Chapter 3 - Advanced Operation

Advanced Operation

Having now become familiar with the *Stereoscan 430* controls, sample insertion and basic micrography the operator may find that there are still a number of effects limiting image quality. This section describes some of these effects and ways to reduce them. Complete elimination of all problems is often impossible, and a compromise is usually reached. In order to get the best results from the *Stereoscan 430* it is important to consider the following:-

- Instrument familiarity
- Photographic considerations
- Choice of instrument parameters
- Specimen charging
- Specimen preparation
- Using the digital store

These factors are treated separately below. In addition the ways in which the user interface can be customised to meet the individual user's needs are described and finally a section on troubleshooting is included.

3.1. Instrument Familiarity

To get the best results from the *Stereoscan 430* it is important to be familiar with the operations described in this document. If possible attend a Leica Cambridge operators course which can be arranged by your local Leica representative listed in Appendix D of this Manual.

3.2. Photographic Considerations

A good micrograph is sharply focused, free of astigmatism, noiseless, with optimum contrast and brightness. These factors are very user dependent but are easily achieved with a little practice. Setting image levels correctly results in an image which is better looking and more readily interpreted. Understanding how to manipulate grey levels allows tailoring of images for specific purposes.

The camera and record CRT or video copier/printer should be correctly calibrated and the signal waveform should be evenly distributed between the upper and lower video level markers, nearly spanning the range but never going outside the limits set by them. Try to select the profile scan position on the image to span the brightest and darkest areas to give a representative sample of grey or video levels. The display look-up table editing facility can be used to create a 'visual' exposure meter that clearly indicates lower and upper levels in the grey scale. This is a modification of the display look-up table, where the black level is changed to blue and the white level changed to red. Hence, an image containing a good grey scale range will just begin to show areas shaded blue and red. With this look-up table active, areas of too low a signal show blue whereas areas of too high a signal show red. See Section 3.7.1 *The Display LUT Editor.*

Other forms of display LUT can be used to enhance the contrast or adjust the brightness of the frozen image.

Many samples exhibit a small amount of charging due to oxide layers or other surface coatings. These samples show changes in signal level and contrast with scan speed. For live micrographs of these samples, the signal should be set up using a scan speed similar to that to be used for the photograph. The image store is particularly useful, as it can be used to save film by allowing the image to be acquired and checked before photographing it. Also, many identical prints may be taken without the need to scan the sample again.

3.3. Choice of Instrument Parameters

Instrument parameters have a large effect upon the final image quality. To some extent the parameters chosen are dedicated by the sample and its interaction with the beam. For each different specimen, some experimentation will usually be required before arriving at the optimum conditions. Having achieved these conditions, they may be saved on disk (as a Macro file) for future reference, see Section 3.7.2 *The Macro Editor.*

3.3.1. Accelerating Voltage (EHT)

The accelerating voltage, or beam energy, is one of the most important factors in achieving a satisfactory sample examination. It will affect:-

- Damage to the specimen by the beam
- Visible surface detail
- The production of X-rays for analysis
- Image resolution

In determining the accelerating voltage, the following factors must be considered:-

Beam Damage

If the specimen is beam sensitive, a higher accelerating voltage will result in a greater risk of the sample being damaged by the beam. This can introduce artefacts into the image which may appear as cracking or darkened areas in the image. The cause of this phenomenon is a localised heating or ionisation of the specimen in the area of examination, hence the problem worsens as the magnification is increased. Beam damage can also be the result of using too high a probe current, thus the accelerating voltage and probe current should both be adjusted together to determine the optimum settings. This problem is usually exhibited by low atomic weight specimens such as plastics or biological samples. Coating the specimen with conducting film such as gold often helps to dissipate the heat generated by the beam. However, better results are usually obtained by operating at lower voltages.

Surface Detail

The accelerating voltage of the electron beam directly affects the amount of surface detail seen in the image. This is most easily understood by considering the simplified beam-specimen interaction volume shown in Figure 3.1.

