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AN AUTOMATIC IMAGE ACQUISITION AND ANALYSIS SYSTEM FOR A SCANNING ELECTRON MICROSCOPE

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Abstract

A restriction on the wider use of image processing and analysis methods in many disciplines has been the need to analyse large numbers of images to provide the necessary statistical basis for comparison between one specimen and another. Many image processing and ana-lysis routines such as image restoration, orientation analysis, objective segmentation for porosity analysis, and granulometric grey-scale morphological methods are well established. Many are readily adapted to batch processing without the need for operator intervention, but the acquisition of the raw images themselves becomes a constraint. An image processing facility has been adapted to control automatically a Hitachi S800 scanning electron microscope and to provide basic image capture facilities. This in turn exchanges data with a separate dedicated image analysis facility running on a personal computer. Basic control of brightness, magnification, focusing and stage position are programmed to include various image capture sequences including regular grid arrays, random acquisition, and acquisition at pre-selected points. The magnification can be varied between one image and another. Up to 400 separate images have been captured in a single operation and this has required the development of a database system and file management system within the image processing facility to record key parametric information such as magnification and specimen co-ordinates. During image capture of one image, the dedicated image analysis facility analyses the previous image in the sequence. Full information is stored about each image and the specimen position can be readily recovered for further image capture under different operating conditions if required.

Key Words: Image analysis, database system, scanning electron microscope.

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Introduction

Image processing and analysis methods on electron micrographs are now in widespread use for image restoration purposes, to extract structural information from features seen in the micrographs, and to quantify features and fabrics within those micrographs. While several methods of processing and analysis require operator intervention for decision making, increasing interest is being shown in several disciplines of fully automatic methods which do not require such intervention. In many cases, the use of subjective methods for thresholding is undesirable as the range of results can be highly operator dependant.

An example of the problem associated with subjective operator decisions is demonstrated in Figure 1. Forty graduate students were each asked to subjectively select a threshold to segment a good quality back-scattered electron image of embedded clay particles (Fig. 1a) into two phases, one representing the pores (dark), and the other the particles (light). The selection of a threshold was done interactively at a computer, and from this information the porosity, as judged by each student, was computed. Their results can be compared with that of a computer generated threshold (Fig. 1b) using the relative contrast histogram method of Kohler (1981) which was extended by Hounslow and Tovey (1992). Two typical student selections are shown as Figures 1c and 1d. The test throws into question the validity of image analysis for porosity measurements at least as far as subjective involvement is concerned. The situation is highlighted by the histogram (Fig. 1e), and the fact that images (Figs. 1b and 1d) appear very similar but in fact represent a porosity difference of over 8%.

There is a clear need for objective methods, and wherever possible these should be completely free from operator involvement to avoid such difficulties. Even if a computer segmentation is not ideal, it is at least consistent, allowing correct relative comparisons to be made, and there are many such methods now available. For instance, the intensity gradient analysis methods of Smart and Tovey (1988) and Tovey *et al.* (1992a, 1992b, 1995) allow for batch processing of large numbers of images without operator intervention. Figure 2a shows a typical back-scattered electron image of an embedded sample of consolidated and sheared kaolin. Of interest was the way in which the particles align with respect to one another following

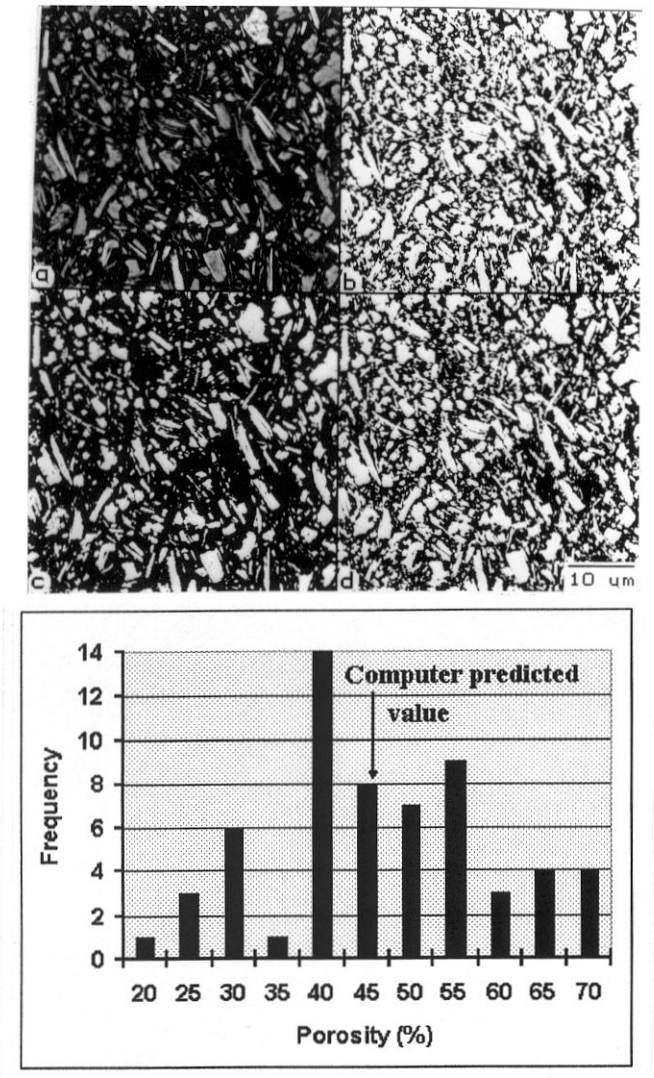

Figure 1. Illustration of problems associated with subjective thresholding. Nearly 40 students were asked to threshold the image shown in (**a**). The distribution of computed porosities is shown in (**e**). Image (**b**) is the objective, computer generated image, while (**c**) and (**d**) are examples of subjective thesholding. Image (**d**) appears little different from (**b**) even though the porosity was 8% different from the computer generated one.

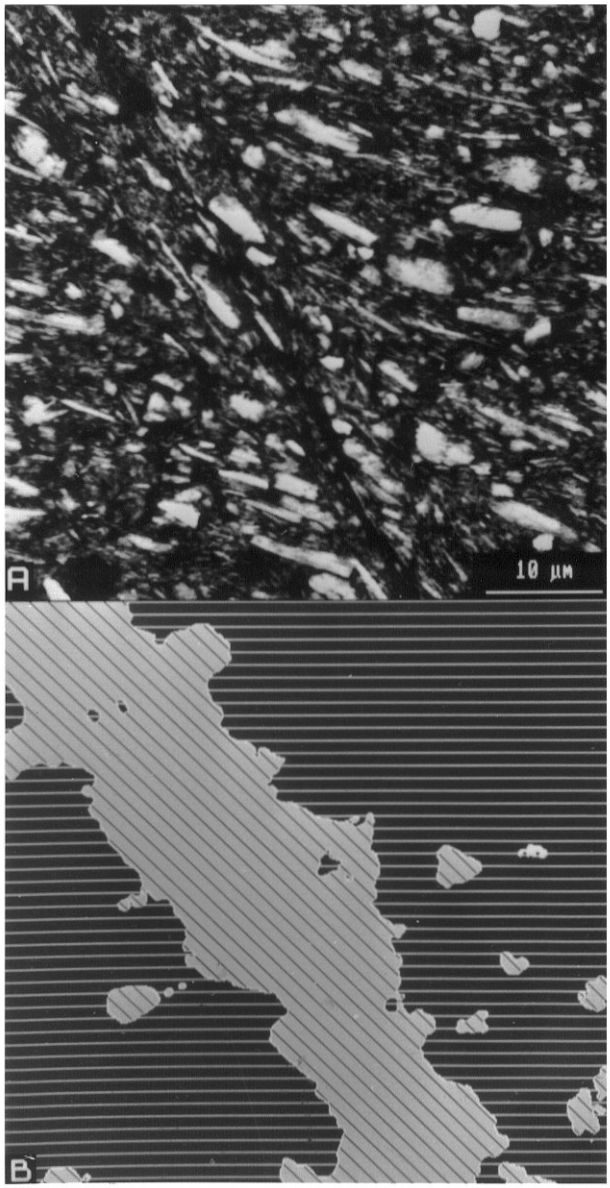

Figure 2. Illustration of domain segmentation using intensity gradient analysis. The failure zone in the original image (**a**) has been highlighted in the segmented image (**b**).

mechanical deformation or the flow of water. In this particular image a shear zone can be seen running from top left to bottom right. The intensity gradient analysis does not require initial segmentation as is the case for many processing and analysis methods. It can also quantify each micrograph by just two parameters, an index of anisotropy (indicating the degree of alignment which is usually measured on a scale from zero as random to unity as perfectly aligned), and an orientation specifying the direction of any preferred alignment. Alternatively, a rosette histo-gram may be displayed to graphically illustrate these two parameters. In addition, an *angles-coded* image may be generated where each pixel represents the orientation of the feature at the corresponding pixel in the original.

