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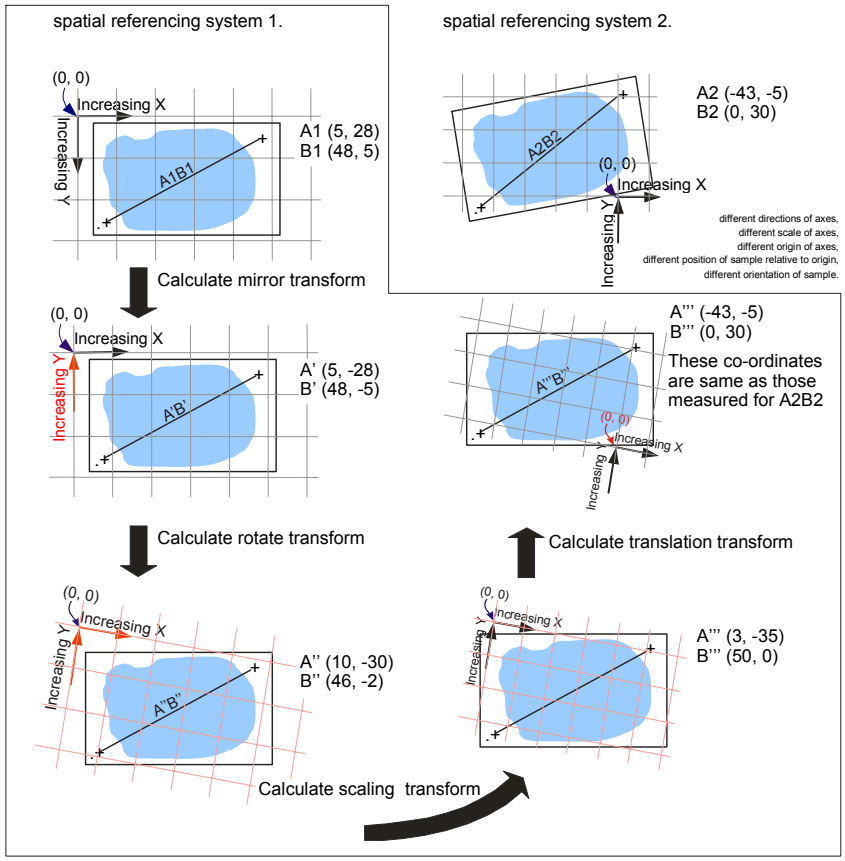
'2DTraC' - a new tool for spatial referencing of samples during microscopy and microanalysis.

Geochemistry, Mineralogy and Hydrogeology Programme.
Development of Capability in Mineralogy and Geochemistry Project ESB61900003.

Internal Report IR/02/063



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INTERNAL REPORT IR/02/063

'2DTraC' - a new tool for spatial referencing of samples during microscopy and microanalysis.

J.E. Bouch, J. McKervey, M.T. Styles and S. Chenery.

Key words

Microscopy, Microanalysis, Spatial Referencing, Sample Transfer..

Front cover

Cover picture details, summary of the steps required to convert co-ordinates between different spatial referencing systems.

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Foreword

This report describes the results of a study by the British Geological Survey (BGS), as part of the Development of Capability Programme, to provide a mechanism by which points of interest on samples can be tracked and readily relocated on different microscopes and microbeam instruments. Of particular interest to this study were optical microscopy, scanning electron microscopy, electron microprobe analysis and laser ablation-inductively coupled plasma-mass spectrometry. However, the routines used to define reference points on a sample and to convert lists of co-ordinates between the spatial referencing systems of different microscopes/instruments could be applied to any situation where co-ordinates need to be converted from one 2-dimensional co-ordinate system to another.

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Summary

This report describes a mechanism that has been developed to enable easy tracking and relocation of points of interest between different microscopes and microbeam instruments. This is a requirement when a small area or series of points of interest need to be examined by several microbeam techniques. The report first considers some generic problems associated with the spatial referencing/co-ordinate systems of different microscopes/instruments, and then:

1) **evaluates the best procedure for marking a sample with reference points that can be used on any piece of equipment.**

   It is recommend that the sample surface be initially marked with two ink-crosses, one in the bottom-left corner and one at some other point on the sample surface (ideally diametrically opposed to the first point). A small pit (10-20 microns) should then burned at the approximate centre of each cross using the laser of the Laser Ablation-ICP-MS instrument. This combination provides a quick means of identifying the general locale of the reference point, and a sufficiently small reference point such that operator bias/error in identification of its centre is reduced.

2) **evaluates the accuracy and reproducibility with which an operator is able to measure the location of reference points and points of interest on each piece of analytical equipment.**

   Tests indicate that the precision with which an operator can relocate the position of reference points and points of interest is largely controlled by the size of the feature of interest. An operator can usually relocate a laser pit of approximately 30 microns diameter with a precision of within 10 microns. The stages of all the microscopes/instruments tested are sufficiently precise to return to within a few microns of a specified pair of co-ordinates.

3) **describes an Excel Add-in '2DTraC' that has been developed to convert co-ordinates measured on one piece of equipment to co-ordinates appropriate to the piece of equipment to which the sample is being transferred.**

   2DTraC (2-Dimensional Transform Calculator) automatically determines the combination of mirror, rotational, stretching and translational transforms that will move the co-ordinates of the reference points as measured using the co-ordinate system of one microscope/instrument into the co-ordinate system of another microscope/instrument. These transforms are then applied to a list of points of interest.

   The development of this add-in has removed the need for development and prototyping of highly engineered sample holders for each piece of equipment. However, in order to generate precise transforms, **2DTraC** requires that the reference co-ordinates are known sufficiently precisely - errors of 10 microns on each of the reference point measurements will readily compound to give errors in the calculated results of >50 microns. This project initially aimed to be able to relocate points of interest on different microscopes/instruments to within 100 microns. This has been achieved provided that the reference points can be located sufficiently precisely (with care it is possible to relocate the reference points to within 10 microns).

1 Introduction

A recurring problem in the microanalysis and microscopy of geological (and other) materials is the relocation of points or areas of interest on a sample as it is transferred between different microscopes/analytical instruments. This report details the results of a project that was instigated in order to generate a set of protocols by which accurate relocation of points of interest would be possible.

Within BGS it is relatively common for the same sample to be analysed/examined using a combination of conventional optical microscopy (OM), scanning electron microscopy (SEM), electron probe microanalysis (EPMA), and laser-ablation inductively-coupled-plasma mass-spectrometry (LA-ICP-MS). These instruments differ in the mechanism of sample mounting in the equipment's sample chamber/stage. Consequently different pieces of equipment display differences both in the overall flexibility of sample positioning, and in the repeatability of sample positioning (i.e. the ability to consistently load a given sample in the same position between different analytical runs). Most of the instruments have stages with digital readouts and in some cases are computer controlled, however, the systematics used by each piece of equipment to record the position of the sample relative to the excitation beam and/or objective lenses (hereafter the 'Spatial Referencing System') vary. Therefore, it is not always possible to directly translate (using simple transforms) the co-ordinates of points of interest as measured on one system into the spatial referencing system of another.

In view of the above, in order to develop a system by which points on a sample can be relocated between analytical runs using different pieces of equipment the following points need to be addressed:

1) evaluation of the best procedure for marking a sample with features that can be used as consistent points of reference for the sample on any piece of equipment.
2) comparison and determination of the compatibility of the different sample mounting and spatial referencing systems used by each piece of equipment.
3) determination of the accuracy and reproducibility to which an operator is able to measure the location of the points of interest on each piece of analytical equipment.
4) creation of a set of routines that allow users to convert co-ordinates measured on one piece of equipment to co-ordinates appropriate to the piece of equipment to which the sample is being transferred.

With these points in mind, this report is divided into three main sections. The first section 'Points of Reference' describes the approach taken in the definition and marking of a consistent set of reference points on each sample (1 above). The second section 'Equipment Specific Considerations' describes each microscope/instrument, with particular reference to the means by which a sample is mounted, the spatial referencing system it applies, and the precision to which it is capable of relocating points (2 and 3 above). The third section '2-Dimensional Transform Calculator (2DTraC)' describes an Microsoft Excel '97 add-in, which has been developed to convert co-ordinates between the spatial referencing systems of difference pieces of analytical equipment (4 above)

2 Points of Reference

In most instances the samples that are likely to be transferred between microscopes/instruments are polished thin-sections (rectangles of typically 48x28 mm, or *c*.75x50 mm) or polished blocks (circles 25 mm or 40 mm diameter). As is explained in later sections, certain microscopes/ instruments have sample holders that prevent features on the very edge of the sample (such as the edge or corners of the sample itself) being used as reference points, and certain microscopes/instruments only image the 'top surface' of the sample (rather than passing light through the sample). Therefore, the reference point(s) need to be away from the edge and on the top surface of the sample.

A blank glass slide of the type routinely used for polished thin-sections was marked up with 7 reference crosses using a permanent marker as indicated in Figure 1 and Plate 1, these lines were of the order of 0.5mm wide. Reference cross 1 was specified as the 'bottom-left' of the sample and identified by a dot adjacent to the cross (Figure 1). Following preliminary measurements of the co-ordinates of these crosses using SEM, OM and EPMA (see discussion relating to individual pieces of equipment in later sections) it became apparent that the largest source of error in measurement of the reference co-ordinates was the consistent identification of the central part of the cross, both by different operators and by the same operator on successive runs. Therefore, during measurement of the cross positions using the LA-ICP-MS, a small laser pit of approximately 30 microns diameter (Plate 2) was made at the centre of each cross. Due to the smaller size of these marking, measurements of their positions using SEM, OM and EPMA proved to be more repeatable. However, due to their small size, it would prove difficult and time consuming to identify and locate these laser marks if they were not positioned in some consistent manner relative to a larger, more readily identifiable feature (in this case the ink crosses).

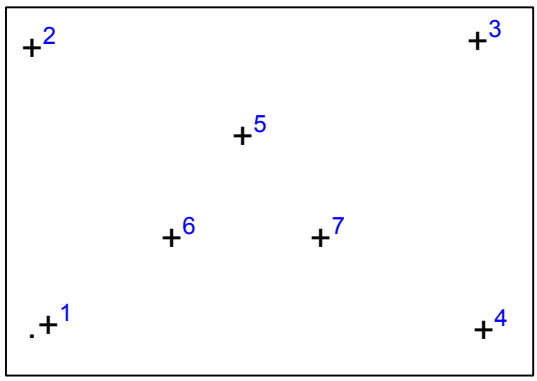

Figure 1. Sketch plan of sample showing relative positions of the 7 reference points. Point 1, defined as being in the bottom-left of the sample, is denoted by an additional dot next to the reference cross.

It was also attempted to burn reference marks into the resin used to mount the samples using the electron beams of both the SEM and the EPMA. The SEM beam is not of sufficiently high energy to volatilise resin in most instances, and therefore proved unworkable. The EPMA beam operates at higher energy and is capable of creating clearly defined micron-scale features which could be used as reference points. However, the sample has to be carbon-coated prior to marking using EPMA, and this coating can interfere with some optical observations. In contrast, the laser can be used to mark uncoated samples, and the sample mounting procedure is less time consuming compared to that of the EPMA system. This makes the laser the method of choice for sample marking.