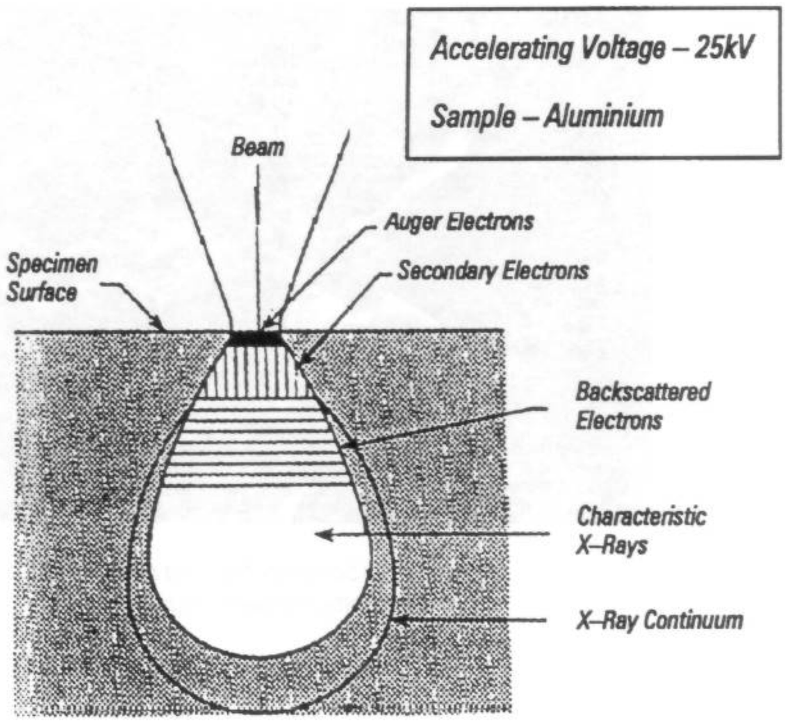

Figure 3.1

The zone of primary excitation increases in depth and diameter with a high accelerating voltage. Backscattered electrons which are generated mainly beneath the specimen surface possess enough energy to ionise one or more specimen atoms before escaping from the specimen surface. Each of these ionisations produces more secondary electrons which are added to the true secondary electron signal and result in a loss of surface detail. The use of too high a voltage also results in parts of the sample exhibiting very bright edges. The 'edge effect' is caused by the electron beam completely penetrating the edge of the feature, so that secondary electrons are produced from a larger effective surface area, see Figures 3.2(a) and 3.2(b).