An alternative approach to orientation analysis is the work of Sokolov (1990) using Fourier methods to determine orientation, and this also allows direct assessments of orientation analysis without intervention. Extensions of both these orientation methods are possible,

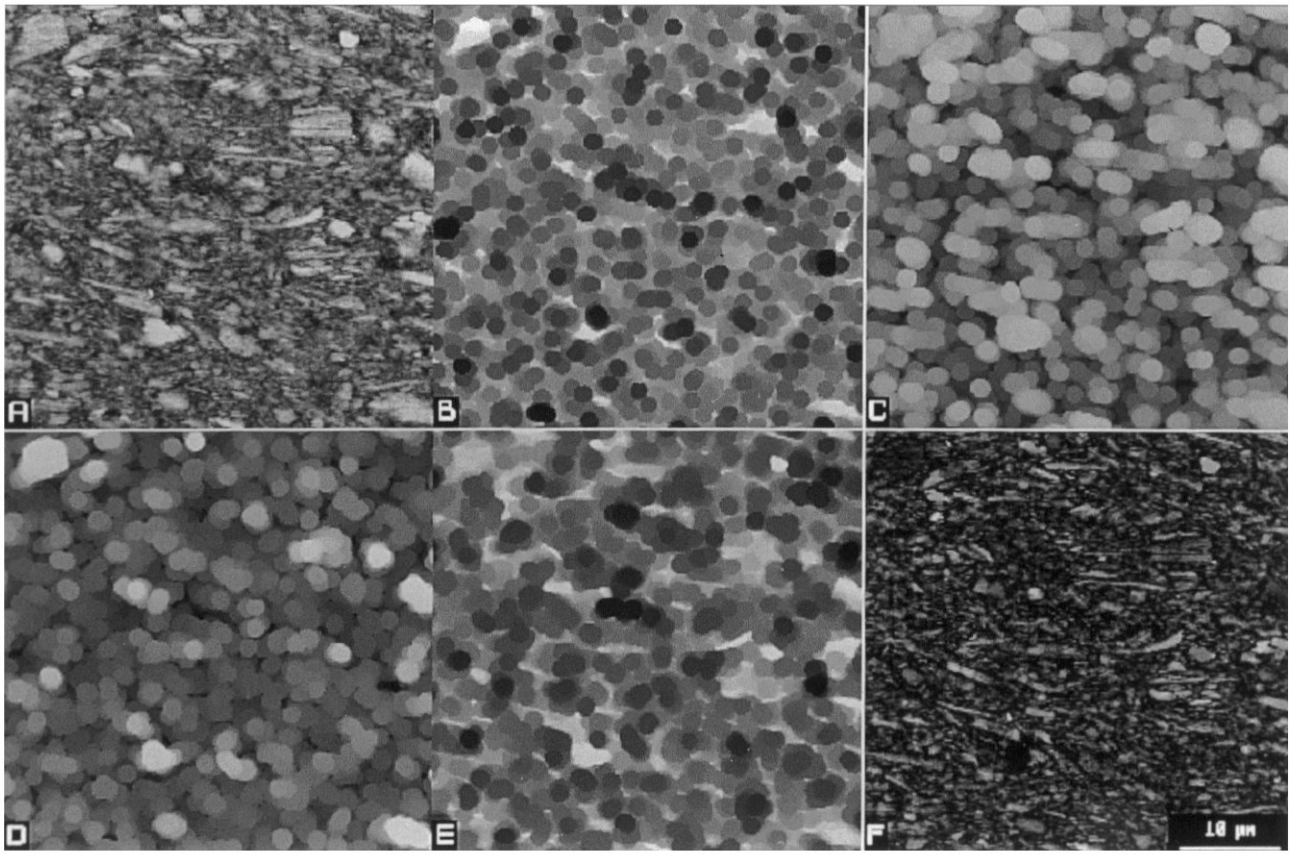

Figure 3. Example of Grey-Level morphological operations on an image. (a) original; (b) result of erosion; (c) results of dilation; (d) results of opening; (e) results of closing; (f) differences between original and "opended" image.

and these can also be done using batch processing methods. In the extension of the intensity gradient method, the *angles-coded* output image from the initial intensity gradient analysis may be used for domain segmentation where the image is automatically segmented into regions with the same general orientation as shown in Fig. 2b. In this way images can be reliably segmented using general orientation patterns as the criterion for segmentation. In the example shown here, the region covered by the failure zone is readily highlighted. The development of this extended orientation analysis has been described in a series of papers, the key ones being Tovey *et al*. (1992a, 1992b, 1995), and Smart and Leng (1993).

In the example shown, it is possible to do further processing by creating a binary mask from the domain segmented image and thereby selecting relevant parts of the original image. In this example, two regions were considered: inside and outside the failure region. By repeating the orientation analysis on the separate regions it was found that the degree of orientation as measured by an index of anisotropy was less within the failure zone: 0.581 at an orientation of 127.1° measured clockwise from the upward vertical compared with 0.666 at an orientation 101.7° outside the zone.

Other methods amenable to direct batch processing include image restoration methods, objective thresholding methods (e.g., Hounslow and Tovey, 1992; Tovey and Hounslow, 1995), morphological methods on binary images to assess pore size and particle size distributions (e.g., Ehrlich *et al*., 1984; Tovey, 1995), and grey level morphological methods for aggregate size distributions (e.g., Prod'homme *et al*., 1992). A development of the latter method is to use structuring elements which can take any form such as circular elements rather than the restricted square form of the original method. Such a method allows feature or aggregate size distributions to be computed on grey-level images and thus obviates the need for thresholding. Figure 3 illustrates various grey-level morphological operations on the original image shown in Figure 3a. These are grey-level erosion, dilation, opening, and closing using a circular structuring element of 7 pixels radius as shown in Figures 3b-3e respectively. Figure 3f shows the effect of subtracting

the "opening" from the original and indicates that much of the original is associated with fine detail. This stage is an important step in granulometric analysis as described by Prod'homme *et al*. (1992).

All the above methods have distinct advantages over many analysis methods as with the absence of operator intervention it can be arranged that large numbers of images can be processed in batch runs. Such an approach is desirable as it can overcome the criticism, often levelled at image analysis, that the detailed investigation of a few images is one thing, but the relevance of the information gained on a micro scale to bulk properties is quite another. Questions as to how representative a structure is when observed at the microscopic scale are not uncommon. Provided that suitable images are available, it is not difficult using suitable file-naming conventions to construct batch runs in which many parameters about a sample can be computed automatically, with the results stored automatically. Typical applications of automatic analysis were reported by Tovey *et al*. (1992a) for orientation analysis, and by Hounslow and Tovey (1992) for image restoration and objective porosity analysis. In theory, there is no reason why several different analyses are not done on the same image, and indeed it is now common in the author's laboratory to combine the porosity and orientation analysis together (Tovey *et al*., 1995). Developments are now also underway to include the normal and grey-level morphological methods as part of the overall batch processing of images.

Whatever the reason for the image processing and/or analysis, there are four basic steps involved to consider:

image acquisition
image processing
image analysis
interpretation

In all cases, the image must be acquired in digital form which may then be processed before analysis and, finally interpretated in which the results are related to the study in hand; however the quality of this interpretation of the results will be dependant on the adequate execution of the three other stages.

Despite the developments towards batch processing and analysis of images, a limiting factor in micromorphological observations is often the rate of acquisition of the digital images themselves. The operator at a scanning electron microscope will acquire these images, and typically a number of images are often acquired from each sample to obtain some indication of variability. There appear to be few applications in which guidance is given as to how many images should be taken for each sample or how they should be distributed. The images can be arranged in a regular grid fashion, such as a straight line across the sample, or in a random fashion. In the author's laboratory, it has been practice to use samples which were approximately 15 mm x 15 mm in size and capture a total of 24 images in two straight lines, vertical and horizontal. The spacing of the images was set at 1 mm. In this way it was ensured that a representative selection of each sample was covered. Others, e.g., Smart and Leng (1993) have acquired 25 images, but this time, arranged as a 5 x 5 grid covering a smaller central area. In yet other cases, it was recognised that several different types of structure existed, and that a random positioning of images was perhaps more relevant to ensure that all type of feature were covered. This latter method can be affected by bias if the regions are chosen while the operator views them, and there is a danger that atypical areas may thus be selected. However, provided that co-ordinate information is recorded, it should be possible to investigate any spatial trends in the results provided that the specimen co-ordinates at the location of each image are recorded at the time of capture. Unfortunately this recording appears to be done rarely.