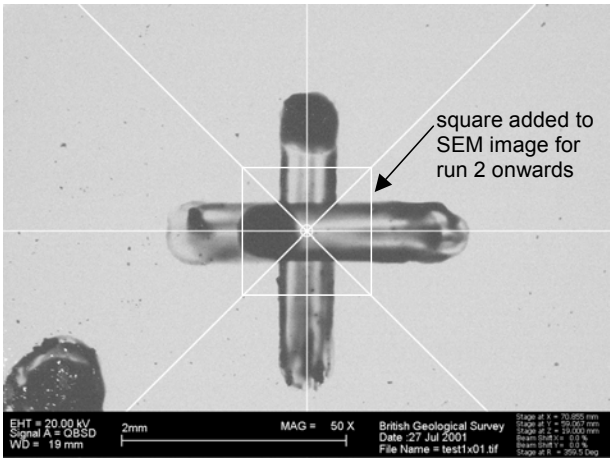

Plate 1. SEM image showing centrally positioned cross mark (reference cross 1). Note, the addition of the square to the default crosshairs aids central positioning of the reference point during SEM measurement.

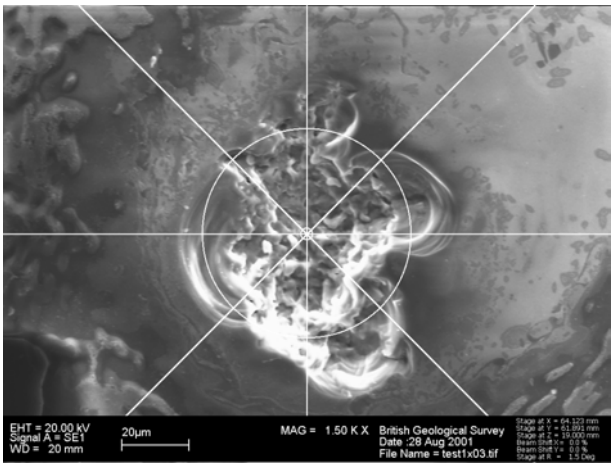

Plate 2. SEM image showing laser burn mark positioned at the centre of reference cross 1. Note, the addition of the circle to the default crosshairs aids the central positioning of the reference point during SEM measurement (a function only available on the SEM).

A final consideration is the fact that the reference point co-ordinates will potentially be required to detect relatively slight differences in sample positioning. Therefore, it is advantageous that the reference points are as widely spaced as possible - such that the magnitude of errors related to the measurement of reference point co-ordinates are minimised relative to true differences in sample positioning (e.g. a 10 microns error in the measurement of a reference point position will be less significant over a total distance of 40,000 microns than over 10,000 microns; see Figure 9 in later section). Therefore, it was decided that two primary reference points should sit in diagonally opposed corners of the sample, and for convention, these should be the bottom-left and top-right corners of rectangular samples (Figure 1).

On the basis of these investigations, it appears that the most satisfactory means of marking reference points on a given sample is to mark the bottom-left and top-right corners of samples with relatively large (*c*.5mm) ink-crosses, followed by more precise marking of smaller reference points at the centres of these crosses using the laser of the LA-ICP-MS. The bottom left corner should be identified by a small dot next to the reference cross, and can be taken as a local zero co-ordinate (Figure 1). Finally, it is desirable to add a third 'check point' to each sample (can be positioned wherever convenient), which can be used as a check on the accuracy of transforms calculated using the routines described in '2-Dimensional Transform Calculator (2DTraC)'.

3 Equipment Specific Considerations

3.1 OPTICAL MICROSCOPES, LA-ICP-MS AND FLUID INCLUSION ANALYSIS

A number of optical microscopes are used in association with the various analytical laboratories, each of which has slightly different characteristics. The simplest sample mounting systems for the optical microscopes are a range of sample holders which allow manual sample movement in the X- Y- directions (example shown in Plate 3). The X- and Y- co-ordinates on these graticules can typically be read to a precision of 1/10th of a mm (100 microns). However, some microscopes are fitted with more sophisticated stages that record precise stage co-ordinates (to 1 micron in some cases; Plate 4 to Plate 6). The co-ordinate systems of the different microscopes display some variability, with origins in different places and different senses of direction for the X- and Y- axes. Furthermore, some systems have fixed origins, whereas others can have "local" origins defined by the user. Repeatability of point re-location depends upon the rigidity with which the sample holder holds the sample, and also on the relative precision of the X- Y- graticule. The stage used in these measurements is that depicted in Plate 4, which is engineered to be highly orthogonal, is accurate to 1 micron, and has a user-defined origin.

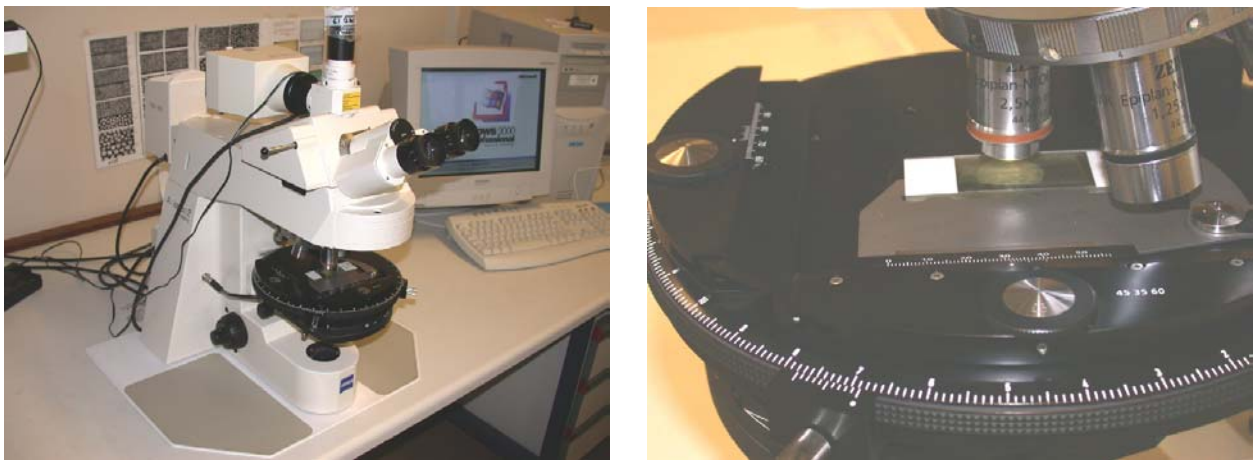

Plate 3 Simple, manual, X-Y sample holder attached to the rotating stage of a polarising microscope. The graticules on this type of sample holder can only be read to a precision of 100 microns.

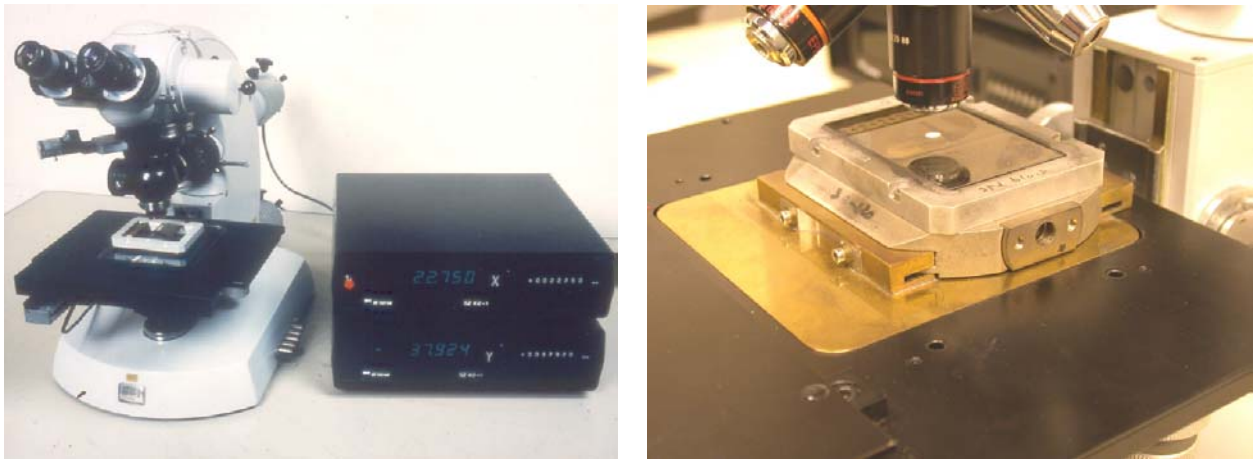

Plate 4 Digital X-Y stage attached to the conventional polarised light microscope used in association with the EPMA instrument. The sample is mounted in the sample block used in the EPMA instrument. This stage is highly orthogonal, reads to a precision of 1 micron and may have a user-defined "local" origin.

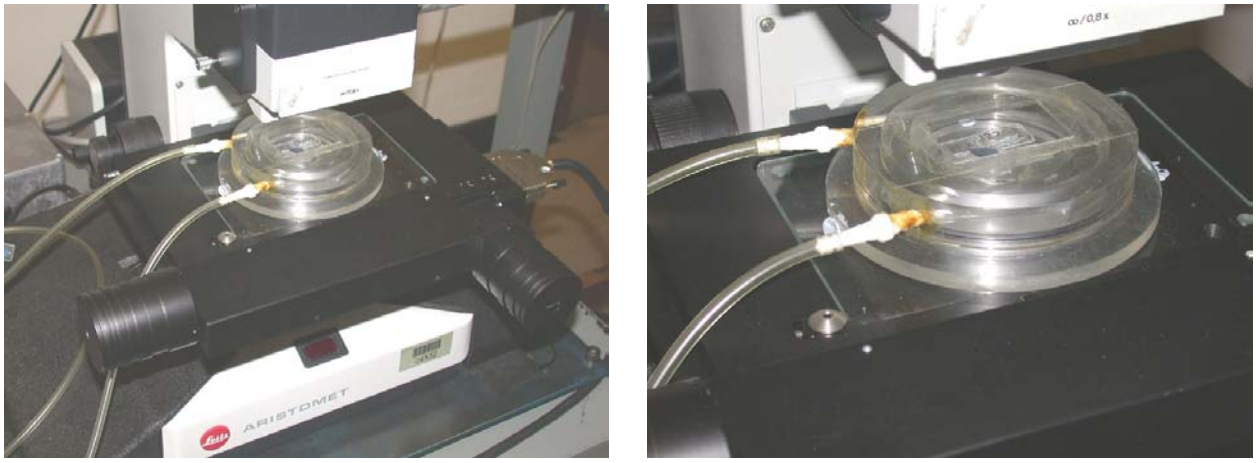

Plate 5 Digital X-Y stage attached to the light microscope used as part of the LA-ICP-MS instrument. This stage reads to a precision of 1 micron, and is under computer control such that the stage can be driven to the co-ordinates of points of interest. However, the sample cell (clear Perspex) offers relatively poor control on the orthogonality and positioning of the sample.

Plate 6 Digital X-Y stage attached to the light microscope used as part of the fluid inclusion microthermometric instrumentation (heating-freezing cell). This stage reads to a precision of 1 micron, and is under computer control such that the stage can be driven to the co-ordinates of points of interest. However, the sample material used in this cell represents sub-samples drilled from larger doubly polished wafers. The stage offers poor reproducibility of sample positioning, and the samples are typically too small for marking with the procedures outlined in the previous section.

3.2 SEM

The SEM under consideration is a LEO 435VP variable pressure instrument. During the earlier runs, the test sample was not carbon-coated and therefore the SEM was operated in variable pressure mode, using the backscattered electron detector for imaging. This was the first instrument in which the points on the test sample were located and spatially referenced. Throughout this work the SEM 'stage backlash' functionality was enabled, as it enables more precise relocation of points by always approaching points from the same direction and therefore eliminating variations in slack within the SEM stage gear-train. The SEM is also able to apply a 'beam offset', which is a more precise way of shifting the field of view at higher magnifications. This electronically offsets the scan area rather than moving the stage, however, in order to record stage co-ordinates only, this was disabled during the work. The centres of the crosses were

located at a consistent magnification of x200 (Plate 1), and the centres of the laser burn marks were located at a consistent magnification of x1500 (Plate 2).