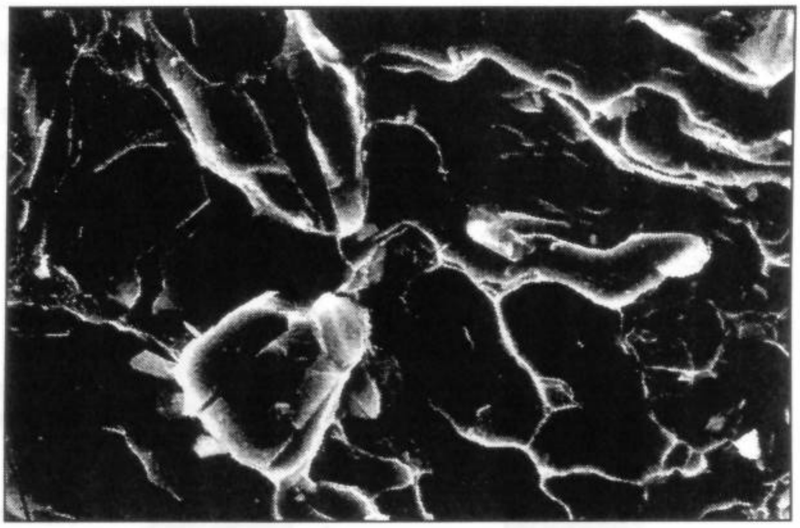

Figure 3.2(a) Sample of fractured aluminium at high voltage showing edge effect

Figure 3.2(b) Sample of fractured aluminium at low voltage without edge effect

X-Ray Analysis

The production of X-rays from the specimen is caused by the removal of an electron from one of the atom's shells and the subsequent re-arrangement of the remaining electrons. The initial removal of an electron is caused by the primary electron beam, therefore the beam energy must be sufficient to overcome the binding energy of the electron to be removed. X-rays may be emitted as a result of ionisations within any of the electron shells - K, L or M and are then termed, K, L or M X-rays. The energy required to stimulate a particular X-ray is known as the critical energy, therefore if the energy of the primary beam is less than the critical energy, no X-ray is produced. Thus, for example if analysis for iron is required, then the beam energy must be at least 6.4KeV (to produce an iron K peak). In fact, for ideal analysis (especially quantitative) the accelerating voltage should be 1.5 to 2 times the critical energy of the highest energy peak to be analysed; this is known as the `overvoltage'. Samples that charge, or are non-conducting should be coated with a suitable material chosen to give electrical and thermal conductivity, but not to interfere with the elements specifically being analysed. For example, if carbon is the element of interest in the spectrum then it would not be a good idea to coat the sample with carbon. In this case, aluminium would be better because it has a sufficiently low atomic number not to attenuate too much of the carbon signal (whereas coating with gold probably would).

Resolution

Resolution can be defined as the minimum distance by which two adjacent features can be seen to be separated, as two discrete points. The best resolution is usually obtained at high accelerating voltages since the shorter wavelength of electrons at high voltages minimises any diffraction.

Other instrument parameters also significantly affect the resolution achievable including Probe Current, Working Distance and Aperture Size.

3.3.2. *Probe Current*

The probe current is defined as the total electron current reaching the specimen surface. The value can be adjusted from the user interface in the range 1pA to 1μA. In most cases, a value of 100pA gives a good video signal without over exposing the sample to electron bombardment. Other settings would be as follows:-

High Resolution:	5pA to 25pA
Backscattered electron imaging:	200pA to 500pA
X-Ray analysis:	600pA to 1nA
Charge or beam sensitive samples:	10pA to 20pA

The improved resolution performance at low probe currents is achieved because the diameter of the beam on the specimen is reduced. A larger beam diameter is acceptable for X-ray analysis because of the larger volume of interaction (see Figure 3.1).

3.3.3. *Working Distance*

Working distance is defined as the distance in millimetres between the bottom of the lens and the sample surface. In common with many of the other SEM parameters, choice of working distance for the specimen may have to be a compromise. The factors to be considered are:-

1. As the working distance is decreased the available resolution increases. The optimum position is in the range 3 to 10mm.

2. If the sample is large, such that areas other than that being imaged come into contact with the lens or backscattered electron detector, it is wise to use a longer working distance.

3. The retractable backscattered electron detector occupies a finite space beneath the final lens. All parts of the sample should therefore be just clear, particularly when the detector is to be moved in or out of position. A safe working distance is 12mm.

4. The geometry used for X-ray analysis, at a 35° take-off angle, dictates that the surface to be analysed should be at a working distance of 25mm.

5. An improvement in depth of focus, the vertical distance over which the sample appears to be in focus, can usually be made by increasing the working distance. An increased working distance has the effect of decreasing the angular aperture of the electron beam. (See Figure 3.3)