Even when there is adequate recording there is a limit to the time that an operator can work effectively in a darkened room, and this limits the number of images that can be captured. The situation is compounded where the instrument is in use by many researchers. Once a sequence which requires careful spatial information has been started, it is difficult to interrupt the sequence to allow others to use the instrument. This leads to inefficient use of an expensive resource.

The developments described in this paper are an attempt to overcome these restrictions and thus enable a more objective view of the analysis of a sample to be gained. While essentially this is an automatic image capture system, it was necessary to evolve methods in several other related topics such as management of the large number of images acquired, the need for spatial co-ordinate recording etc., and the ability to recover the exact position an image was captured from a specimen at some later date, usually after the specimen has been removed from the microscope and reinserted. The full procedure is known as Automatic Digital Image Acquisition and Analysis System (ADIAAS).

While the methods were evolved for the observation of polished, resin embedded sediment samples in the back-scattered electron mode, provision was also made for capture in the secondary electron mode and many of the procedures developed are of general applicability. For impregnated sediment samples, a carbon coating of approximately 100 nm was used while observation was typically done at an accelerating voltage of 20 kV in a Hitachi (Tokyo, Japan) S800 scanning electron microscope (SEM). This microscope has facilities for automatic focusing merely by the press of a button. The microscope has a motorised stage, the position of which can be controlled to the nearest 1 μm. The electron gun for this microscope is of the field

emission type.

Within this paper, there is a discussion of the new automated image acquisition system, some illustrations on what becomes possible in image analysis with such a set of images, which at times have been captured at the rate of over 1000 a day.

While it is possible to devise a single system which both captures and processes and analyses images this is undesirable when SEM usage is at a premium. Further it increases the time interval between image capture which makes capture more prone to instability problems.

Requirements of the System

For an automatic system of image analysis, the microscope must be automatically controlled, and store images in a form compatible for direct analysis in an automatic sequence by the image analysis facility. Several key aspects can be identified for such a system:

(1) The system must be able to automatically adjust most of the key microscope functions such as magnification, or focusing,

(2) The specimen position must be controlled automatically by the capture system, and the co-ordinate at each capture position must be recorded,

(3) It must be possible to capture the images from any number of pre-determined positions on a specimen which may be:

(a) spaced as a rectangular grid array (a straight line is a special case of this),

(b) a single point,

(c) derived from co-ordinates previous specified by the user and stored in a file.

(4) It must be possible to record the co-ordinates of a random selection of points for later retrieval.

(5) It must be possible to change the magnification from one image to another in the sequence, and also from the back-scattered mode to the secondary electron mode (at present these are the only detection methods available to the authors, but clearly the option for other modes as alternatives can be considered).

(6) It must be possible to relocate a position on a specimen up to 25 mm x 25 mm at a later date after the specimen has been removed and reinserted even in a specimen where few details are visible at magnifications lower than about 500x.

(7) It must be possible to start analysing the images directly without affecting the digital acquisition system.

(8) Images should be stored digitally and in separate files, and an automatic system to track file name is necessary.

(9) A facility to archive the large number of potential images must be available.

(10) A warning system to alert the user of problems with the image acquisition should be present.

At the time of writing, all these functions are in place although a little development is still needed to streamline the system and make it more user-friendly particularly when faults are detected.

Description of the System

The system has been designed to capture any number of images at any spacing and has been tested in runs of 400 images. In practice, the management file naming system limits the total number of images that can be captured in a single session to 999 and this is also compatible with available disk storage as this number of images would require over 260 Mbytes of storage for 512 x 512 images. There is some flexibility with regard to image sizes for capture, but standard sizes used are 512 x 512, 768 x 512, 1024 x 1024, and 1536 x 1024. These are conveniently related to the frame grabbing facilities available.

The digital acquisition system is based around 2 personal computers (PC); for convenience, one is an older 8086 computer whose function was to provide timing pulses for the various functions, while the other, a 486 (66 MHz) computer which controls the overall system and runs the algorithms for image capture and also does preliminary processing of images. This latter computer is networked with several other PC computers any one of which may be used for the image processing and analysis simultaneously with the image capture. The images are acquired using the Synergy frame grabbing facility (manufactured by Synoptics, Cambridge, UK). This provides the opportunity for slow scan image capture as well as normal video rate input from two separate channels. Facilities for different real time filtering, and for positional offset of image capture are also available on the Synergy board.

Three separate interface units were constructed. The first provided an interface between the microscope and the Synergy Board. This takes pulses from the timing computer to control the start of each line, the start of each frame, and the dwell time on each pixel. The start of the timing sequence is initiated by a suitable command from the controlling computer via a link between the COM1 ports on both computers (see Fig. 4). A second link is provided between the controlling computer and the interface via the LPT1 port. This link receives the end of field pulse from the microscope via the interface and informs the controlling computer that the image capture sequence is complete.

The second interface unit is associated with the motorised stage. For convenience this was connected to the COM2 port of the controlling computer. New software was written to allow the stage to be moved either according to the absolute set of co-ordinates or relative to the present position. The command also allows the system to interrogate

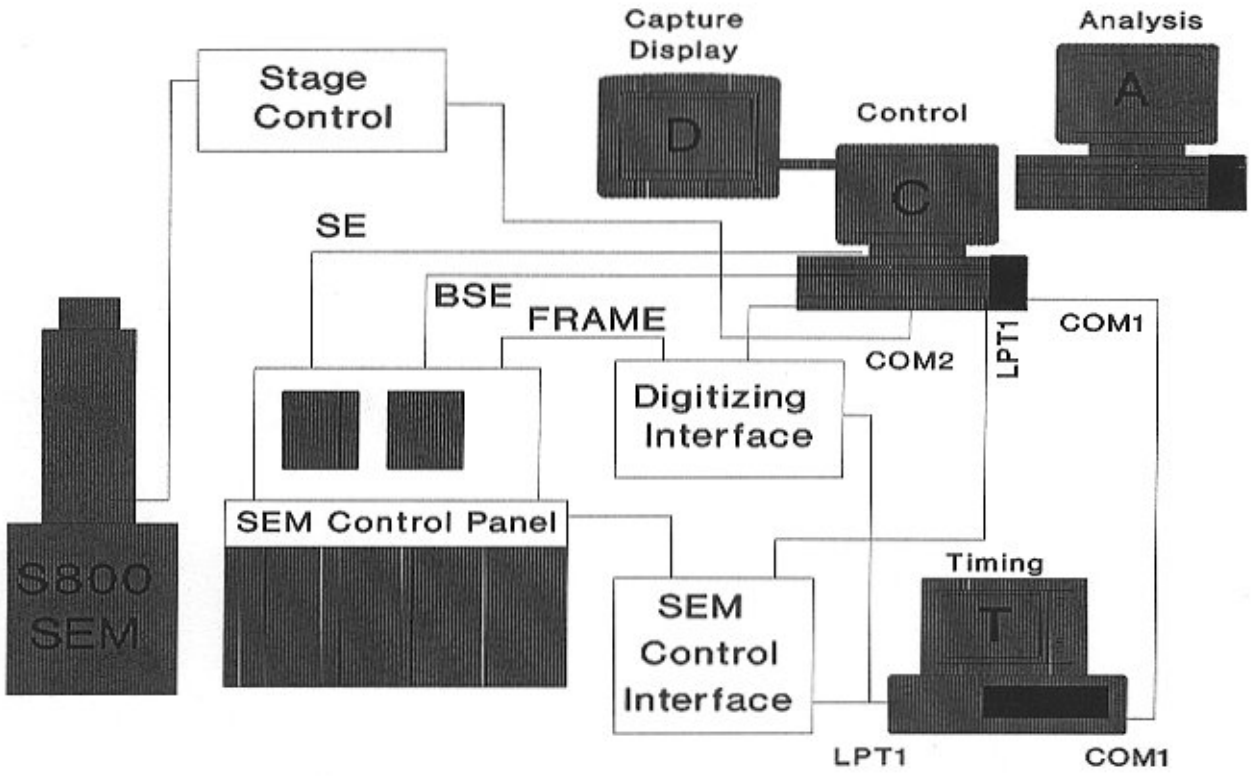

Figure 4. Schematic diagram of automatic image capture facility.

the current position of the stage, lock the stage to prevent vibration, and it also performs a backlash check after each move of the stage. During stage movement, image capture is automatically disabled.