3.2.1 SEM Sample Mounting

The sample was mounted on the 'normal' SEM stub-holder in the usual manner using double-sided carbon adhesive tape (Figure 2). Visual cues were taken from symmetrical features of the stage, such that the sample orientation was as close to orthogonal to the SEM chamber X-Y directions possible. Using these visual cues alone it proved possible to position the sample to within a couple of degrees of orthogonal to the SEM chamber.

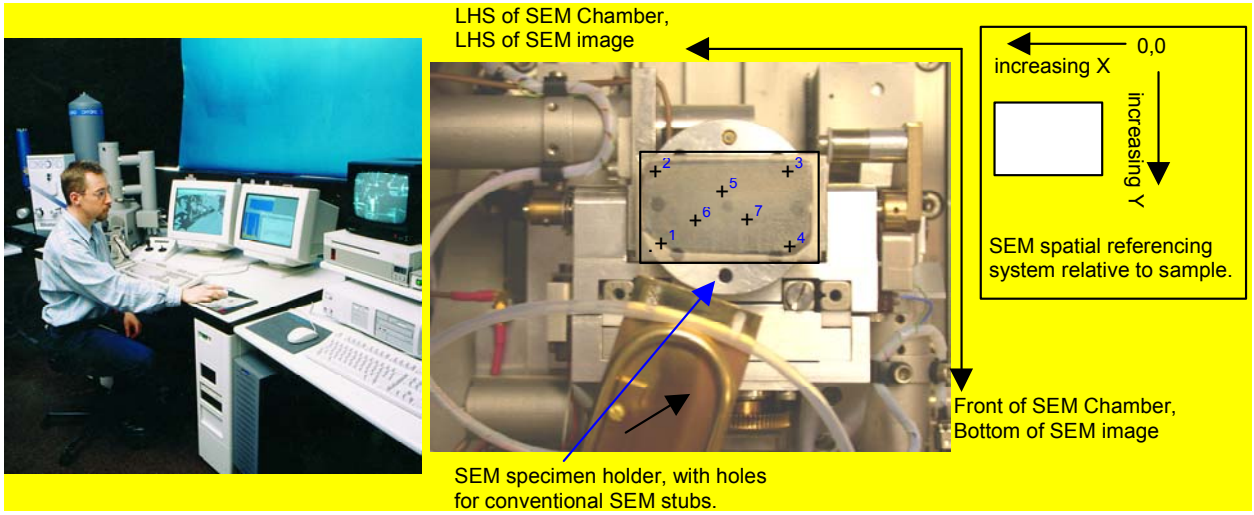

Figure 2. Position of the test sample as mounted in the SEM chamber (plan view), orientation of SEM image relative to SEM chamber, and SEM co-ordinate system (origin in top right).

The SEM stage rotation control was used in conjunction with SEM imaging of the sample edge to adjust the stage orientation in what was hoped would be a more controlled manner (the adjustment required was <1 degree in this case). However, it actually proved difficult to align the sample any more precisely with this method, because it was not possible to achieve a low enough magnification such that irregularities in the sample could be differentiated from a non-orthogonal sample orientations.

3.2.2 SEM Co-ordinate System

SEM images appear on screen in the orientation indicated in Figure 2 (i.e. left and right directionality is preserved (the image is not inverted), and the back of the chamber is the top of the image). However, when the stage is positioned in the (50mm, 50mm) position, the centre of the stage is at the centre of the SEM scan area. As the stage is moved towards its origin at the front-left corner of the sample chamber, the back-right part of the sample is moved into the scan area. Therefore, relative to points on the sample, X-increases from right to left, and Y increases from top to bottom (see Figure 2). Finally, it is possible to set up a 'data zone' at the bottom of the SEM image to record the current stage position, which enables the easy capture of point co-ordinates.

3.2.3 Accuracy and Precision of Point Location using SEM

The seven marks on the test sample were located and centred on the SEM image and the SEM stage co-ordinates at each point were recorded. The SEM 'graticule' display, which gives a precise indication of the centre of the SEM image was utilised (Plate 2; note that the graticule marks the centre of the entire image, including the lower part of the image which may be masked by the data zone). This procedure was repeated four times using the ink crosses and four times

using the laser marks. Details of each run can be located in Appendix Table 1. 1, with the results tabulated and compared in Appendix Table 1. 2.

Repeatability of co-ordinate measurements was notably better with the laser marks (typically within 10 microns) than with the ink crosses (typically 20-40 microns). This demonstrates that the SEM stage is able to reliably return to a specified location, and that the larger source of error in these tests was the consistent identification of the centre of the reference mark. This confirms the requirement that the reference marks on the samples need to be small.

Comparison of data from SEM runs 2 and 3 (Appendix Table 1. 2) highlights the importance of precise orthogonal positioning of the sample, or the ability of the sample transfer and/or co-ordinate conversion procedure to correct for these errors. A rotation of <0.5degrees was sufficient to move the points marked on the test sample by up to 100 microns.

3.3 EPMA

The electron probe under consideration here is a Cameca SX-50, equipped with both an optical imaging system and an electron (SEM) imaging system. The optical image proved to be of no use in locating the centres of the ink crosses because the intersection area of the crosses was greater than the field-of-view of the optical image (approximately 250 µm). The centres of the crosses were therefore located using SEM imaging at a magnification of approximately x120.

3.3.1 EPMA Sample Mounting

The test sample was loaded into a standard Cameca sample holder as illustrated in Figure 3. The test sample was loaded in the '1st' sample position. This sample holder is able to hold the sample to a high level of orthogonality, however, the mounting mechanism is such that approximately 2mm of side of the sample is obscured beneath a lip on the stage. This prevents the sample corner being used as a reference point.

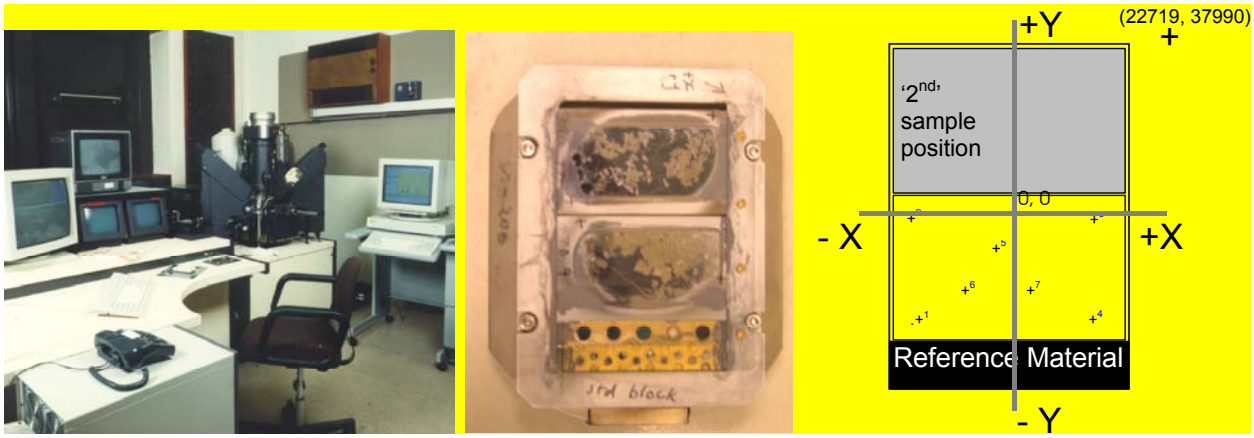

Figure 3. Position of the test sample holder within a Cameca sample holder, and relative directions of X- and Y-axes of the EPMA spatial referencing system.

3.3.2 EPMA Co-ordinate System

As evident from Figure 3 the origin of the EPMA spatial referencing system equates to the centre of the sample holder, with X-increasing from left to right, and Y-increasing from bottom to top of the sample holder. This co-ordinate system is fixed.

3.3.3 Accuracy and Precision of Point Location using EPMA

Following digitisation of the positions of the points using a conventional optical microscope (see above), the ink crosses were located in the EPMA using the amov x and amov y commands four

times: twice on one day and then twice on the following day after the machine had been shutdown and restarted (the measured co-ordinates are presented in Appendix Table 1.2).

The crosses were easily relocated during each analysis run, however the centre of each cross had to be relocated each time by eye and proved to be a significant source of error. The measured co-ordinates typically varied between 0 and 32 μm on successive runs, with an average variation of 9 μm. A significant part of this range must be due to the user having to choose the centre of the cross.

3.4 SUMMARY

The investigations outlined above indicate that all the pieces of analytical equipment under consideration have the capability to accurately relocate points on a given sample provided the sample does not move relative to the equipment's spatial referencing system (i.e. it is possible to precisely relocate points during a single 'run' of a sample on a given piece of equipment). Furthermore, certain pieces of equipment have sample holders that consistently place samples in the same position relative to the equipment's spatial referencing system (EPMA, and certain sample holders for OM). This means that orthogonality is generally preserved between runs, and that co-ordinates of points between successive runs are broadly comparable.

However, differences in the spatial referencing systems of different pieces of equipment are observed: most notably differences in the relative positions of origins, and senses of direction of the X- and Y-axes. These differences are compounded by the difficulty with which it is possible to consistently position a sample relative to the spatial referencing system of certain pieces of equipment (certain sample holders for OM, SEM, LA-ICP-MS).

4 2-Dimensional Transform Calculator (2DTraC)

An add-in for Microsoft Excel '97 called **2DTraC** ('2-Dimensional Transform Calculator') has been developed using Visual Basic for Applications. **2DTraC** converts sets of co-ordinates between the spatial referencing systems of different microscopes/instruments, and has been designed as generically as is possible, such that it should be able to convert co-ordinates between any pair of 2-dimensional spatial referencing systems.

2DTraC relies on a sample having two reference points whose positions within one spatial referencing system are known. Points of interest are then located in the same spatial referencing system (relative to these reference points). *This system means that it doesn't matter which microscope/instrument the sample is placed on first, so long as the two reference points are measured, and subsequent points of interest are located relative to these*.

When the sample is transferred to a different microscope/instrument, the positions of the two reference points need to be measured using the spatial referencing system of the new equipment. These co-ordinates are then input into **2DTraC**, which calculates the required mirror, rotational, scaling and translational transforms required to convert from the old to the new spatial referencing system. It then applies these transforms to a list of the co-ordinates of points of interest, and generates a new list showing the positions of the points of interest in the new co-ordinate system.

The following sections provide installation and user instructions ('Instructions'), details of the mechanism by which **2DTraC** calculates and applies transforms ('Application Methodology'), and an assessment of the quality of the resulting transforms ('Quality Of Calculated Versus Measured Co-ordinates'). The individual functions in **2DTraC**, which can be used by other worksheets provided that the **2DTraC** add-in is open, are documented in more detail in Appendix 2.

4.1 INSTRUCTIONS

4.1.1 Installation

2DTraC consists of two files. The first is the actual add-in ('**2DTraC.xla'**) which contains all the relevant functions and routines required to calculate and apply the transforms. This is supported by a worksheet template ('**2DTraC.xlt'**), which provides the means of inputting the reference co-ordinates and lists of points of interest. These files must be kept in the same directory on the PC on which they are to be used, and should not be renamed.

The add-in can be loaded in two ways:

1. for 'one off' usage double click or open the file 2DTraC.xla as you would for any other Excel file. If asked, 'enable macros'.
2. for the add-in to be available every time Excel is opened, select 'Add-ins…' from the Excel 'Tools' menu. In the dialog box click the 'Browse…' button and navigate to the folder in which the file 2DTrac.xla is located. Select the file, and the add-in will be added to the list of add-ins available to your particular installation of Excel. Once you have completed this procedure the files should not be moved as Excel will be unable to locate them.