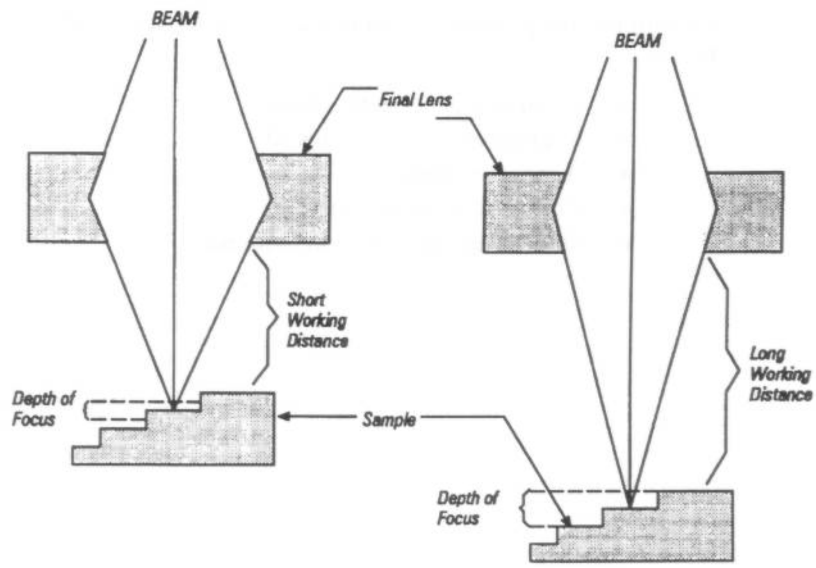

Figure 3.3 Enhancement of Depth of Focus

Item 5 above directly conflicts with item 1. However, improvements in depth of focus are usually required at relatively low magnifications where the minimal loss of resolution caused by using a longer working distance would not be noticed. Depth of focus can also be improved by using a smaller aperture, say 30µm or 20µm, or by selecting *Optibeam* depth of focus mode. This selects the lens settings which give the greatest depth of focus while maintaining the selected probe current.

3.3.4. Specimen Tilt

Tilting the specimen can greatly affect the SEM image, not only by changing the area excited but also by altering the zone of secondary electron emission. The size of this zone varies with the angle at which the primary beam strikes the specimen. When the specimen surface is perpendicular to the beam, the zone from which secondary electrons are emitted is smaller than when the specimen surface is tilted. Since more secondary electrons are emitted, the tilted surface gives a brighter signal. Tilting in the range 20 to 40° gives the best result. Very often, a small amount of tilt adds some depth to the final image which gives more visual appeal than a flat image.

3.3.5. Other Parameters

In addition to the parameters mentioned previously, the following should all be checked:-

- Electron gun correctly aligned
- Stigmators correctly adjusted
- Aperture centred
- Correct film speed selected
- Gamma and derivative as required

3.4. *Specimen Charging*

Electrons incident upon the sample which do not escape as backscattered or secondary electrons are absorbed by the sample. Unless these absorbed electrons can find their way to ground (as in a conductive sample) they will remain in the sample resulting in a negative charge build up exhibited as one of the following image defects:-

- Loss of image contrast (see Figure 3.4)
- Very bright or dark areas (see Figure 3.5)
- Image or beam shift (shearing) (see Figure 3.6)

Dust and other debris on the sample surface can also charge up, and appear as a bright area on the image surrounded by a dark region. The charged debris may deflect secondary electron emission and sometimes the incident beam, causing dark region and possibly image 'shearing' (see Figure 3.6). If the sample itself is charging, beam deflection will occur during scanning, the effect being seen as a slow image drift followed by a jump back to its original position.