The third interface is connected to the controlling computer via the LPT1 port and provides the control of several microscope functions. The majority of operations on the S800 are through the use of push buttons (including the automatic focusing), and this unit sends pulses to simulate a button push as and when required. Some operations such as automatic focusing require a few seconds to complete, and all other functions are temporarily disabled until the confirmation signal is received that the current operation has been completed. Some operations such as the beam monitor current indicator normally require the operator to press the button when the system starts to become unstable, but this should not be done when an image is being captured as changes in brightness can suddenly occur when this happens. In automatic control, this warning signal is interrogated before each image is captured. It typically occurs once or twice in a sequence to capture 400 images.

Image Management and File Naming

Each image capture run, whether it be for the capture of a single image or for large numbers, automatically records a log file which contains key information about the operating conditions and the position and magnification of each image. This file can be accessed directly in subsequent runs so that, if required, the image may be recaptured or the location re-visited for a more detailed look at a different magnification. When acquiring individual digital images, the system automatically writes a file which is compatible for later interrogation within Dbase or an equivalent program. This facility is also available for photographic image recording. Within this file it is possible to directly add notes about each image as it is captured. For fully automatic capture, the operator would not normally be present for each image, and this facility is disabled as the general information about the batch run is usually more relevant.

A database of registered users is automatically accessed whenever digital or normal photographs are recorded. This ensures that file naming conventions by the many users do not conflict. Within the 8 alpha-numeric file nomenclature of files suitable for storage on a PC, the convention adopted is as follows.

The filenames are all of the form AAxxyyyC, where:

AA is a 2 letter/number combination identifying each user. These may be initials, or reflect a project name. Many users have several such identifiers.

xx is a two digit sample number (00 - 99), thereby allowing up to 100 different samples to be observed within each project.

yyy is a three digit number indicating the image number in the sample. This allows up to 999 images to be captured on each sample. As discussed above, storage limits make 999 images a sensible upper limit as does the time to capture such a run within the normal stable operation time of about 8 hours of the field emission microscope.

C is a suffix to denote mode of operation of microscope (e.g., S for secondary electron, B for back-scattered, C for cathodoluminescence etc.).

The file naming convention is generally consistent with image capture on a second SEM with X-ray mapping facilities except that since the number of images acquired is much less, the maximum number is limited to 99 which allows for a two letter suffix which can relate to the particular elemental map. For these images, the conventional abbreviations (SE for secondary electron and BS for back-scattered images are used).

The method for naming files is unique for each person or project and is flexible enough to meet the varying demands of users. However, storage becomes critical and all digital images are recorded on a compact disk (CD) as soon as practical. For heavy users with large numbers of images this is usually within 24 hours. For other users, files are stored temporarily on disk, and when 50 - 100 Mbytes are ready these are archived on CD with each user having a separate directory. In the near future it is hoped that a multi-disk CD reader will be attached to the system so that the recent CDs can be accessed directly in a new session as a reference.

Operation of the Image Acquisition System

The majority of the image processing software and algorithms used in the authors laboratory have been written using the SEMPER (Synoptics) image processing analysis language. This has included the incorporation of many new image processing and analysis commands as described elsewhere (e.g. Tovey *et al*., 1995). It was thus sensible to use this language as the basis of the work here and to write new algorithms as relevant. These new algorithms were written in a mixture of FORTRAN, Assembler, and "C". The aim has been to provide the user with a convenient method for operating the system.

On immediate start-up, the user is confronted with a general menu from which to select several options such as retrieving images previously stored, but in most cases a new sequence will be selected when the menu (Fig. 5) is displayed. This is typical of the subsequent menus and is operated by moving the highlighting bar up and down as appropriate and then selecting the option with the normal "Enter" key.

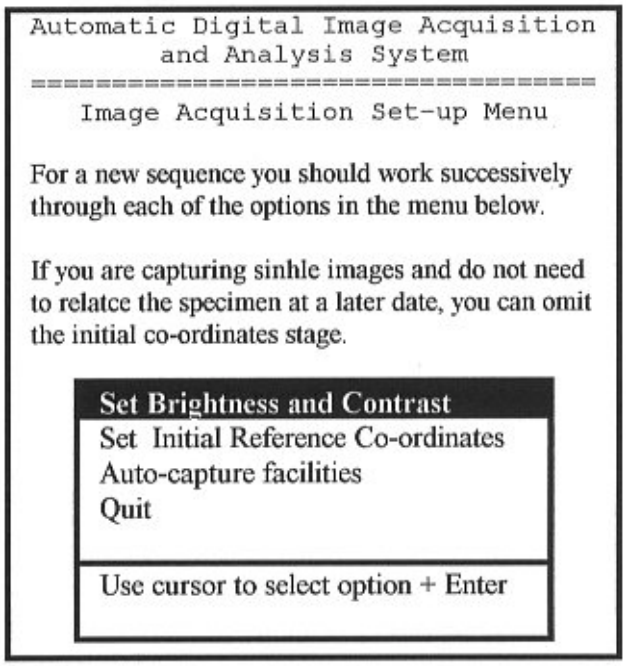

Figure 5. A typical menu.

Setting the initial contrast and brightness

Initially, the user must switch on the microscope, select a typical area of interest and focus the image (manually or automatically, and if at high magnification correct for astigmatism). Selecting the "Set Brightness and Contrast" option in the set-up menu displays a sub-menu which first permits selection of the detection mode (e.g., secondary electron, back-scatter), the resolution (e.g., 512x512) and then allows a digital image to be captured. It is essential that the digital image has the correct dynamic range of intensities which may often be different from those on the microscope screen. After capture, a histogram of intensities is displayed on the screen, and if not satisfactory the contrast and brightness on the SEM can be captured again. Automatic brightness and contrast facilities are available on the SEM for the secondary electron imaging mode, but not for the back-scattered mode used for most of the research done by the authors. The adjustment of contrast and brightness is thus done manually with reference to the histogram. Normally this operation has to be done just once in a particular sequence of image capture.

With some frame grabbing cards, the aspect ratio of

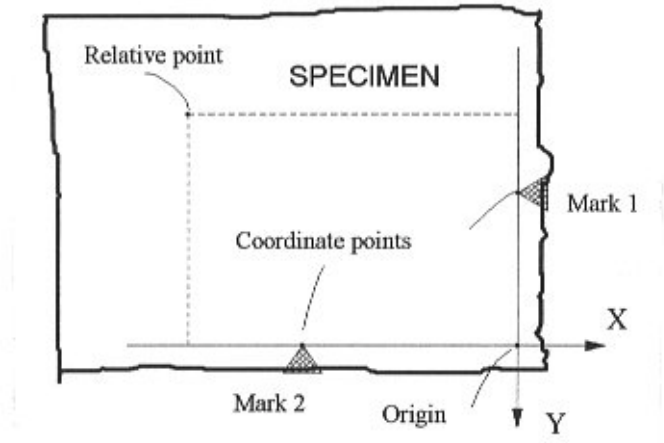

Figure 6. Co-ordinate referencing system on specimen.

the pixels may be rectangular or incompatible with the scan sequence on the microscope. The consequence is to distort the image in one direction. Provision to compensate for this is provided by an improved tilt compensation unit for the SEM which will provide the necessary affine transformation to ensure correct aspect ratio during image capture.

The range of signal intensities is usually adjusted to give the full range 0 - 255 with a small amount of saturation at each end (<1%). New facilities to be incorporated soon will include a choice of different real time filtering options.

Setting specimen reference co-ordinates

In many types of specimen, the orientation of the image relative to the bulk specimen is important particularly when microfabric observations are to be related to macroscopic properties such as stressing, etc. It is thus essential to provide a clearly identifiable reference so that observations in the SEM are correctly related to these directions. This is particularly important since the scanning raster will be rotated about the column axis of the SEM by the magnetic field from the lenses. Further, it is desirable to relocate the position on a specimen.

For many types of specimen, this may not be a significant problem as low magnification location images can be taken, and provided that there are distinct features, these can be used for reference purposes. For polished, back-scattered electron images, the surface is very smooth and devoid of features. Further, for sediments consisting predominantly of clay-sized particles, the features themselves only become visible above 500x magnification, and sometimes magnifications in excess of 1000x must be used. The field of view in such instances is very small, and since each area looks so like another in overall texture, it becomes very difficult to relocate a specimen unless the field of observation is deliberately restricted in which case any possible variation within a specimen cannot be observed.