4.1.2 User Instructions

Once the add-in is loaded, a new item '2DTraC' will have been added to the 'Tools' menu. Within this menu item are the following options (Figure 4):

- **New 2DTraC Sheet** creates a new worksheet based on the template 2DTraC.xlt onto which reference point and point of interest co-ordinates can be entered and saved (Figure 5).
- **Update** performs a set of transform calculations.
- **Clear Results** deletes any co-ordinates calculated for the points of interest in spatial referencing system 2, but will preserve the measured co-ordinates of the reference points and points of interest (in spatial referencing system 1).
- **Clear All** clears current worksheet of results **and** any reference point/points of interest co-ordinates.
- **Help** shows/hides help comment boxes.
- **About** shows add-in information/version number.

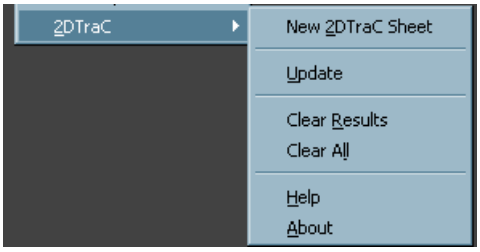

Figure 4. 2DTrac custom menu (on Excel Tools menu).

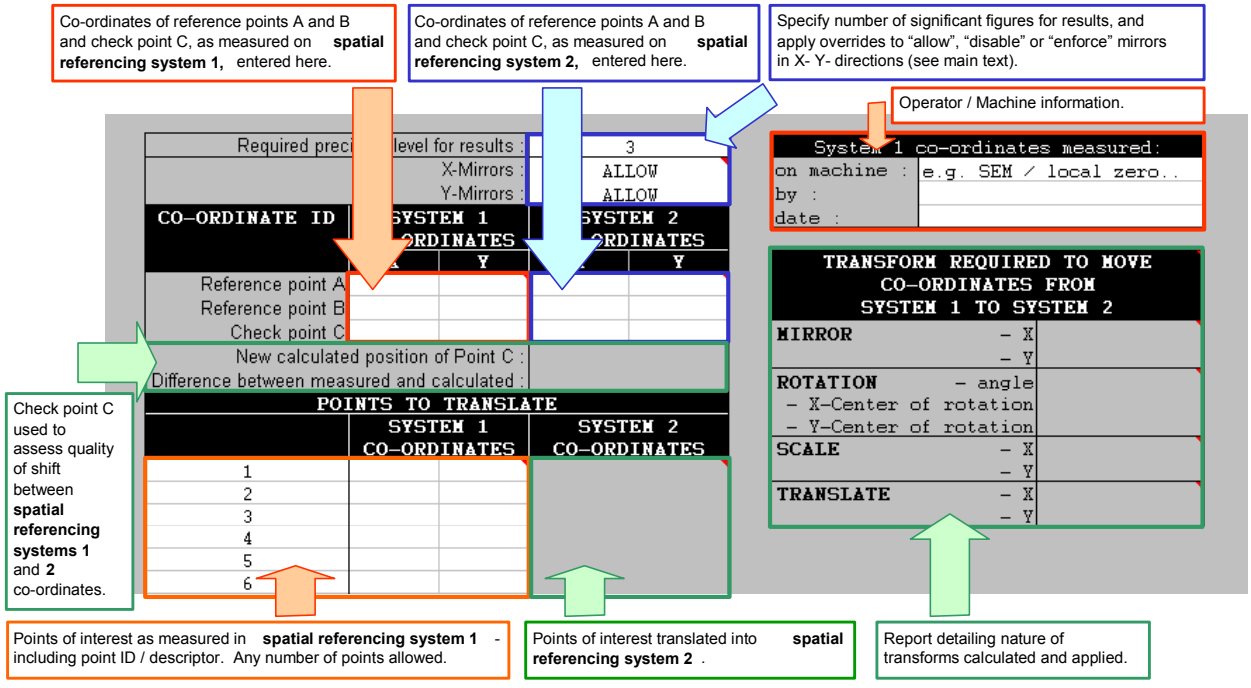

Figure 5. Layout of transform calculator worksheet (2DTraC.xlt), showing ranges where data are input, and results calculated.

Figure 5 shows the layout of the transform calculator worksheet. 2DTraC expects to find data in the cells with white fills. Enter the co-ordinates of the 2 reference points and a check point (if available) as measured in the spatial referencing systems of both microscopes/instruments, and the co-ordinates of the points of interest as measured using the first microscope/instrument. At

this stage you can also specify the number of significant figures to which the results will be reported to. The pull down lists for X-mirrors/Y-mirrors allow the user to override mirror transforms that may be incorrectly determined by the add-in if there is a high angular difference between the two spatial referencing systems (see footnote, and below for more details). "Allow" enables 2DTraC to determine and apply mirror planes automatically. "Enforce" forces 2DTraC to apply a mirror plane, and "Disable" prevents 2DTraC from applying a mirror plane in the specified direction. Once the co-ordinates have been input and these selections made, select "update" on the 2DTraC menu to calculate the co-ordinates of the points of interest in the spatial referencing system of the new microscope/instrument

4.2 APPLICATION METHODOLOGY

The functions in **2DTraC** use the co-ordinates of two reference points (A and B) measured in the two spatial reference systems of interest to work out the transforms required to convert the co-ordinates measured in analytical system 1 to those of analytical system 2 (Figure 6A and F), in the following manner:

1. Reference points A and B on the sample define the start and end points of line AB, which in any given spatial referencing system has:
   - a sense of direction (start A — end B).
   - a gradient (angle relative to horizontal).
   - a length.
   - an absolute position relative to the origin.

   The measured co-ordinates of reference points A and B on the same sample in two different spatial referencing systems are illustrated in Figure 6A and F.

2. **2DTraC** compares the vector of line AB in spatial referencing system 1 (line A1B1) with that in spatial referencing system 2 (line A2B2) to calculate if any **mirror transforms** are required. These mirror transforms account for differences in the sense of direction of X-Y axes in different spatial referencing systems. The nature of any required mirror transforms is stored and applied to the co-ordinates of points A1B1 to give co-ordinates A' and B', and hence new line A'B' (Figure 6B).

   The algorithm **2DTraC** uses to calculate mirror transforms uses the gradients and senses of direction of lines A1B1 and A2B2 to determine which quadrant the line's vector is in (Figure 7) and, on the basis of this, determines whether X-axis parallel and/or Y-axis parallel mirror transforms are required. However, in cases where there is significant rotation of the sample between two spatial referencing systems, it is possible that **2DTraC** may incorrectly interpret rotation as a mirror plane (Figure 6C). This may lead to the application of mirror plane(s) where none are required, or to their omission where they are required. Although these errors only occur at relatively high angles of rotation, **2DTraC** contains a mechanism by which the user can override the software and specify that mirror planes be independently enforced or disabled in the X- and/or the Y- directions. This means that, in cases where there is significant[1] rotation of the sample, the user can undertake a relatively simple comparison of the senses of direction of the X- and Y- axes in both spatial referencing systems and then enforce/disable the mirror planes accordingly.

[1] The magnitude of rotation required to cause these errors is dependent upon the relative positions of the reference points on the sample. In the case of the samples shown in Figure 6 and Figure 7, either *c*.30°-clockwise or *c*.60°-counter-clockwise rotation would be required to cause the algorithm to misinterpret rotation as mirror planes. This is unlikely to occur in most applications, as alignment of samples by eye is sufficiently sensitive to reduce errors in rotational positioning to <5°.

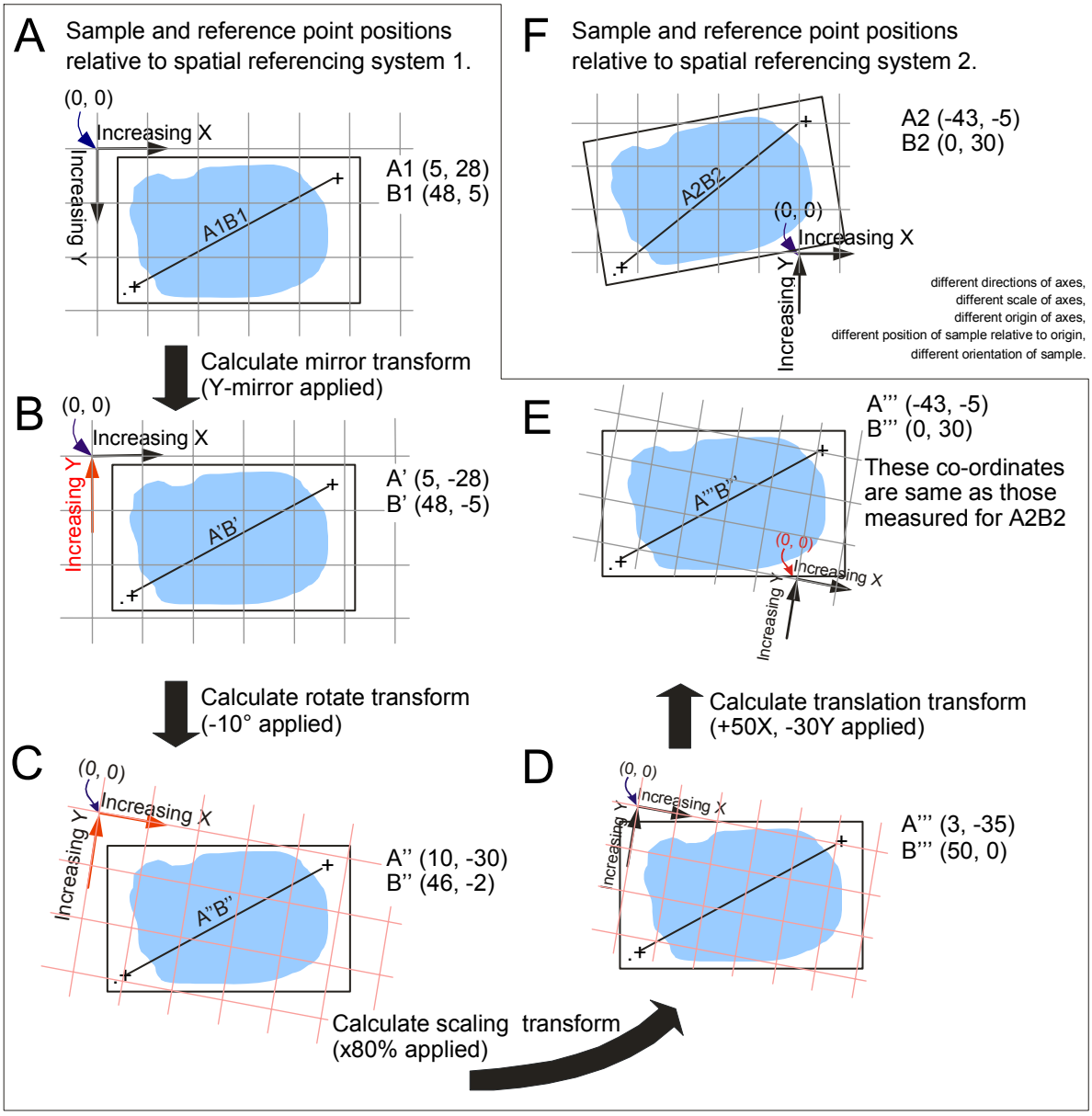

Figure 6. Illustration of the mechanism by which 2DTraC calculates and applies a series of transforms to convert co-ordinates between spatial referencing systems 1 (A) and 2 (F). B-D illustrate the effect of each of the transforms on the new calculated co-ordinates of reference points A and B.

