orientation. As the maximum phi was used to increase convergence there was very little movement available for common phi.

Because of the narrow angle geometry and the small format available, tilts have little effect anyhow. If any particular object must be observed in specific attitude, it should be planned for in the setting-up procedure adopted for retaining the object on the tilting stage. Care here will render absolute orientation probably unnecessary.

5. DIGITAL TREATMENT AND ACCURACY

Where Principal Distance cylinder deformation cannot be totally removed, because of instrument limitations, useful work may still be done as much measurement in the field seems likely to be relative rather than absolute. Where cylinder must be removed subsequently, a second order polynomial adjustment approximates the error curve; (more work will be done on this aspect). This technique suffers the disadvantage of working on discrete points only; the distorted "model" of the haemacytometer is adjusted onto a level plane grid.

An alternative approach is to work in such a small area that cylinder distortion becomes negligible. This may be anathema to most practising photogrammetrists who (understandably) need an undistorted flat model datum to which the geodetic height datum may be referred.

Early 3-dimensional linear transformations achieved lesser accuracies because the observers tried to process too much of the model. The results suffered because of lack of definition, cylinder deformation and other causes.

6. RESULTS

The most recent series entailed observations of three square arrays, each containing 25 points, the array size being 0.2 mm x 0.2 mm sub-divided at 0.05 mm intervals. The best array yielded mean square errors in X, Y and Z of 0.48 um, 0.4 um and 0.94 um after 3-dimensional linear transformation onto "control" in micrometers representing the real size of that part of the haemacytometer. No observations were rejected; the largest residual was less than two standard deviations.

These results are compared with previous results from 1977 and 1979.

	Microscopic .77	Microscopic .79	Microscopic .80
Negative Scale	11:1	4.3:1	21.7:1
Model Scale	40:1	10.3:1	22.9:1
Plotting Scale	200:1	102:1	Not plotted.
Formline Interval	7.4 micrometres	10 micrometres	Not plotted.
Standard Deviations in X	1.4 micrometres	1.6 micrometres	0.48 micrometres
Standard Deviations in Y	1.2 micrometres	1.8 micrometres	0.46 micrometres
Standard Deviations in Z	1.3 micrometres	2.0 micrometres	0.94 micrometres

7. CONCLUSIONS

There is very little parallax when the normal aerial survey case (near vertical, 60% forward overlap) is translated into microscope terms. This is related to the extreme narrow-angle characteristic caused by the long principal distance. A further problem is the unavailability of 40% of the format. Hence one must look elsewhere for usable, measurable parallax. Convergent photography, using the tilting stage was tried, found successful, and (importantly) offered near 100% overlap.

One is advised not to use a stereo-microscope, with or without the tilting stage. The X parallax was found insufficient for proper measurement; convergent photography yielded better stereoscopy. The uncalibrated built-in convergence of the stereo microscope interracted with the carefully calibrated tilt of the tilting stage to produce erroneous, unknown total convergences which lead to vertical model-scale problems, tilted models and some unsolvable relative orientations. Some stereomicroscopes appear by the nature of their optics to achieve lesser definition than a monocular microscope, used at the same magnification.

The achieved standard deviations of statistically significant series of observations on stereograms of an appropriate calibration object are below one micrometre in both planimetry and height. This achievement justified past frustrations, and further pursuit of this research project with more emphasis on like work in the future. Now that such accuracies can be validated, it is justified to attempt the calibration of some camera-microscope systems. Studies, as seem appropriate to academic photogrammetrists, covering such matters as interior orientation parameters, relative and absolute orientation procedures, and other matters dear to the theorists, can be initiated. There is now a real and PRACTICAL justification for the further study of systems capable of producing appropriate stereograms.

Camera-microscope systems other than that used for the past recent work must also be tried out. It may well be that some other available systems will be found to produce even more satisfactory results. Those already produced are satisfactory to these authors at this time. A field of research appears to be opened to many, at both practical and theoretical levels into such use of photogrammetric techniques, procedures and equipment. These authors would welcome others who may be motivated to join them in this fascinating field of study.

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