To overcome this difficulty, two fiducial marks are scratched at the edge of the sample. For reasons associated with the geometry of the S800 microscope, these are to the right and the bottom of the specimen. The marking convention is such that the fiducial mark at the bottom corresponds with the vertical direction in the bulk specimen, and if this is important, it will be the lower edge. The marks are conveniently scored on the surface using a sharp razor blade. At a magnification of 100x, the tip of these cut marks is usually less than 10 pixels in size.

Even when the specimen is placed in the SEM with the lower edge in an orientation which should cause it to be horizontal on the viewing screen, this is rarely the case because of scan raster rotation and during co-ordinate set-up, the first step is to ensure that the lower edge of the specimen is indeed approximately horizontal. This is achieved using the mechanical specimen rotation control on the stage. The first reference mark is then brought to coincide with a particular point on the viewing screen on the SEM (e.g., the centre), and the co-ordinates corresponding to this position are automatically recorded by the system. The second point is then located similarly and from this the system will automatically compute the origin position and drive the stage to that location. This is illustrated in Figure 6. For a typical set of observations, the specimen is then moved to a starting point, and all future image capture is done from that point. It should be noted that because of the geometry of the S800, all co-ordinates will be in the second quadrant. If the specimen is reinserted in the microscope, and if orientation is important, then the original orientation can be recovered as described in the next section.

Recovery of exact orientation of specimen in subsequent observations

Once the initial reference co-ordinates have been set up, these are automatically stored in the header of log file for that run, and no further such reference is required. Nor for that matter will it be necessary to change the set up provided that the specimen remains in the microscope and there has been no angular movement of the specimen. On the other hand, if the specimen is removed from the microscope and re-inserted for further observation in exactly the same orientation, then an additional stage is necessary. It is very difficult to ensure exactly the same orientation when the specimen is re-inserted as the scan raster and effective viewing orientation, will be dependant on several factors such as accelerating voltage, the exact position of the specimen in the specimen holder, and the working distance chosen. However, by using the new reference coordinate data and comparing it with the original set-up as stored in the log file it is possible to compute the necessary

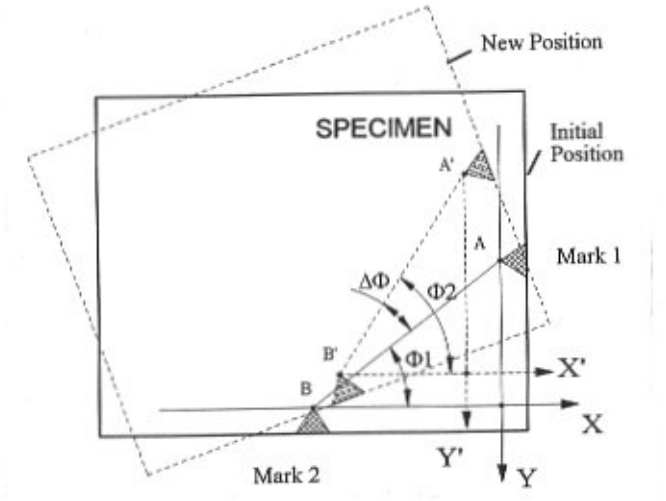

Figure 7. Rotated co-ordinates after specimen has been re-inserted in SEM.

angular rotation needed to bring the two sets of images into correct alignment. Figure 7 illustrates this point.

If the initial set-up and the current set-up are mismatched, then the following information about both co-ordinate set-ups will be displayed on the screen as shown in Figure 7. The value of the angle difference is calculated as follows:

Between the two marks A and B on a specimen a line can be drawn, as shown in Figure 7. The length of the line is constant for a solid specimen, and assuming the relative co-ordinates of the marks A and B are $(0,Y_A)$ and $(X_B,0)$, respectively, the angle, Φ_1, of the line relative to the X-axis can be obtained by

$$\Phi_1 = \tan^{-1} (Y_A / X_B) \quad (1)$$

If the relative orientation of the specimen is different from the initial set-up, then the co-ordinates of the marks A and B become $(0,Y'_A)$ and $(X'_B,0)$, as shown in Figure 7. Similarly, the angle of the line AB is now Φ_2 and can be calculated by

$$\Phi_2 = \tan^{-1} (Y'_A / X'_B) \quad (2)$$

The angular difference $\Delta\Phi$ will be

$$\Delta\Phi = \Phi_2 - \Phi_1 \quad (3)$$

By computing the value of $\Delta\Phi$ and displaying it on

	Origin		Mark 1		Mark 2	
	x	y	x	y	x	y
Current Position	13906	14471	0	-2396	-1100	0
Position in File	13258	12628	0	-503	-319	0
Adjustment required - 6.881 degrees						

Figure 8. Information displayed to observer to realign specimen to conform to original orientation.

the screen as indicated in Figure 8, the operator can rotate the specimen by the appropriate amount and then re-record the current reference co-ordinates which should now be close to the original values, and normally sufficiently close for the new run. Normally as long as the angular difference is less than 0.5°, the orientation is sufficiently close that any position on the specimen can be relocated within a maximum of 1 frame width even at 2000x magnification. Obviously if the microscope has motorised control of the rotation control, then this adjustment could be done automatically, but it is somewhat less common to find such control than it is to find motorised control for translational movements.

Automatic-Capture Facilities

The final and most important option in the menu displayed in Figure 5 is the automatic image capture system. A sub-menu is displayed with the following four options:

(a) Grid points method,
(b) Pre-set points method,
(c) Single point method,
(d) Points from a file.

Grid points method

This option allows the automatic acquisition of the images from a specimen at each point in a rectangular grid array, parameters for which are defined by the user as follows:

(1) Digital image resolution (512 x 512 or 1024 x 1024 pixels),

(2) Image type (secondary electron or back scatter),

(3) Number of points for image capture in each row and also number of rows,

(4) The spacing between the grid points which may be different in the two directions,

(5) The start point (position co-ordinates): this can be done by manual movement to a particular point, or

by controlling the stage automatically to move to a given point.

(6) Magnification.

For image management, two further items are defined:

(7) Sample identification - a two letter reference unique to each observer,

(8) Sample number.

As indicated above, all co-ordinate values are within the second quadrant. The images are captured from left to right in the first row, each image separated by the pre-specified amount. The stage is then moved downward by the row spacing and the next row is captured in reverse order and so on. This minimises the specimen movement time compared to the situation if all rows of images were captured in the same direction. This sequence is illustrated in Figure 9. The co-ordinates of each successive image point are simply computed from the known starting point and also the grid spacing in both the X- and Y-directions.

Parameters 7 and 8 in the above list do not affect the operation of the system, but these are needed to construct the file names for storing the digital images.

Once the parameters have been defined, the sequence will run automatically. As a preliminary, the stage is controlled to move to the initial starting point (in case it has been moved after parameter set-up). Since the magnification on the S800 microscope is changed by a push button, it is possible to change and record the correct magnification provided the initial setting is correct. To ensure this, the magnification is reduced by sending a signal to automatically simulate many presses of the demagnification button. This ensures that the reference magnification is x40 after which the sample is automatically focused by sending pulses to operate the in-built coarse and fine focusing sequences. This is then followed by sending sufficient pulses to the magnification enlargement button to ensure that the correct magnification is selected after which further focusing (both coarse and fine) is initiated. Following this initial start routine, the first image is captured and when complete the controlling computer sends the command for the stage to move to the next position while at the same time the captured image is surveyed for intensities and stored on disk. At the new location there is no need to select the 40x magnification as it is already at the operating magnification and all that is needed before the capture of the image at the second position is further focusing.

While the above summarises the sequence of automatic commands sent out by the controlling computer there are several other features which were needed to ensure that the operation continued as reliably as possible without the need for any operator intervention.

(1) It is necessary to minimise backlash effects and this is achieved by ensuring that the final approach to each grid position was always from the same direction. Further a command is also sent to lock and disable the normal manual control to prevent inadvertent movement of the stage during image capture.

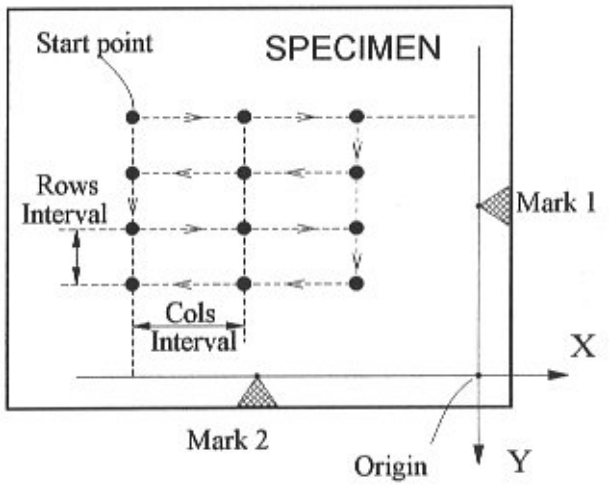

Figure 9. Sequence for recording images in a grid point manner.