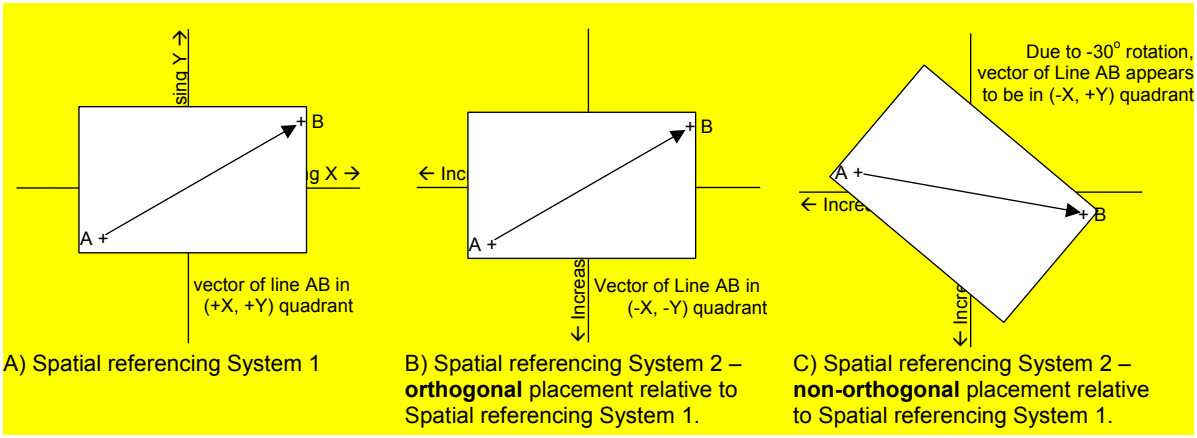

Figure 7. Illustration of the quadrants of line AB in different spatial referencing systems.
In spatial referencing system 1, line AB is oriented in the +X, +Y quadrant (A). In spatial referencing system 2 line AB is oriented in the -X, -Y quadrant (B). Conversion between spatial referencing systems 1 and 2 would therefore require application of mirror transforms parallel to both the X- and Y- axes. C shows the sample placed non-orthogonally in spatial referencing system 2. The rotation of the sample has been sufficient to move the apparent vector of line AB from the (-X, -Y) to the (-X, +Y) quadrant. The algorithm in 2DTraC would misinterpret this, and in converting between spatial referencing systems 1 and 2, would only apply a mirror plane parallel to the X-axis, rather than the required X- and Y- mirror planes.

3. The gradient of line A'B' is then compared with that of line A2B2 to work out the rotational correction required. **2DTraC** stores the required angle of rotation, and applies it to co-ordinates A' and B' to give co-ordinates A'' and B'' (Figure 6C).

   Uncorrected errors in rotational placement of less than 1° would generate errors of 100's microns in calculated positions over the distances appropriate to the sizes of typically analysed samples (typically up to *c*.4 cm; Figure 8). Therefore, this rotational correction has removed the requirement for the manufacture of highly engineered orthogonal specimen holders (which would have needed to be capable of orienting a sample to a precision of much better than 1°).

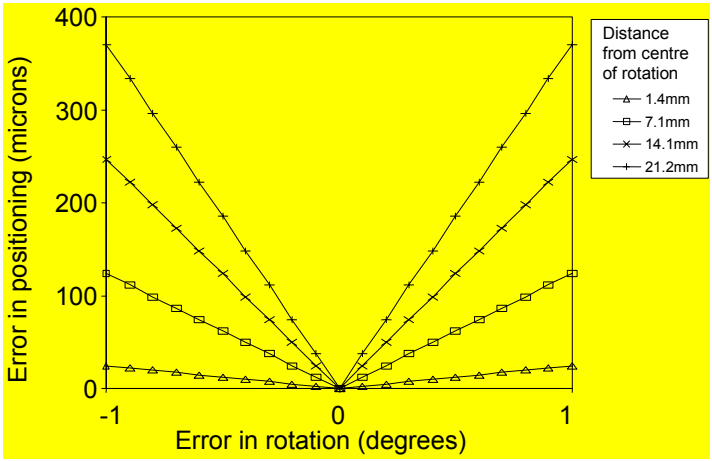

Figure 8. Graph showing the impact of small uncorrected rotational errors on the calculated positions of features of varying distance from the centre of rotation. On the scale of a polished section (c.50 mm in length, it is easy to generate errors of several hundred microns, for relatively small errors in rotation.

4. **2DTraC** then calculates X- and Y- scaling factors such that the lengths of the X- and Y- components of line A''B'' are the same as those for line A1B1. These scaling factors are stored, and applied to co-ordinates A'' and B'' to give co-ordinates A''' and B''' (Figure 6D). This correction accounts for differences in units of measurement between pieces of equipment, and also for any differences in absolute terms that may occur between different pieces of equipment (*i.e.* one piece of equipment's idea of a micron may slightly differ from that of another!).
5. **2DTraC** then calculates and stores the X- and Y- translations required to move line A'''B''' to the co-ordinates of line A2B2. By definition, application of these translations to points A''' and B''' gives the co-ordinates of points A2 and B2 (Figure 6E).

Finally, **2DTraC** applies the stored (mirror, rotational, stretch and translational) transforms to the check point (C), and to all the other co-ordinates in the list of points of interest to calculate the co-ordinates of these points in analytical system 2. If the transforms are correct then the calculated position of check point C should closely match it's measured position.

4.3 QUALITY OF CALCULATED VERSUS MEASURED CO-ORDINATES

In order to assess the quality/accuracy of transforms calculated by **2DTraC**, the transforms required to convert between the different spatial referencing systems of the analytical equipment tested have been calculated using points 1 and 3 of the test sample as the two reference points. Table 1 gives a summary of the average, minimum and maximum differences between the calculated and measured positions of the other reference points on the test sample (points, 2, 4, 5, 6, and 7). Point by point comparisons are included in Appendix Table 1. 1. The calculated differences between measured and calculated positions range between 0.2 and 68 microns, with the average difference between the calculated and measured position for a given conversion between 12.6-25.1 microns. These differences are well within the 100 microns range which was the target this work was originally aiming to achieve.

		system converted **TO**			
		OPT-7	SEM-7	CAM-5	LAS-1
system converted **FROM**	OPT-7	AVE	15.7	25.1	25.0
		MIN	0.7	5.4	1.7
		MAX	41.3	67.5	54.6
	SEM-7	15.6	AVE	23.5	23.9
		0.4	MIN	0.2	1.0
		41.3	MAX	48.0	43.0
	CAM-5	25.1	23.3	AVE	12.6
		5.4	0.1	MIN	7.1
		67.3	48.0	MAX	20.6
	LAS-1	24.9	23.8	12.6	AVE
		1.6	1.4	7.1	MIN
		54.4	42.9	20.6	MAX

Table 1. Average, minimum and maximum magnitude of difference between calculated and measured values (in microns, ignoring points 1 and 3 which are used to calculate the transform). LAS-1, CAM-5, SEM-7 and OPT-7 refer to the test runs from which the co-ordinates used in the comparison have been taken (see Appendix Table 1. 1 for detailed data).

The quality of the results generated essentially reflect the precision to which the positions of the reference co-ordinates were measured. The individual algorithms used to calculate transforms in **2DTraC** are robust, however the application assumes that the reference co-ordinates are correct

and then works through the conversion process in a series of steps (in the order mirrors→rotation→scaling→translation). Therefore, an error in measured co-ordinate(s) for a given reference point(s) will be interpreted as some combination of rotational, +/- scaling, +/- translational transforms, which are then applied to all the points of interest, resulting in systematic errors across the sample proportional to the original error of measurement. Figure 9 demonstrates the effect that small errors in the measured positions of the reference points can have on the positions of calculated points over the scale of a typical sample (up to 50mm).

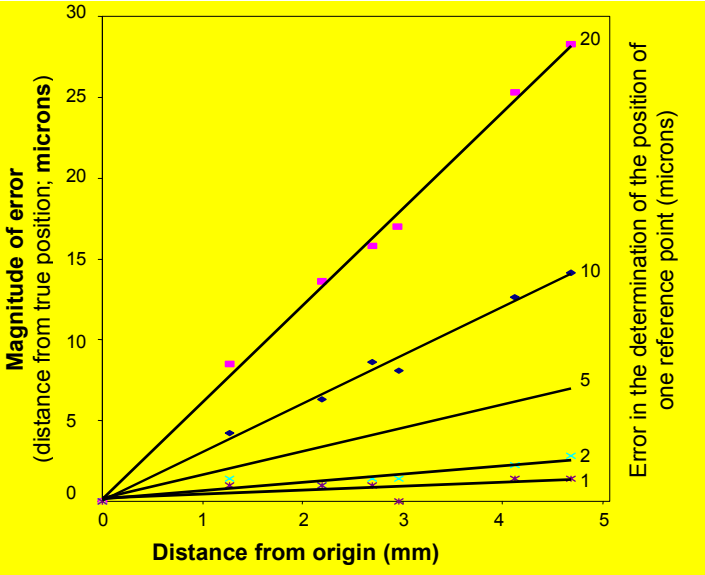

Figure 9. Graph showing the impact of small errors in the measured positions of reference points on their calculated positions when transformed between spatial referencing systems.

Figure 9 was constructed using data calculated by assuming that the co-ordinates of the two reference points have been measured correctly in spatial referencing system 1 (co-ordinates A1 and B1 of Figure 6), and that reference point A has been measured correctly in spatial referencing system 2 (co-ordinate A2). A progressively increasing error was applied to the position of reference point B in system 2 (co-ordinate B2), with curves shown for errors of 1, 2, 5, 10 and 20 microns in both X- and Y-directions. The magnitude of the resulting error is seen to be proportional to the error of the measurment and the distance from point A. *However, in this model only one of the four pairs of co-ordinates used to calculate transforms is erroneous: errors in the measurements of all four reference point positions are likely to compound.* Using laser markings *c*.30 microns in diameter, measurements of the test sample indicate that the positions of the test markings are repeatable to within about 10 microns. Inspection of Figure 9, indicates that measurement errors of this magnitude can easily compound to give errors in the calculated results of >50 microns.

4.4 SUMMARY

In summary, a robust Excel Add-in ('**2DTraC**') has been developed that will calculate and apply the transforms required to convert co-ordinates of points of interest between the spatial referencing systems of different pieces of analytical equipment. The development of this application has removed the need for development and prototyping of highly engineered sample holders for each piece of equipment. However, in order to generate precise transforms, **2DTraC** requires that the reference co-ordinates are known sufficiently precisely - errors of 10 microns on each of the reference point measurements will readily compound to give errors in the calculated results of >50 microns.

5 Conclusions

5.1 POINTS OF REFERENCE

- differences in the spatial referencing systems of different pieces of equipment include differences in the relative positions of origins, and senses of direction of the X- and Y-axes. Furthermore, it is not possible to consistently and precisely load a sample in the same position on all instruments/microscopes between analytical runs. This means that a machine specific feature (such as the corner of the sample holder) can not be used as a reference point.
- limitations in the sample holders/imaging systems of some instruments/microscopes also prevent the use of a consistent feature of the sample, such as its corner as a reference point.

Therefore, any reference point for spatial referencing and point relocation must be marked on the sample surface.

5.2 SAMPLE MARKING

- tests were conducted using a 'blank' glass slide marked using both ink crosses (approximately 2 mm in size) and laser-burned pits (approximately 30 microns diameter).
- as they are visible to the naked eye, the ink crosses are easy to locate and can be readily brought within the field of view of the various pieces of analytical equipment. However, they are too large to enable sufficiently accurate repeat measurements of their positions due to operator bias/error in determining the centre of the feature.
- it proved possible to relocate the laser-burned pits with a reasonable degree of precision, as ambiguity as to the centre of the feature was reduced. However, the small size of these features means that they would be relatively difficult to locate without some larger scale feature that can be used to bring them into the field of view.