Figure 3.4 Loss of Contrast Due to Charging

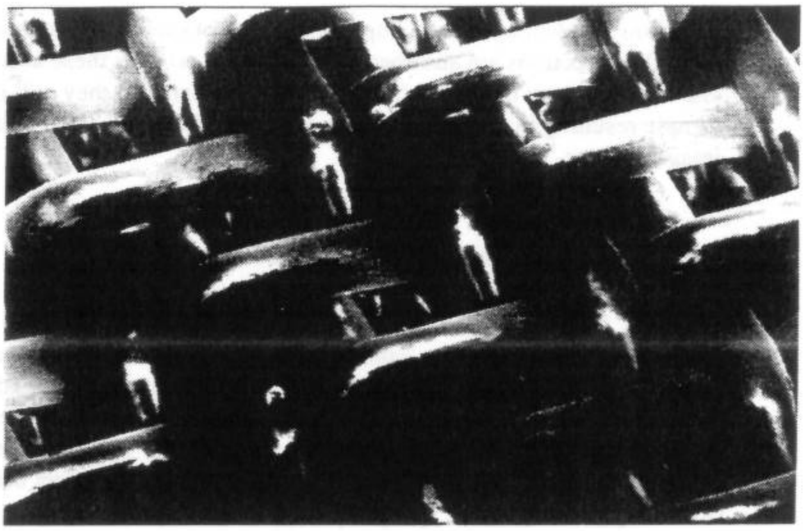

Figure 3.5 Bright/Dark Areas Due to Charging

Specimen charging of non or badly conducting specimens is dependent on the charge equilibrium inside the sample. The secondary electron yield (S) is defined as the number of secondary electrons which leave the sample for one incoming primary electron. The corresponding number for backscattered electrons is the backscatter coefficient (B). If the total electron yield T=B+S equals 1, the charge balance is zero and therefore no charging occurs. If T increases above 1, the sample should charge up positively because more electrons leave the sample than primary electrons enter it. If T falls below 1, the sample will charge up negatively. One of the most important parameters for changing the total electron yield T is the primary beam energy. This is shown in Figure 3.7 which shows the typical dependence of T on the primary beam energy Eo. If the primary = Ec_2, then no specimen charging occurs. Typically, Ec_2 lies between 500eV and 2KeV.

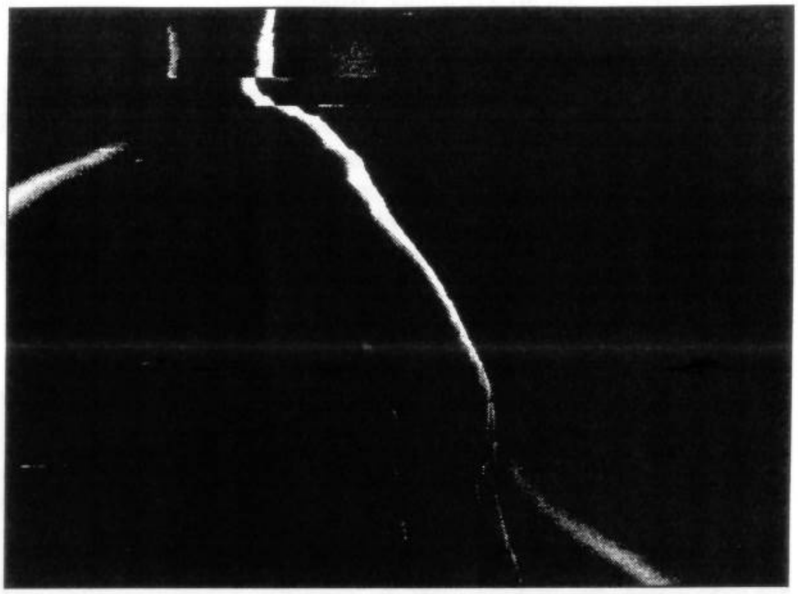

Figure 3.6 Image Shearing Due to Charging

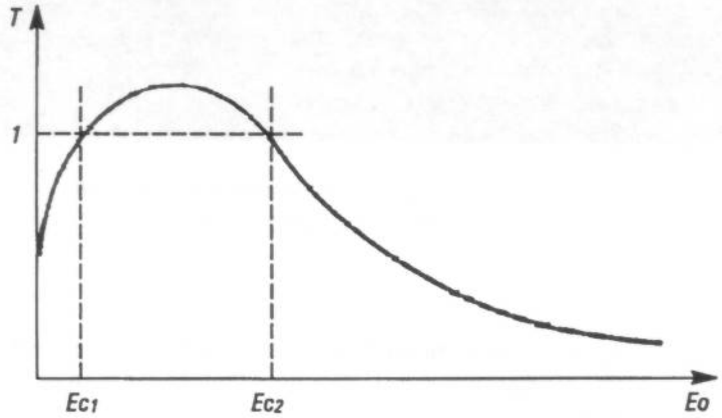

Figure 3.7 Relationship Between Primary Energy Eo and Total Electron Yield T

The total electron yield can also be increased by tilting the specimen. A tilt in the region of 20 to 40 will greatly reduce charging.

The other most important factor affecting charging is electron "dose rate". This can be defined as the total number of primary electrons per unit area of the specimen as a function of time. In other words, the scan speed of the beam is critically important. It can be seen that on some beam sensitive materials a fast scan speed gives a good screen image with good contrast and topography (see Figure 3.8(a)) whilst a slow scan speed, ie for live photography, (see Figure 3.8(b)) causes the image to display the defects mentioned previously. The following diagrams illustrate this effect.