(2) There are two separate auto-focus buttons on the SEM: both coarse and fine. In the ADIAAS system, however, when the magnification is greater than 1000x only fine auto focusing is used provided that the sample is already reasonably in focus as it always will be when scanning in a grid fashion. Only on very rare occasions was it found that the auto-focus failed to work correctly, and the majority of the failures occurred when attempts were made to coarse auto-focus an image which was already close to focus. This was another reason why coarse auto-focus was omitted for high magnification operation in the grid capture mode.

(3) Any field emission scanning electron microscope will have a stable operating period, but the current will decrease as gas molecules are adsorbed onto the field emission tip. The SEM has a detector for testing the emission current, and when this reduces to approximately 1 μA or less, the HV ON button on the SEM will start flashing. In normal manual operation, this is cured by depressing the button, but not when an image is photographed. When the emission current falls as low as this, noisy images can result. In the ADIAAS system the button pressing is simulated if it is detected that the lamp on the button is flashing and this will cause the emission current to increase again. If after simulating the button push the flashing does not stop, the system will make a further two attempts. After this, the program will pause as it is necessary for the operator to "flash" the gun before another period of stable operation can be achieved. The operating sequence can be resumed once the gun is stable again, although it is normal to capture

the image at the point at which the sequence was interrupted several times to ensure that correct contrast has once again been achieved. During this time, a histogram of the grey-levels will be automatically displayed on the screen after each image capture to check that the settings are correct. Once this is satisfactory, the automatic sequence can resume uninterrupted.

(4) In addition to the checks provided in (3) above it is also necessary to interrogate the Beam Monitor Current (BMC) button as this may also flash if the beam current moves outside pre-determined limits. This can also affect the contrast and brightness, and in normal manual operation, the operator will press this button before taking a photograph with the ADIAAS system, the lamp on the BMC button is checked periodically to see if it is flashing, and like the procedure in (3) above, this will be "pressed" if required. Once again, if repeated attempts fail to stop the lamp flashing, the system will pause for operator intervention.

(5) For auto-focusing it is important that scan speed is relatively fast, but for image acquisition, it should be relatively slow to improve the signal to noise ratio. The controlling computer thus automatically switches from one speed to another as appropriate in the sequence. In addition a different scanning speed is required if images of 1024 x 1024 resolution are required. This selection of the correct scanning speed once again is controlled by the main computer.

(6) Two separate digitising channels are available on the frame grabbing facility and the computer selects the correct detection mode just prior to the initiation of a digitisation scan.

(7) The digitised image is stored line by line directly in the frame store and displayed simultaneously on an auxiliary monitor.

(8) The final sequence is to save the digital image in the frame store as a disk file and to generate the relevant positional and magnification information for storage in the log file. Finally the specimen is moved to the next digitisation point.

Pre-set points method

This option allows the user to select points on a specimen while the user is browsing across the specimen. At each point from which an image is to be captured, the user presses the "Enter" button (and if necessary enters the required detection mode and magnification if they are different from the previous image). This information is stored in a file, and when complete, the computer will automatically reposition the stage at the previously visited points and capture images with the desired detection mode and magnification without further operator intervention. The automatic capture procedure is the same as in grid point method, except that the position sequence will not be regular but follow the sequence of co-ordinates selected by the user.

Unlike the grid point method, this function does allow the user to change magnification, image type and image resolution for any individual image (point). That means the user has an opportunity to take the pictures at a point with different magnifications (such as to have a close up view), different image detection modes, and different resolutions. The system is intelligent in that any attempt to take two sequential images with exactly the same location and operating conditions will be skipped.

Single point method

For some applications only a few digital images are required, and this option allows for the field of view, the magnification, detection mode, and resolution to be selected at will. This is similar to the previous method except that the stage is moved manually between each point and the digitisation and storage of the image is done directly after the position has been selected. Unlike the previous method there is no automatic control of focus and this is left to the observer. Because the observer needs to wait while the image is captured and stored, particularly if the resolution is 1024 x 1024, this method is less efficient in operator time, although for a few images or if special effects of contrast and brightness are needed this method can be effective.

As with the previous methods, a log file containing all the pertinent information is stored so that the previous locations can be revisited in an automatic mode should the need arise using the "points in file method" of operation.

Points in file method

All the three previous methods, (i.e., Grid points, Pre-set points and Single point), will generate a log file for each session of observations. This log file is a ASCII code file and has a uniform format with a name which is associated with the digital image filename generated from the information generated during the set-up sequence. The log file takes the same root name as the first image file but the extension ".DAT". It is possible to edit this log file at any stage during a session apart from during the automatic capture sequence so that extra points can be added. Alternatively, the co-ordinates, magnification, resolution, or detection mode in the existing file can be changed if required. In theory, it is also possible to generate a suitable log file so that any user defined system of images can be captured automatically. All that is needed is for the relevant log file to be associated with a particular session when the control will automatically follow the instruction of position, and magnification, as stored in that file.

With the log file loaded, it is an easy matter to drive the stage to the position of any one image and re-capture the image under different operating conditions. Alternatively the complete sequence of images may be recaptured

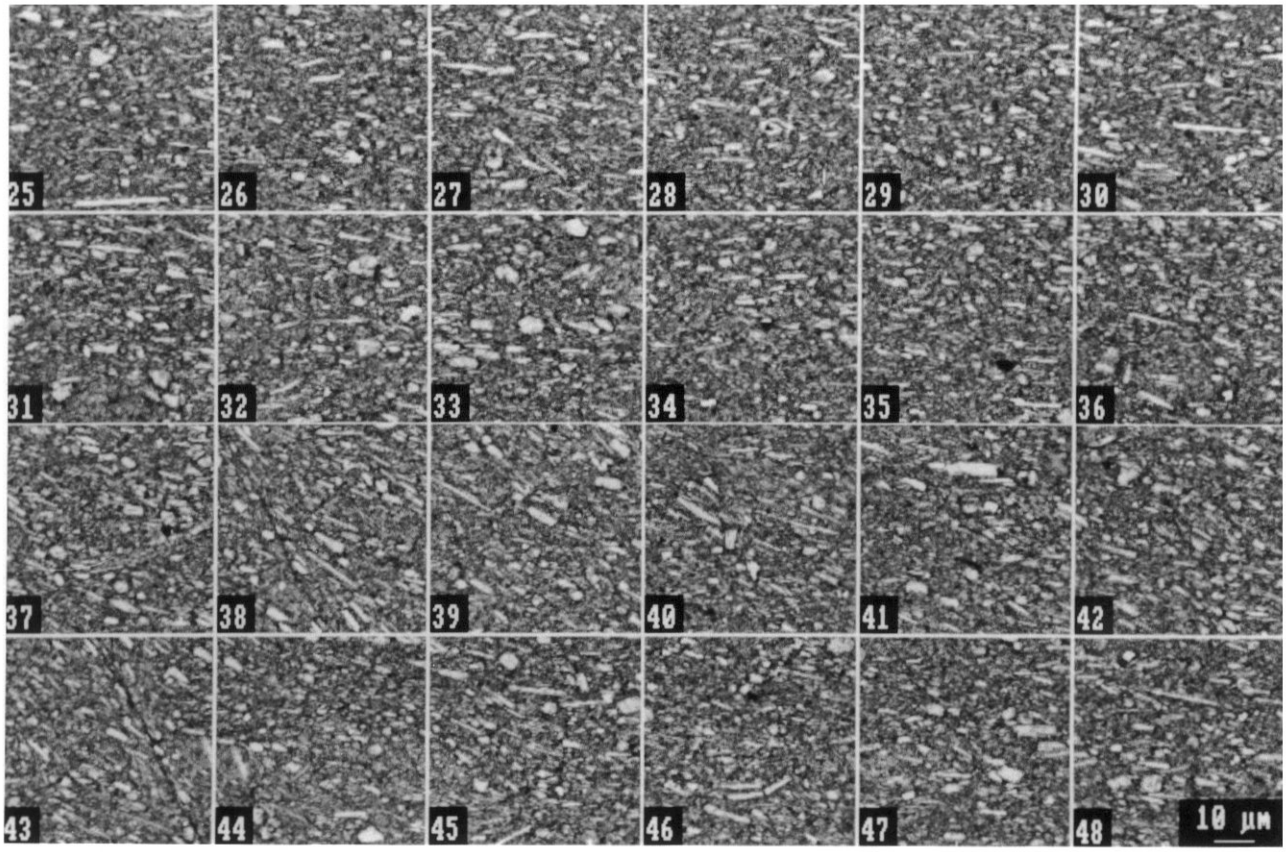

Figure 10. Summary images captured as part of a sequence of 400. The contrast variation between images is small.

and is, in fact, the method used in the "Pre-set" points method.