Therefore, it is suggested that the best means of marking reference points is to use ink crosses and then to add laser-burned pits to the centres of the crosses for more precise co-ordinate measurement. For consistency it is recommended that these marked be placed in the 'bottom-left' of the sample (which can be set as a 'local zero' [co-ordinates (0,0)]), and at some other point as far from the bottom-left corner as the sample will permit (e.g. the 'top-right' corner of the sample surface).

5.3 SAMPLE TRANSFER AND SPATIAL TRANSFORMS

Once the sample surface has been marked with two reference points their co-ordinates should be measured on the first instrument the sample is loaded onto. Points of interest can then be located using this first instrument, and their co-ordinates recorded.

When the sample is transferred to a 'new' instrument, the co-ordinates of the points of interest need to be converted into the spatial referencing system of the new instrument. To do this, the co-ordinates of the reference points should be measured using the new instrument. 2DTraC (a custom Excel Add-in) can then be used to calculate and apply the transform(s) required to convert the points of interest into the spatial referencing system of the new instrument.

The development of this application has removed the need for development and prototyping of highly engineered sample holders for each piece of equipment. However, in order to generate precise transforms, **2DTraC** requires that the reference co-ordinates are known sufficiently precisely - errors of 10 microns on each of the reference point measurements will readily compound to give errors in the calculated results of >50 microns.

Appendix 1 Results of Test Runs

Appendix Table 1. 1 Details of conditions for each co-ordinate determination run

Run	Date	Operator	Target	Comments
SEM-1	27/07/01	JEB	ink crosses	Central points of the cross marks were located and recorded in a relatively crude manner.
SEM-2	28/07/01	JEB	ink crosses	the stage was sent (using the "stage goto" function) to the co-ordinates of the centre of each cross as recorded during run 1. This relocated the central points satisfactorily. The positions of the centres of the crosses were then located and recorded more precisely. The addition of a square to the default crosshairs at this stage made it possible to centre the cross to a greater perceived level of accuracy.
SEM-3	29/07/01	JEB	ink crosses	during runs 1 and 2, it became apparent that the stage was not quite positioned truly orthogonal to the SEM sample chamber. Therefore, the stage orientation was adjusted using the SEM rotation control, and the seven crosses were relocated using the co-ordinates from run 2. Although the degree of rotation was small (less than 1 degree), this introduced a shift in the positions of the points of between 20 to 100□m depending on the position of the point relative to the centre of the SEM stage. this highlights the importance of precise orthogonal positioning of samples, without a means of correcting for rotational errors.
SEM-4	30/07/01	JEB	ink crosses	the sample was left in the SEM chamber over the weekend between run 3 and run 4. The centres of the crosses were relocated from the co-ordinates recorded during run 3 (using "stage goto" function), followed by manual adjustment of the stage position. This gave x-y co-ordinates within c.50 microns (typically <20 microns) of the co-ordinates recorded during run 3.
SEM-5	28/08/01	JEB	laser points	centre point function set to not allow beam shifts for minor re-positioning of scan area ("stage only"). Something wrong with these values (?sample shift on stage). Data excluded from compiled data list
SEM-6	28/08/01	JEB	laser points	
SEM-7	28/08/01	JEB	laser points	centres of each point relocated as precisely as possible using photos taken during run 2 (any variation in co-ordinates between runs 2 and 3 is machine rather than operator effect)
SEM-8	28/08/01	JEB	laser points	BSEM (relatively poor images). Points located using outline of laser hole rather than centre of burn mark. Makes a slight difference with the more irregular shaped burn marks.
SEM-9	07/09/01	JEB	laser points	Secondary SEM, points located using images generated on 28-08-01 to identify same "centres" of points. Reference points measured and used together with co-ordinates from optical microscope (OPT-9) to calculate transform and position of other points.
SEM-10	07/09/01	JEB	laser points	Secondary SEM, points located using images generated on 28-08-01 to identify same "centres" of points. Reference points measured and used together with co-ordinates from optical microscope (OPT-9) to calculate transform and position of other points. Sample rotated by 30 degrees from orthogonal.
SEM-11	07/09/01	JEB	laser points	Secondary SEM, points located using images generated on 28-08-01 to identify same "centres" of points. Reference points measured and used together with co-ordinates from optical microscope (OPT-9) to calculate transform and position of other points. Sample rotated by 30 degrees from orthogonal.
OPT-1	july-01	JMcK	ink crosses	Cameca co-ordinates
OPT-2	july-01	MTS	ink crosses	Cameca co-ordinates
OPT-3	july-01	JMcK	ink crosses	local co-ordinates
OPT-4	july-01	MTS	ink crosses	local co-ordinates
OPT-5	28/08/01	JEB	laser points	Cameca co-ordinates
OPT-6	28/08/01	JEB	laser points	local co-ordinates
OPT-7	aug-01	MTS	laser points	Cameca co-ordinates
OPT-8	aug-01	MTS	laser points	local co-ordinates
OPT-9	05/09/01	MTS	laser points	local co-ordinates
OPT-10	05/09/01	MTS	laser points	Cameca co-ordinates, co-ordinates from run OPT-9 used together with measured positions of points 1 and 3 to calculate co-ordinates, which were then located and measured.
OPT-11	05/09/01	MTS	laser points	Cameca co-ordinates, co-ordinates from run OPT-9 used together with measured positions of points 1 and 3 to calculate co-ordinates, which were then located and measured.
CAM-1	july-01	JMcK	ink crosses	based on co-ordinates recorded during run OPT-1
CAM-2	july-01	JMcK	ink crosses	
CAM-3	july-01	JMcK	ink crosses	
CAM-4	july-01	JMcK	ink crosses	
CAM-5	aug-01	MTS	laser points	
CAM-6	05/09/01	MTS	laser points	co-ordinates from run OPT-9 used together with measured positions of points 1 and 3 to calculate co-ordinates, which were then located and measured.
LAS-1	aug-01	SCh	laser points	

Appendix Table 1. 2 Compilation and comparison of the co-ordinates of the reference points on the test sample.

3 pages

		COMPARISON OF MEASURED SEM DATA ON DIFFERENT RUNS														COMPARISON OF MEASURED AND CALCULATED SEM DATA													
	Run	SEM-1		SEM-2		SEM-3		SEM-4		SEM-5		SEM-6		SEM-7		SEM-8		SEM-9 calculated		SEM-9 measured		SEM-10 calculated		SEM-10 measured		SEM-11 calculated		SEM-11 measured	
	Target	Ink Crosses		Ink Crosses		Ink Crosses		Ink Crosses		Laser Points		Laser Points		Laser Points		Laser Points				Laser Points				Laser Points				Laser Points	
	Position	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
MEASURED CO-ORDINATES	1	70.834	59.043	70.834	59.070	70.858	59.002	70.864	59.021	64.122	61.782	64.123	61.891	64.123	61.891	64.122	61.894	71.951	61.049	71.951	61.049	66.515	65.342	66.515	65.342	62.317	68.829	62.317	68.829
	2	71.057	37.138	71.050	37.140	70.999	37.054	70.981	37.032	64.388	39.881	64.393	39.990	64.395	39.992	64.396	39.992	72.009	39.103	72.015	39.113	72.400	44.208	72.394	44.229	73.156	49.745	73.142	49.788
	3	29.817	36.667	29.811	36.665	29.769	36.762	29.756	36.808	23.188	39.248	23.193	39.361	23.194	39.360	23.200	39.358	30.748	38.882	30.773	38.882	32.696	33.036	32.696	33.036	37.330	29.269	37.330	29.269
	4	29.642	58.586	29.656	58.601	29.700	58.696	29.719	58.746	22.978	61.189	22.987	61.300	22.988	61.300	22.988	61.300	30.754	60.883	30.773	60.873	26.858	54.240	26.877	54.196	26.520	48.433	26.570	48.370
	5	50.546	41.438	50.552	41.443	50.522	41.443	50.509	41.425	43.861	44.177	43.868	44.192	43.868	44.190	43.868	44.192	51.478	43.532	51.492	43.535	51.438	43.023	51.497	43.040	53.098	43.509	53.150	43.528
	6	59.656	53.036	59.654	53.031	59.656	52.988	59.677	52.966	52.971	55.824	52.977	55.835	52.977	55.835	52.981	55.832	60.694	55.090	60.701	55.090	57.250	56.610	57.276	56.614	55.443	58.106	55.457	58.116
	7	41.993	52.775	42.001	52.770	42.022	52.788	42.027	52.803	35.287	55.482	35.289	55.510	35.291	55.503	35.291	55.503	43.041	54.933	43.056	54.928	40.279	51.769	40.294	51.758	40.145	49.291	40.163	49.281
LOCAL CO-ORDINATES - POSITION 1 AS ORIGIN, (SEM data converted to microns)	1	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000
	2	00223	-21905	00216	-21930	00141	-21948	00117	-21989	00266	-21901	00270	-21901	00272	-21899	00274	-21902	00058	-21946	00064	-21936	05885	-21134	05879	-21113	10839	-19084	10825	-19041
	3	-41017	-22376	-41023	-22405	-41089	-22240	-41108	-22213	-40934	-22534	-40930	-22530	-40929	-22531	-40922	-22536	-41203	-22167	-41203	-22167	-33819	-32306	-33819	-32306	-24987	-39560	-24987	-39560
	4	-41192	-00457	-41178	-00469	-41158	-00306	-41145	-00275	-41144	-00593	-41136	-00591	-41135	-00591	-41134	-00594	-41197	-00166	-41178	-00176	-39657	-11102	-39638	-11146	-35797	-20396	-35747	-20459
	5	-20288	-17605	-20282	-17627	-20336	-17559	-20355	-17596	-20261	-17605	-20255	-17699	-20255	-17701	-20254	-17702	-20473	-17517	-20459	-17514	-15077	-22319	-15018	-22302	-09219	-25320	-09167	-25301
	6	-11178	-06007	-11180	-06039	-11202	-06014	-11187	-06055	-11151	-05958	-11146	-06056	-11146	-06056	-11141	-06062	-11257	-05959	-11250	-05959	-09265	-08732	-09239	-08728	-06874	-10723	-06860	-10713
	7	-28841	-06268	-28833	-06300	-28836	-06214	-28837	-06218	-28835	-06300	-28834	-06381	-28832	-06388	-28831	-06391	-28910	-06116	-28895	-06121	-26236	-13573	-26221	-13584	-22172	-19538	-22154	-19548
	Runs compared			SEM-1 & SEM-2		SEM-2 & SEM-3		SEM-3 & SEM-4						SEM-6 & SEM-7		SEM-7 & SEM-8				SEM-9 calc & meas				SEM-10 calc & meas				SEM-11 calc & meas	
	Target			Ink Crosses		Ink Crosses		Ink Crosses						Laser Points		Laser Points				Laser Points				Laser Points				Laser Points	
	Position			X	Y	X	Y	X	Y					X	Y	X	Y			X	Y			X	Y			X	Y
COMPARISON OF EQUIVALENT RUN DATA	1			0	0	0	0	0	0					0	0	0	0												
	2			-7	-25	-75	-18	-24	-41					2	2	2	-3			6	10			-6	21			-14	43
	3			-6	-29	-66	165	-19	27					1	-1	7	-5												
	4			14	-12	20	163	13	31					1	0	1	-3			19	-10			19	-44			50	-63
	5			6	-22	-54	68	-19	-37					0	-2	1	-1			14	3			59	17			52	19
	6			-2	-32	-22	25	15	-41					0	0	5	-6			7	0			26	4			14	10
	7			8	-32	-3	86	-1	-4					2	-7	1	-3			15	-5			15	-11			18	-10
	Comments			Same operator on successive runs. Poor repeatability of positioning (20-50micron error) due to large target size.		Same operator on successive runs. Poor repeatability of positioning (20-50micron error) due to the compounded effects of a small change in rotation (<0.5 degrees) and large target size.		Same operator on runs separated by 3 days. Poor repeatability of positioning (20-50micron error) due to large target size.						Same operator on successive runs. Very good repeatability of results (<10 micron error) with smaller target size.		Same operator on successive runs. Very good repeatability of results (<10 micron error) with smaller target size.				Sample approximately orthogonal, rotation correction of 0.2 degrees applied. Calculated points generated using data from run OPT-9, and measured reference positions of points 1 and 3. Good agreement between calculated and measured positions				Sample rotated c.15degrees. Calculated points generated using data from run OPT-9, and measured reference positions of points 1 and 3. Good agreement between calculated and measured positions, but less good than for run SEM-9. Probably due to compounding of errors over increased magnitude of transform.				Sample rotated c.30degrees. Calculated points generated using data from run OPT-9, and measured reference positions of points 1 and 3. Good agreement between calculated and measured positions, but less good than for run SEM-10. Probably due to compounding of errors over increased magnitude of transform.	