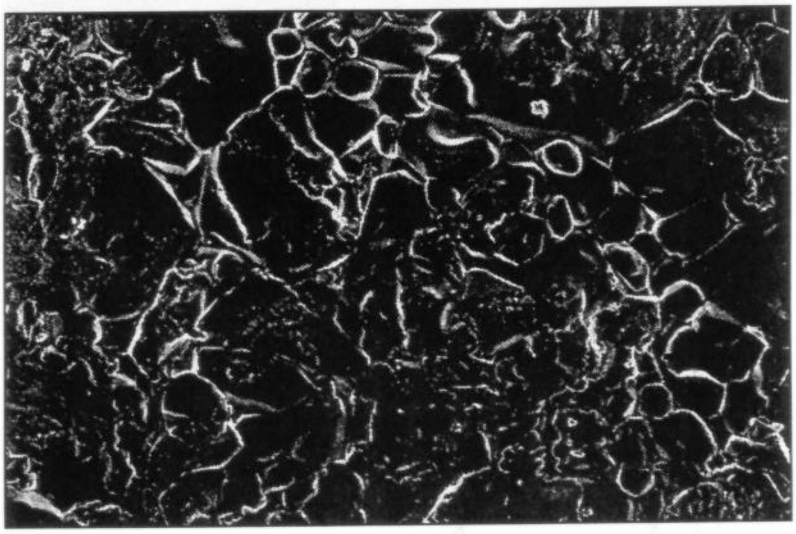

Figure 3.8(a) Taken at a fast scan speed
The above shows a sample of Ceramic

In this case, the image store can be used to acquire the image, with noise reduction if necessary, at a fast scan speed. The stored image can then be photographed or stored to disk.

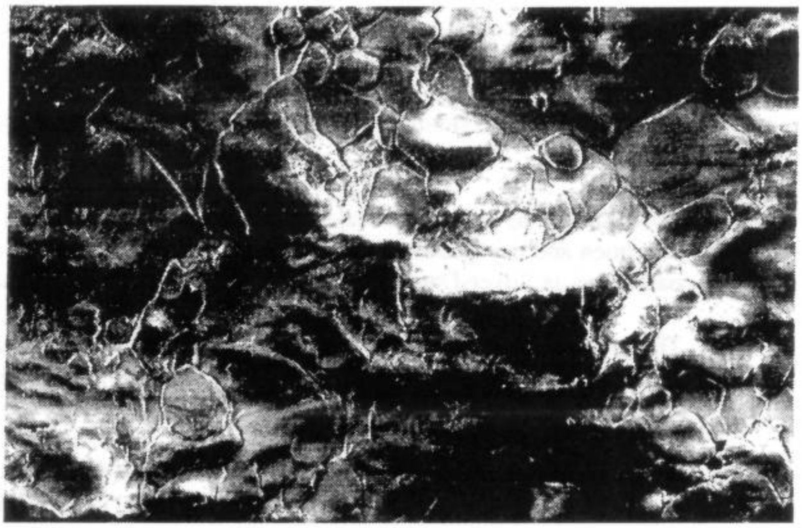

Figure 3.8(b) Taken at a slow scan speed
The above shows a sample of Ceramic

3.5. Specimen Preparation

Specimens which are studied in the SEM can be divided into two main categories, namely conductors and non-conductors. Factors to consider during specimen preparation are given below:-

1. The size of the specimen must be reduced (if necessary) to fit the available specimen holders and specimen stages, but there is often an advantage in selecting an even smaller sample size for ease of observation.

2. The object must be able to withstand being in the high vacuum of the SEM; it must not change its shape, and it must not outgas. A cold stage may help here.

3. It should be clean, ie free of dust, oils and greases. (Their presence can lead to charging and contamination effects.)

4. It may be treated to improve the secondary yield if this is low, eg coating of the sample with gold.

5. Any disturbance in the surface structure caused by preparation procedures should give rise to surface details which are too fine to be resolved at the magnification used to record the images.

6. If an artefact is suspected as a result of a preparative procedure, a control specimen should be utilised.

7. The specimen stub should be in good electrical contact with ground potential.

8. There should be good electrical contact between the surface of the specimen and the specimen stub, ie attach sample to stub with conducting paint such as Silver-Dag and coat the specimen adequately if necessary.