At the completion of an automatic sequence of image capture, the stored images are recalled to generate a summary image in which 24 separate images are stored as small reduced resolution versions such as shown in Figure 10. In this example images 25-48 of a sequence of 400 images are shown. What is clearly apparent is that the contrast of the separate images is very similar for all images. Constructing the images in this way allows a rapid check for any problem images such as artefacts, incorrect focusing, or incorrect contrast and brightness. In none of the tests done so far has the number of unsatisfactory images exceeded 2%, and usually it is much less. Since the co-ordinates and collection information of each is stored, it is easy to recapture the few unsatisfactory images again at the end of the sequence.

A typical capture sequence using the grid-point method involving 400 separate images was completed in under 3 hours.

Simultaneous Image Processing and Analysis

The image capture sequence is dedicated to that end with minimal image processing apart from a check of the grey level range. This means that it can complete the sequence efficiently and within the normal stable time frame of the field emission gun on the SEM. Once capture is underway, a second automatic sequence can be started to process the images appropriately. This analysis can be done on any one of several computers networked to the image capture computer, and may be by any of the methods which do not require operator intervention as discussed in the introduction. None of these algorithms when running on a 486 PC, can be completed in less time than a capture sequence, and in some cases, the processing time is several times the capture time. In this way, provided that the analysis sequence is arranged to start when about 5 images are stored, it is possible to have capture and analysis running parallel so that the completed results will be available in some cases only a short while after the capture is finished. This is particularly true of the intensity gradient orientation analysis which, including statistical analysis of the results

to generate an index of anisotropy etc. takes just under 30 seconds per image.

Example of Automatic Image Capture and Analysis

In the past, relating microfabric structures to macroscopic properties has normally required the capture of a limited number of micrographs from which key parameters such as index of anisotropy, direction of preferred orientation, micro-porosity have been computed. Results from all the images have been averaged arithmetically (sometimes vectorially in the case of orientation data) in attempts to relate these to external stress, strain or fluid flow. Such averaging was the only method available, although Tovey and Martinez (1991), and Tovey *et al*. (1992a) both show linear plots of the variation of parameters such as anisotropy and orientation along traverses of a sample. With the additional information available using the automatic capture method, it is possible to display the results in a spatial sequence to reveal sequences which would otherwise not be evident.

Two separate samples were studied as a test of the system. In the first, a sample of kaolin which was consolidated and then partially sheared was observed. Kaolin particles are typically plate-shaped around 0.4 μm thick and up to 5 μm in diameter. In an impregnated back-scattered image, the kaolin particles appear bright against the darker embedding matrix. To ensure that adequate resolution is achieved, the particles should be at least 3 pixels wide (see Tovey *et al*., 1995), and a magnification of 2000x is ideal as it is within the resolution achievable for this type of specimen, and yet not too high that the area covered is so small as to be unrepresentative. For a 512 x 512 image this means that each pixel covers an area of the specimen approximately 0.11 μm x 0.11 μm. In the second sample, the shearing was taken until well established failure zones had developed. To ensure detailed coverage, the grid point method of image capture was used with 20 rows of images each with 20 images spaced on a square grid with 50 μm between image centres. This provided complete coverage of a 1 mm x 1 mm area, with a small 5 μm overlap between images which would allow accurate compilation of a large mosaic if needed.

In the analysis stage both the orientation and index of anisotropy were computed at each point. These are displayed for the first sample in Figure 11, where each grid point is depicted by a small cross, and both the direction and strength of orientation are shown by a vector drawn from that point. The direction of this vector reflects the direction of preferred orientation, while its length is related to the index of anisotropy (the length of an index $I_a = 1.0$ is shown as a scale bar for comparison). This figure shows that the orientation of the clay particles is consistent within

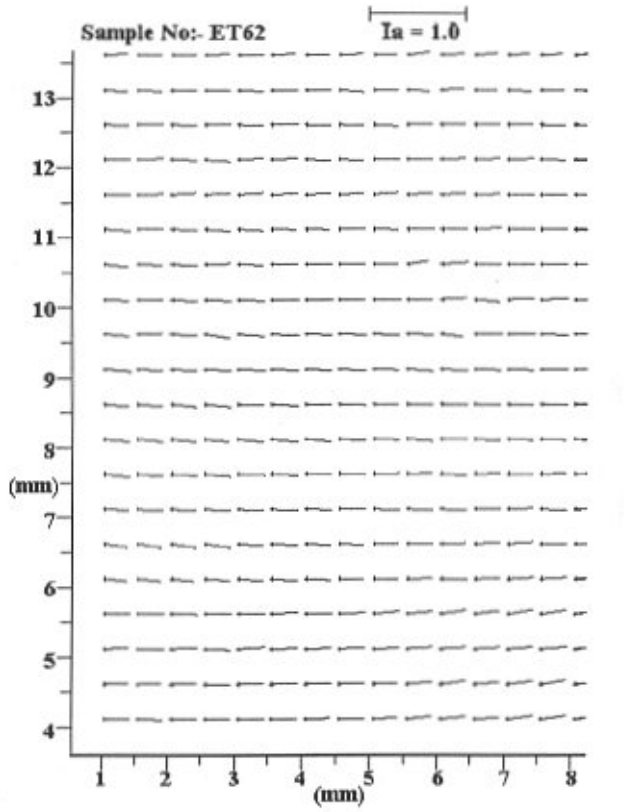

Figure 11. Orientation from over 400 images shows that this sample is homogeneous. The display has been truncated at the right hand side to fit within the column width.

the field of view. Contrast this with the situation in Figure 12 from the second sample, where there is clear evidence of a discontinuity associated with the failure surface. If a smaller area had been covered, or a linear or random set of observations had been taken, then this effect would well have gone unnoticed. Since careful record of the orientation of the sample is kept, it is relatively easy to relate the direction of this discontinuity to the external stressing conditions on the sample at the time of failure.

Conclusion

A fully integrated automatic image capture and analysis facility has been developed which makes efficient use of limited SEM resources but allows large numbers of images to be automatically analysed effectively. This is achieved by using two separate computers one dedicated to image capture, and the other to processing and analysis.

Associated with this development has been the need to incorporate image management schemes so that key parameters at the time of capture, and in particular the spatial

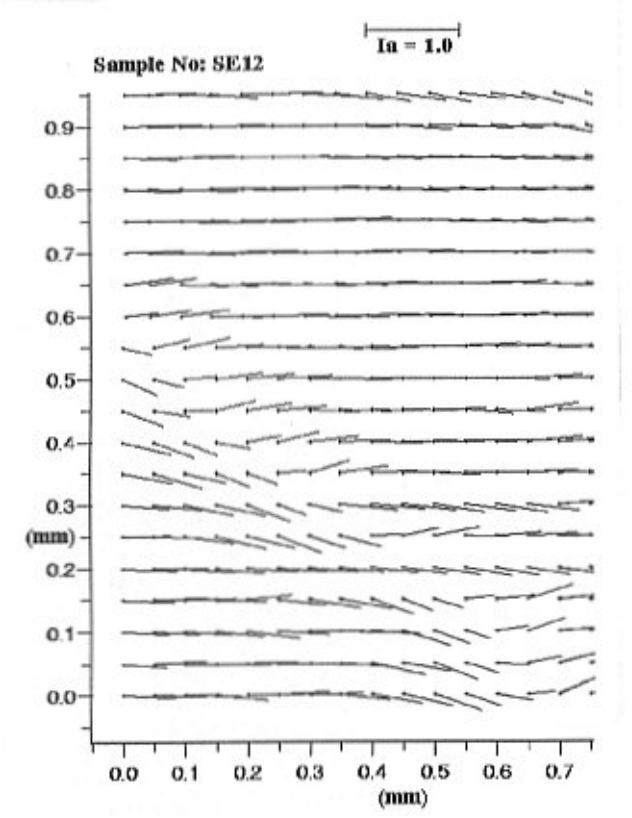

Figure 12. As Figure 11, but in this case there is clear evidence of a discontinuity.

position of the stage, are recorded. This allows rapid relocation of a position on a specimen even if it has been removed from the microscope. This image management includes user information as well as a convenient and automatically generated but unique file naming system suitable for easy automatic batch access at a later date. Such a system requires a suitable archiving method of the large storage volume, and CDs have been found to be the most convenient and reliable.