Appendix Table 1.2. Compilation and comparison of the measured and calculated co-ordinates of marked points on the test sample.

	COMPARISON OF MEASURED OPTICAL MICROSCOPE DATA ON DIFFERENT RUNS																COMPARISON OF MEASURED AND CALCULATED OPTICAL MICROSCOPE DATA							
Run	OPT-1		OPT-2		OPT-3		OPT-4		OPT-5		OPT-6		OPT-7		OPT-8		OPT-9		OPT-10 calculated		OPT-10 measured		OPT-11 calculated		OPT-11 measured	
Target	Ink Crosses		Ink Crosses		Ink Crosses		Ink Crosses		Laser Points		Laser Points		Laser Points		Laser Points		Laser Points		Laser Points		Laser Points		Laser Points		Laser Points	
Position	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	X	X	X	X	X	X	X	X	X
1	-19989	-26443	-19988	-26015	00000	00000	00000	00000	-20047	-26035	00000	00000	-20019	-25988	00000	00000	00000	00000	-20180	-26070	-20180	-26070	-20073	03226	-20073	03226
2	-19991	-04494	-20023	-04039	-00015	21937	00025	21932	-20027	-04115	00020	21922	-20010	-04060	00008	21919	00012	21925	-20160	-04148	-20160	-04138	-20139	25141	-20134	25148
3	21231	-04494	21247	-03973	41206	21937	41228	22017	21200	-04052	41240	21979	21210	-03980	41229	22006	41234	22014	21075	-04062	21075	-04062	21082	25375	21082	25375
4	21129	-26428	21180	-25955	41126	00009	41199	00041	21108	-26014	41145	00011	21120	-25947	41156	00039	41158	00034	20992	-26037	20992	-26048	21094	03418	21080	03398
5	00512	-09004	00507	-08549	20486	17430	20510	17437	00476	-08609	20519	17423	00489	-08556	20510	17430	20509	17435	00339	-08636	00339	-08636	00372	20724	00372	20724
6	-08736	-20508	-08703	-20090	11261	05927	11315	05912	-08768	-20120	11282	05911	-08751	-20071	11277	05913	11265	05917	-08897	-20155	-08909	-20155	-08814	09177	-08829	09180
7	08903	-20415	08891	-19945	28855	05997	28922	06009	08858	-20034	28897	05996	08877	-19971	28904	06015	28902	06018	08744	-20063	08734	-20059	08820	09339	08805	09340
1	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000
2	-00002	21949	-00035	21976	-00015	21937	00025	21932	00020	21920	00020	21922	00009	21928	00008	21919	00012	21925	00020	21922	00020	21932	-00066	21915	-00061	21922
3	41220	21949	41235	22042	41206	21937	41228	22017	41247	21983	41240	21979	41229	22008	41229	22006	41234	22014	41255	22008	41255	22008	41155	22149	41155	22149
4	41118	00015	41168	00060	41126	00009	41199	00041	41155	00021	41145	00011	41139	00041	41156	00039	41158	00034	41172	00033	41172	00022	41167	00192	41153	00172
5	20501	17439	20495	17466	20486	17430	20510	17437	20523	17426	20519	17423	20508	17432	20510	17430	20509	17435	20519	17434	20519	17434	20445	17498	20445	17498
6	11253	05935	11285	05925	11261	05927	11315	05912	11279	05915	11282	05911	11268	05917	11277	05913	11265	05917	11283	05915	11271	05915	11259	05951	11244	05954
7	28892	06028	28879	06070	28855	05997	28922	06009	28905	06001	28897	05996	28896	06017	28904	06015	28902	06018	28924	06007	28914	06011	28893	06113	28878	06114
Runs compared			OPT-1 & OPT-2				OPT-2 & OPT-4		OPT-5 & OPT-6		OPT-5 & OPT-7		OPT-7 & OPT-8		OPT-6 & OPT-8						OPT10 calc & meas				OPT11 calc & meas	
Target			Ink Crosses				Ink Crosses		Laser Points		Laser Points		Laser Points		Laser Points						Laser Points				Laser Points	
Position	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y					X	Y			X	Y
1			0	0			0	0	0	0	0	0	0	0	0	0										
2			-33	27			60	-44	0	2	-11	8	-1	-9	-12	-3					0	10			5	7
3			15	93			-7	-25	-7	-4	-18	25	0	-2	-11	27										
4			50	45			31	-19	-10	-10	-16	20	17	-2	11	28					0	-11			-14	-20
5			-6	27			15	-29	-4	-3	-15	6	2	-2	-9	7										
6			32	-10			30	-13	3	-4	-11	2	9	-4	-5	2					-12	0			-15	3
7			-13	42			43	-61	-8	-5	-9	16	8	-2	7	19					-10	4			-15	1
Comments			Different operators on different days. Poor repeatability largely due to large target size plus possible operator bias/ instrumental drift?				Same operator different days. Poor repeatability due to large target size - but errors comparable with those seen for different operators on different days (see comparison OPT-1 & OPT-2).		Same operator on successive runs. Good repeatability due to reduced target size.		Different operators on different days. Note reduced repeatability, compared to same operator on successive runs (see comparisons OPT-5 & OPT-6, and OPT-7 & OPT-8).		Same operator on successive runs. Good repeatability due to reduced target size.		Different operators on different days. Note reduced repeatability, compared to same operator on successive runs (see comparisons OPT-5 & OPT-6, and OPT-7 & OPT-8).						Good agreement between calculated and measured co-ordinates.				Good agreement between calculated and measured co-ordinates.	

Appendix Table 1.2. Compilation and comparison of the measured and calculated co-ordinates of marked points on the test sample.

	COMPARISON OF MEASURED EPMA DATA ON DIFFERENT RUNS										COMPARISON OF MEASURED AND				COMPARISON OF MEASURED LAMP-	
Run	CAM-1		CAM-2		CAM-3		CAM-4		CAM-5		CAM-6 calculated		CAM-6 measured		LAS-1	
Target	Ink Crosses		Ink Crosses		Ink Crosses		Ink Crosses		Laser Points		Laser Points		Laser Points		Laser Points	
Position	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
1	-20058	-26515	-20063	-26515	-20038	-26527	-20040	-26519	-20149	-26121	-20230	-11760	-20230	-11760	0.000	0.000
2	-20104	-04588	-20087	-04579	-20086	-04583	-20077	-04585	-20233	-04220	-20348	10149	-20333	10206	0.105	21.900
3	21278	-04437	21258	-04442	21226	-04426	21257	-04435	21080	-03936	20965	10512	20965	10512	-41.196	22.190
4	21207	-26365	21195	-26366	21200	-26363	21202	-26361	21083	-25875	21012	-11439	21005	-11509	-41.229	0.247
5	00441	-09046	00458	-09040	00457	-09037	00459	-09047	00317	-08614	00207	05795	00207	05795	-20.433	17.519
6	-08787	-20586	-08781	-20571	-08763	-20577	-08784	-20583	-08890	-20166	-08973	-05775	-08975	-05773	-11.261	5.964
7	08891	-20450	08874	-20441	08876	-20441	08881	-20439	08773	-19989	08692	-05558	08694	-05579	-28.916	6.148
1	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000
2	-00046	21927	-00024	21936	-00048	21944	-00037	21934	-00084	21901	-00118	21909	-00103	21966	00105	21900
3	41336	22078	41321	22073	41264	22101	41297	22084	41229	22185	41195	22272	41195	22272	-41196	22190
4	41265	00150	41258	00149	41238	00164	41242	00158	41232	00246	41242	00321	41235	00251	-41229	00247
5	20499	17469	20521	17475	20495	17490	20499	17472	20466	17507	20437	17555	20437	17555	-20433	17519
6	11271	05929	11282	05944	11275	05950	11256	05936	11259	05955	11257	05985	11255	05987	-11261	05964
7	28949	06065	28937	06074	28914	06086	28921	06080	28922	06132	28922	06202	28924	06181	-28916	06148

Runs compared		CAM-1 & CAM-2		CAM-2 & CAM-3		CAM-3 & CAM-4			CAM-5 & CAM-6		CAM-6 calc & meas	
Target		Ink Crosses		Ink Crosses		Ink Crosses			Laser Points		Laser Points	
Position		X	Y	X	Y	X	Y		X	Y	X	Y
1		0	0	0	0	0	0		0	0		
2		22	9	-24	8	11	-10		-19	65	15	57
3		-15	-5	-57	28	33	-17		-34	87		
4		-7	-1	-20	15	4	-6		3	5	-7	-70
5		22	6	-26	15	4	-18		-29	48		
6		11	15	-7	6	-19	-14		-4	32	-2	2
7		-12	9	-23	12	7	-6		2	49	2	-21
Comments		Same operator on successive runs. Reasonable repeatability in this case.		Same operator on successive runs. poor repeatability in this case.		Same operator on successive runs. Poor-moderate repeatability in this case.			Same operator on successive runs. Poor-moderate repeatability in this case.		Calc data derived using data from run OPT-9, and measured reference points 1 and 3. Reasonable agreement between measured and calculated positions, but some fairly large errors.	

Appendix Table 1.2. Compilation and comparison of the measured and calculated co-ordinates of marked points on the test sample.