9. The specimen stub should give rise to as few backscattered and secondary electrons as possible. Aluminium stubs are normally used, although carbon stubs are used for some X-ray microanalysis applications.

10. Very small particles are best mounted on a low mass foil to give rise to minimal background signals, eg nylon film stretched over an aluminium ring.

11. The sample must be attached to the specimen holder (stub) so that it does not move whilst being irradiated by the electron beam.

12. The sample should be attached to the specimen holder (stub) so that all the surface can be studied using the existing stage movements (eg tilt, rotate, X, Y, Z).

3.5.1. Conductors

These fall into two groups:-

- Metallic: these are generally excellent conductors and need no preparation
- Semi-conducting samples with a resistance of less than 10^{-10} ohms can be examined without special preparation

3.5.2. Non-Conductors

This group includes all samples which are not electrically conducting, eg those generally not containing volatiles, eg fibres, plastics, polymers, semi-conductors with a resistance greater than about 10^{-10} ohms.

Those generally containing volatiles, eg biological and botanical material.

3.5.3. Non-Volatile, Non-Conductors

If it is not possible to obtain suitable resolution by using a low accelerating voltage and leaving the sample uncoated, then the following applies:-

For most non-conductors which contain no volatile components, eg water, that would outgas in the vacuum system it is sufficient to coat the sample with a thin layer of conducting medium such as Au, C, Au/Pd, Al etc. This layer is typically 20-30nm in thickness. There are several reasons for this coating:-

- Increased conductivity of the sample, thus minimising sample charge up, which results in deflection of the incident beam and severe degradation of the final image. (see Section 3.4 *Specimen Charging*)
- Increased mechanical stability of the sample due to increased heat condition.
- Increase in primary and secondary electron emission.
- Decrease in beam penetration, resulting in better spatial resolution. The two important current techniques of applying a coating are vacuum evaporation and ion sputtering.

Gold is generally used for the following reasons:-

- High secondary emission co-efficient.
- High conduction of electrons and heat.
- Does not oxidise.
- Good granularity of evaporated or sputtered particles.

Carbon coating by evaporation is generally used if X-ray microanalysis is to be undertaken on the sample unless, of course, the element under investigation happens to be carbon. Aluminium could be used in this case.

More recently Pt/Pd and Au/Pd have been used since their granularity is smaller. Aluminium can also be used, but it has low mechanical strength and can oxidise.

3.5.4. Non-Conductors (Volatile)

Biological and botanical samples, by their nature, require relatively more complex preparation procedures. The samples fall into two main categories: a) hard, b) soft.

a. **Hard samples (eg bone, teeth, wood).** These, if necessary, can be washed to remove extraneous fluids such as blood and mucus, dried in air and coated in the normal way.

b. **Soft Samples**

- **Untreated** - Soft tissue needs more specialised treatment. Most soft tissue contains up to 90% water which must be removed without altering the structure. If this is not done, there will be difficulty in achieving adequate vacuum in the SEM, and complete or partial sample collapse and distortion will occur.

Some botanical specimens can be observed successfully for short periods provided that thought is given to the selection of instrument parameters, eg using a low accelerating voltage and beam current.

• **Replication -** Although it is usually only adequate for low magnification work and for comparatively simple surface topography, replication has the advantage that the sample can be totally preserved. One method of replication uses an elastomeric material such as silicone rubber to obtain an impression. A positive replica is then obtained from the impression by coating it with low-viscosity polymethylmethacrylate solution, allowing this to dry and then stripping the resultant film away. Coating and examination follow in the usual manner.

With suitable modification, transmission electron microscope replication techniques can be utilised.