The increased data available from such analysis also provides information not otherwise available, particularly when spatial trends within an sample are under investigation.

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Discussion with Reviewers

V.N. Sokolov: In the author's pervious paper (Smart and Tovey, 1982), K. Tovey pointed out that reliable information

on sand grain microstructure (size and shape distribution) can be obtained after SEM observations on not less than 30 grains (usually from 30 to 50 grains). In this article, the authors believe 24 different images to be enough to study the microstructure of a soil specimen which is 15 mm x 15 mm in size, while Smart and Leng (1993) recommend 25 images to be studied for this purpose. What is the authors' background to validate such numbers of SEM areas required to get reliable quantitative information about soil microstructure? How do the authors estimate the soil microstructure homogeneity, and take into account this factor when they choose a certain number of SEM images for analysis?

Authors: The original reference in 1982 referred to several discussions which were held at meetings where the question of reliability of interpretation was discussed in connection environmental reconstruction from evidence seen from the microtextures on sand grains. The figures cited were the general consensus of opinion at the time, and were in general agreement with the size analysis investigation carried out by Tovey *et al*. (1978).

With regard to soil microstructure, the situation is more complex, as structural variations may be consistent over relatively large areas. Evidence using 24 images (e.g., Tovey *et al*., 1992a) indicates that there is usually broad agreement in the quantitative estimates between adjoining images taken at spacings up to 2 mm as used previously in the authors' laboratory. This figure of 24 was also convenient as it reflected the maximum storage available on the SEM available at the time. However, after distances of about 8-10 mm, there may often be a significant change. The question about reliability and number thus depends on the scale of variations in which one is interested. Even though there is often broad agreement at the scale of 1 mm, it is quite possible to miss discontinuities in the form of shear zones which may be only 0.1 mm wide. To identify such effects it is necessary to take a continuous sequence covering the whole width of a sample with overlapping images. Tovey and Martinez (1991) showed such a discontinuity in distances of about 0.05 mm, while Figure 12 reinforces this aspect.

In summary, the question of numbers relates to the scale of interest. It would appear that about 20-30 images uniformly spaced can give an adequate indication of structure at the scale of 1mm, but at smaller scales, or situations where discontinuities are to be located, then a higher number of images is required. While arrays of 20 x 20 images (i.e., 400 images in total) were used in this investigation, the presence of the discontinuity in Figure 12 could have been identified with the same number of images per row, but only perhaps 3-5 rows (making 60-100 images).

V.N. Sokolov: Actually all the rocks and clay soil are polydispersed mineral systems and it is impossible to embrace the entire size range of structural elements by considering only one fixed magnification of SEM images. How does your method account for this problem and is it possible by your analysis system to carry out quantitative soil microstructure investigation by a sequence of diverse scale SEM images within a very wide range of structural element sizes and after that get the integral data on pore and particle sizes in the soil specimen?

Authors: To date we have concentrated on the automation aspects. However, the facilities do allow us to specify different magnifications either individually or as part of a predetermined sequence so that we could examine the changes in parameters as the magnification is changed, and thereby examine an overview at a range of scales at a particular location as well as observing the changes in that parameter across wide areas of the specimen. Some of the parameters - e.g. orientation analysis need special consideration when large particles (about 1 mm in diameter) are included with clay size particles. The former are approximately equant, while the latter are elongate, and noise issues effects within the larger particles will need to be addressed. In porosity studies using embedded samples in the back-scattered electron mode, specimen beam interactions limit the ultimate resolution (even with image reconstruction), and so the maximum magnification is probably around 10000x. However, using fracture surfaces and the secondary electron mode should allow information up to higher magnifications to be obtained.

Reviewer II: The authors have found workable solutions to the challenges posed by automating a SEM for routine work by many different users. It would be useful for the paper to have a section listing alternative solutions that the authors would have liked to try as well as ones they believe would not be workable — in other words, a look into the future.

Authors: This is a particularly interesting suggestion. One thing that has to be considered is the availability of technology and likely changes in the future. We made a start on this project in 1993 and the initial specification was done in terms of likely requirements of the users and the technology then available. We revised our specification during the project which was completed in mid 1995. One problem we found that many of the users themselves did not appreciate the potential and so we were continually projecting what they may wish to do. In this way we developed a system with a high degree of flexibility for the different types of users. Essentially, we initially identified three key types of use; all others applications could be accommodated within these:

(i) automatic unattended capture of large numbers of images in a pre-set grid array,

(ii) automated capture of images the specimen position of which had been manually located previously,

(iii) automated capture of images from a random set of locations defined in a file.

One deficiency which we have now implemented is the ability to take a preselected sequence of magnifications at a particular location. Previously, the user could separately identify a separate image at each magnification, but this became tedious, and occasionally prone to error. Instead the user can define a standard set of magnifications. Secondly, something which we have not implemented, but are considering is the situation where there are individual features (e.g., sand grains, forams) scattered randomly over a specimen stub. This is something of limited interest to the present authors and so has had low priority. It should however be a relatively simple matter to take a few images at low magnification to cover the whole specimen. From each of the images using cross correlation, it will be possible to identify the co-ordinates of each feature, and then translate these into specimen stage movements for observing the feature at higher magnifications.

We are conscious of the requirement for basic image processing on line (e.g., the cross correlation mentioned above), and that was a reason for adapting image processing software rather than developing stand alone facilities. However, we strongly believe that the approach we have taken to use a separate image processing facility to process images rather than do it on a single dedicated facility attached to the SEM is the way forward. In the latter approach, it will often be the processing time in an image analysis which will dictate the overall time of operation, and this could become lengthy for long runs, and lead to inefficient use of the SEM.

Reviewer II: Although the sections containing the authors' descriptions of microscope operation read somewhat like an instruction manual, I believe that it will be useful for the interested reader to learn about the details involved. On the other hand, the description of the file-naming conventions could be shortened without too much loss.

Authors: We generally agree with your sentiments, however, it became apparent during the work with the acquisition of large numbers of images that a key issue was a reliable and consistent method for naming files. In a case where there are many users, this can be difficult to get an agreement. Thus we have imposed an automatic method of naming as described. This is our solution to the problem and it is effective. That is why we have described it in full. Further, it was an issue raised by many visitors as to how we would actually achieve this, and thus appears to be of interest to some readers.

P. Smart: Would it be possible to check the stability of the microscope by repeating an image at the same location and under the same operating conditions? Does the computer warn, but permit this?

Authors: This is an important issue, and we are still experimenting with this. If repeated images are taken then this will increase the acquisition time, and thus it is not practical to do this for every image in a sequence of many. At present we find that on our instrument the critical problem is a stepwise change in parameters, particularly in brightness. At present we use the image processing facility to extract a histogram of intensities every five images. If the mean and standard deviation of intensities is significantly different from previously set then a warning sound is broadcast to the laboratory with the option for an operator to intervene. We do not have facilities on our instrument to then readjust the contrast and brightness in the back-scattered mode under software control, but with newer generation instruments this should be possible. Our experience is that in a run of 400 images, we may receive the warning once or twice in the 3 hour period required for capture.

P. Smart: Are any automatic artefact finders built into the software, for example to find bad charge-coupled device (CCD) elements, missing scan lines, truncated images, charged areas, mechanical damage, or atypical parts of the specimen.

Authors: The issue of CCD elements does not arise as there is direct capture from the SEM itself. Also since the image capture for each line is triggered by a pulse generated by the software, the question of missing scan lines is also not a problem. Charged areas would be manifest by bright areas within a region of otherwise normal contrast, and at present we do not reject those images at time of capture, but subsequently during processing. As we generate a summary image (Fig. 10, which is automatically printed on the laser printer), we can quickly see manually any images so affected, but it would be a simple matter to determine this automatically. Since we are normally using the back-scattered electron mode, the problem of charging is less severe than for secondary electron capture. Mechanical damage in general would be difficult to identify automatically, but it should be possible to identify some aspects of damage such as score marks from polishing, although we do not have such working at present. Once again, since a full set of images is typically 400, and these can be displayed in summary form (Fig. 10) in just 11 images, so this is a quick way of identifying such effects.

Finally, with regard to atypical parts of a specimen, this was one of the main driving forces behind this project. Thus by mapping the results as shown in Figure 12, it is possible to identify the discontinuity within the specimen.

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