Appendix 2 2DTraC Functions

Provided the 2DTraC add-in is loaded, the functions it contains are available to any Excel worksheet. The syntax of the functions input arguments, and outputs of the individual functions are documented below. Optional inputs are printed in *Italics*.

```
================================================================================
CalcTransform
SYNTAX:  CalcTransform(Line1X1, Line1Y1, Line1X2, Line1Y2, Line2X1, Line2Y1
         Line2X2, Line2Y2, [Result])

CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   will take 2 lines defined by start and end coordinates, and calculate mirrors,
   rotation, scaling and translation required to move line 2 to position of line 1
NOTES:
   reads state of gblDisableXMirror and gblDisableYMirror during execution
   030102 updated to account for disabling/enforcing of X-Y mirror transforms
          gintmirror has become too complex as a bitmask, therefore empahsis for
          storing if mirrors are allowed/disabled/enabled shifted to global
          variables gintXMirrorState and gintYMirrorState, which are repeatedly
          tested during this function
   030102 other minor tweaks
INPUTS:
   Line1X1,Line1Y1       [required] start (x,y) coordinates of line 1
   Line1X2,Line1Y2       [required] end (x,y) coordinates of line 1
   Line2X1,Line2Y1       [required] start (x,y) coordinates of line 2
   Line2X2,Line2Y2       [required] end (x,y) coordinates of line 2
   Result                [optional] how result is returned
                           0   (default) returns string containing information
                               on required translation
CALLS:
   Function CalcMirror            (this module)
   Function CalcRotate            (this module)
   Function Rotate                (this module)
   Function CalcScaling           (this module)
   Function Scaling               (this module)
   Function CalcTranslate         (this module)
   Function Translate             (this module)
OUPUTS:
   string containing information about how to transform line1 to position of line 2
   also updates global variables:
       gintmirrors, gdblRotation, gdblScaleX, gdblScaleY, gdblShiftX, gdblShiftY
   which are used by update function to present user with this information in a
   more digestible format.
```

==========

Transform

SYNTAX: Transform(posX, posY, Mirror, Rotation, XCenter, YCenter, XScale, YScale, XShift, YShift)

```
CREATE DATE:   29-08-01
BY:            Jon Bouch
PURPOSE:
   will apply rotational, scaling and translational transforms using parameters
   calculated with CalcTransform (and associated functions)
NOTES:
   reads state of gblDisableXMirror and gblDisableYMirror during execution
   030102 updated to account for disabling/enforcing of X-Y mirror transforms
          gintmirror has become too complex as a bitmask, therefore emphasis for
          storing if mirrors are allowed/disabled/enabled shifted to global
          variables gintXMirrorState and gintYMirrorState, which are repeatedly
          tested during this function
   030102 other minor tweaks
INPUTS:
   posX, posY            [required] co-ordinates of point to be transformed
   Mirror As Integer     [required] nature of mirror transforms to be applied:
                                 0 = no mirror required
                                 1 = apply X-mirror
                                 2 = apply Y-mirror
                                 3 = apply Y-mirror and X-mirror
   rotation              [required] angle of rotation in degrees (anticlockwise)
   XCenter, YCenter      [required] centre of rotation in same co-ordinate system
                           as posX and posY
   XScale, YScale        [required] scaling factors for X- and Y stretching.  <1
                           will squash, 1 = no change, >1 will stretch.
   XShift, YShift        [required] magnitude of shift in X- and Y- directions
CALLS:
OUPUTS:                  string containing new x-y co-ordinates of point specified
                               posX and posY, following application of specified
                               transforms
```

==========

CalcMirror

SYNTAX: CalcMirror(Line1X1, Line1Y1, Line1X2, Line1Y2, Line2X1, Line2Y1 Line2X2, Line2Y2)

```
CREATE DATE:   02-09-01
BY:            Jon Bouch
PURPOSE:
   takes start and end co-ordinates of two lines, and works out which (if any)
   mirror transforms are required to get the lines to lie within same quadrants
NOTES:
INPUTS:
   Line1X1,Line1Y1       [required] start (x,y) coordinates of line 1
   Line1X2,Line1Y2       [required] end (x,y) coordinates of line 1
   Line2X1,Line2Y1       [required] start (x,y) coordinates of line 2
   Line2X2,Line2Y2       [required] end (x,y) coordinates of line 2
CALLS:
   Function Angle (this Module)
OUPUTS:
   which mirror transforms are required as an integer which can be read as:
   0 = no transform required,  1 = x-transform required
   2 = y-transform required,   3 = both x-y transforms required.
```

==========

CalcRotate

SYNTAX: **CalcRotate(Line1X1, Line1Y1, Line1X2, Line1Y2, Line2X1, Line2Y1, Line2X2, Line2Y2)**

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   calculates angular difference between two lines, each defined by a start
   and end set of x,y coordinates
NOTES:
INPUTS:
   Line1X1,Line1Y1       [required] start (x,y) coordinates of line 1
   Line1X2,Line1Y2       [required] end (x,y) coordinates of line 1
   Line2X1,Line2Y1       [required] start (x,y) coordinates of line 2
   Line2X2,Line2Y2       [required] end (x,y) coordinates of line 2
CALLS:
   Function Angle (this Module)
OUPUTS:
   rotation angle (anti-clockwise) relative to horizontal required to rotate line 2
   into orientation of line 1 (double)
```

==========

Rotate

SYNTAX: **Rotate(xStartPos, yStartPos, Angle, *[xOffset], [yOffset], [Result]*)**

```
CREATE DATE:   01-08-01
BY:            Jon Bouch
PURPOSE:
   calculates X and Y components of rotation, and returns new pair of coordinates
NOTES:
INPUTS:
   xStartPos, yStartPo   [required] X- Y- coordinates of start point
   Angle                 [required] rotation angle (in degrees), rotation applied
                           anti-clockwise 0-360 degrees
   xOffset, yoffset      [optional] position of centre of rotation.
                           using same x-y space as StartPos, default = 0, 0
   Result                [optional] how result is returned
                           0   (default) returns coordinates as an array containing
                               x- and y- coordinates
                           1   returns new x- coordinate only
                           2   returns new y- coordinate only
                           >2  returns both new x- and y- coordinates as string
CALLS:
   Function Radians (this Module)
   Function RotateX (this Module)
   Function RotateY (this Module)
OUPUTS:
   one or both of new x-y coordinates following rotation of specified point through
   angular displacement about a defined centre. (see "result" input for available
   output formats)
```

==========

RotateX

SYNTAX: **RotateX(xStartPos, yStartPos, Angle)**

```
CREATE DATE:   20-08-01 (based on earlier function)
BY:            Jon Bouch
PURPOSE:
   calculates new X-coordinate following rotation through specified angle
NOTES:
   based on older RotateX, created on 30-07-01 by Jon Bouch
INPUTS:
   xStartPos,yStartPos   [required] X-Y coordinates of start point
   Angle                 [required] angle of rotation (radians, anti-clockwise)
CALLS:
OUPUTS:
   X-coordinate of point following rotation (double)
```

==========

RotateY

SYNTAX: RotateY(xStartPos, yStartPos, Angle)

```
CREATE DATE:   20-08-01 (based on earlier function)
BY:            Jon Bouch
PURPOSE:
   calculates new Y-coordinates following rotation through specified angle
NOTES:
   based on older RotateY, created on 30-07-01 by Jon Bouch
INPUTS:
   xStartPos, yStartPos  [required] X-Y coordinates of start point
   Angle                 [required] angle of rotation (radians, anti-clockwise)
CALLS:
OUPUTS:
   Y-coordinate of point following rotation (double)

Function RotateY(xStartPos As Double, yStartPos As Double, _
                    Angle As Double) As Double
```

==========

CalcScaling

SYNTAX: CalcScaling(Line1X1, Line1Y1, Line1X2, Line1Y2, Line2X1, Line2Y1, Line2X2, Line2Y2, *[Result])*

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   calculates x- and y-scaling factors required to scale "line 2" so that it is
   same length as "line 1".  Both lines are initially defined by start and
   end pairs of x, y coordinates.  To be applied once "Line 2" has been rotated
   using the "CalcRotation" and "Rotate" functions
NOTES:
INPUTS:
   Line1X1,Line1Y1       [required] start coordinates (x,y) of line 1
   Line1X2,Line1Y2       [required] end coordinates (x,y) of line 1
   Line2X1,Line2Y1       [required] start (x,y) coordinates of line 2
   Line2X2,Line2Y2       [required] end (x,y) coordinates of line 2
   Result                [optional] how result is returned
                           0   (default) returns coordinates as an array
                               containing x- and y- coordinates
                           1   returns x- scaling factor only
                           2   returns y- scaling factor only
                           >2  returns both x- and y- coordinates as string
CALLS:
   Self contained
OUPUTS:
   Factors for stretching in X- and Y- directions (see "result" input for
   available output formats)
```

==========

Scaling

SYNTAX: Scaling(XStart, YStart, XScale, YScale, *[Result])*

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   Applies x and y scaling factors to (x,y) coordinates
NOTES:
INPUTS:
   XStart, YStart        [required](x,y) coordinates of point
   XScale,YScale         [required]scaling factors for x-y directions
   Result                [optional] how result is returned
                           0   (default) returns coordinates as an array
                               containing new x- and y- coordinates
                           1   returns new x- coordinate only
                           2   returns new y- coordinate only
                           >2  returns both new x- and y- coordinates as string
CALLS:
   Self contained.
OUPUTS:
   Scaled (x,y) coordinates (see "result" input for available output formats)
```

==========

CalcTranslate

SYNTAX: **CalcTranslate(x1, Y1, X2, Y2, *[Result]*)**

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   calculates required X- and Y-shifts to move "line 2" so that its start and end
   points coincide with those of "line 1".  To be applied after Line 2 is oriented
   and scaled so that it is same orientation (use "CalcRotate" and "Rotate"
   functions) and length (use "CalcScaling" and "Scaling" functions)
NOTES:
INPUTS:
   X1, Y1               [required] coordinates (x,y) of point 1
   X2, Y2               [required] coordinates (x,y) of Point 2
   Result               [optional] how result is returned
                          0   (default) returns coordinates as an array
                              containing x- and y- coordinates
                          1   returns x- translation value only
                          2   returns y- translation value only
                          >2  returns both x- and y- coordinates as string
CALLS:
   Self contained
OUPUTS:
   X- and Y- shift values required to move point 1, to position of point 2
   (see "result" input for available output formats)
```

==========

Translate

SYNTAX: **Translate(x1, Y1, XShift, YShift, *[Result]*)**

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   moves (x,y) coordinates by specified translation amounts
NOTES:
INPUTS:
   X1, Y1               [required] coordinates (x,y) of point 1
   XShift, YShift       [required] magnitude and sign of shifts in X- and Y-
   Result               [optional] how result is returned
                          0   (default) returns coordinates as an array
                              containing x- and y- coordinates
                          1   returns x- translation value only
                          2   returns y- translation value only
                          >2  returns both x- and y- coordinates as string
CALLS:
OUPUTS:
   new x- y- coordinates in a form dependant upon nature of optional "result"
   flag variable
```

==========

Angle

SYNTAX: **Angle(XStart, YStart, Xend, YEnd)**

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   calculates angle between 2 points defined by (x,y) coordinates
NOTES:
INPUTS:
   xStart, yStart       [required] start coordinates (x,y) of line
   xEnd, yEnd           [required] end coordinates (x,y) of line
CALLS:
   Function Degrees (this Module)
OUPUTS:
   angle of line relative to horizontal at start point (double)
```

==========

Radians

SYNTAX: **Radians(Degrees)**

```
CREATE DATE:   30-07-01
BY:            Jon Bouch
PURPOSE:
   converts angle in degrees to angle in radians
NOTES:
INPUTS:
   Degrees              [required] angle in degrees
CALLS:
   Self contained
OUPUTS:
   angle in radians (double)
```

==========

Degrees

SYNTAX: **Degrees(Radians)**

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   converts angle in radians to angle in degrees
NOTES:
INPUTS:
   Radians              [required] angle in radians
CALLS:
   Self contained
OUPUTS:
   angle in degrees (double)
```

==========

Distance

SYNTAX: **Distance(XStart, YStart, XEnd, YEnd)**

```
CREATE DATE:   14-08-01
BY:            Jon Bouch
PURPOSE:
   calculates linear distance between points defined by 2 sets of coordinates
NOTES:
INPUTS:
   xStart, yStart       [required] start coordinates of line
   xEnd, yEnd           [required] end coordinates of line
CALLS:
   Self contained
OUPUTS:
   straight line distance between start and and points (double)
```

==========

GetFilePath

SYNTAX: **GetFilePath(strFileAndPath)**

```
CREATE DATE:   12-05-00
BY:            Jon Bouch
PURPOSE:
   takes an input string containing the current filename and path, and removes the
   filename
NOTES:
INPUTS:
   strFileAndPath       string containing filename and path.
CALLS:
OUPUTS:
   GetFilePath          string containing filepath only
```