• **Chemical Pre-Treatment -** This technique involves chemical fixation of the material to strengthen the tissue. There is a large range of chemicals used in this process (eg glutaraldehyde and osmium tetroxide) and there are numerous publications discussing the benefits of each. After fixation, it is necessary to displace the water in the sample by a solvent to aid drying. The method must be such that the specimen suffers no physical change. The commonest drying agent used is a series of ethanol/water mixtures through to 100% ethanol. Having replaced the water present in the sample there is a choice of three methods for drying:-

i) Freeze Drying
This is a complicated procedure, and may not be too successful. The sample is quench-frozen and maintained at low temperature (about -130°C) until the sublimation process is complete.

ii) Air Drying
It is worthwhile first transferring the specimen to a solvent of low volatility (eg amyl acetate) as the last stage of dehydration. The solvent is then allowed to evaporate from the sample under carefully controlled temperature conditions. As this is a gentle process, there is little chance of any specimen damage.

iii) Critical Point Drying
The specimen is dehydrated as previously described and the solvent replaced with a liquified gas in small pressure vessel. The vessel is then heated to above the critical temperature of the selected gas. Under these conditions the liquid and vapour phases have the sample physical properties, so that on venting, the liquid

vapourises across cell boundaries and therefore minimum sample distortion occurs.

The sample may however, undergo some mechanical shock during the venting stage. The choice of gas for this process is limited by the number of available gases which have a critical temperature relatively close to ambient, a safe pressure level and low toxicity, whilst at the same time being completely miscible in all proportions with the solvent selected in the final stages of dehydration. It is important to replace the solvent completely with the liquified gas (usually carbon dioxide) before venting.

General Considerations
The methods described above for soft tissue preparation are mainly for secondary electron imaging. The problems facing the biologist or botanist who wishes to undertake X-ray microanalysis are different in that the requirements in this case are to maintain the element(s) of interest in their original position in the sample. There is not one major preparative technique for biological/botanical samples. Where possible, several combinations should be tried for a particular type of sample, giving prime consideration to the information sought. Once a technique has been established, instrument parameters and specimen coating methods must be carefully considered.

3.6. Using the Digital Image Store

3.6.1. Noise

The image store of the *Stereoscan 430* is particularly useful in situations where use of poor signal-to-noise conditions is forced. In these circumstances, noise can be removed from the image before photography. For example, samples that exhibit charging often require the use of a very small probe current. Noise arises from the electronics of the video system and gives a grainy appearance to the image. This occurs in situations where there is little signal detected from the specimen and the photomultiplier gain (or amplifier gain for BSD) is increased to compensate. A low signal can be the result of a small electron emission co-efficient of the sample or of the choice of microscope operating conditions, eg low probe current. The amount of signal compared to the noise content is known as the signal to noise ratio.

3.6.2. Noise Reduction

Prior to the use of digital techniques the only way to reduce the noise in the signal was to use a slower scan speed. This allows the beam to dwell longer on each scanned point of the specimen so that more signal is collected. Digital methods of noise reduction utilise the fact that the noise is random in nature. If the signal from a single specimen point is sampled repeatedly and the average taken, noise will be averaged out and the signal will be reinforced.

On the *Stereoscan 430* the concept of scan rate is replaced by pixel averaging. The pixel average parameter determines the number of times each pixel is sampled with the average making up the signal to the image store. Increasing the pixel averaging has an effect similar to using a slower scan rate (thus the image takes longer to acquire, but will have less noise in it). Reduce raster mode does not change the pixel averaging, but does reduce the cycle time (the time taken to perform an image acquisition) which makes adjusting certain parameters easier.

Additional noise reduction of two other types may be applied.

Frame Average

When frame average is selected the live signal is proportionally mixed with the store signal so that the image reflects the average of the recent frames. The proportion of live to stored can be adjusted with the parameter **N** which represents the number of frames to be averaged. Frame average can be selected with any level of pixel average, but is generally most useful at the faster speeds (lower pixel average) where a larger amount of noise reduction can be obtaineed without introducing a long cycle time.

Line Average

Selecting the line average causes each line to be scanned a number of times before the scan moves on. The average line signal is stored and displayed.

Choice of Technique

The decision as to which noise reduction technique is to be used will depend to some extent on the specimen being imaged. If it is sensitive or prone to charging then frame average may well be the best option because the beam does not dwell at one place for a long period of time. Line average is used when the result of the noise reduction needs to be seen without waiting for the cycle to complete. Increased pixel average is most useful when doing live photo records (where the image goes directly to the HRRU without being processed in the image store).

On the Noise Reduction panel there are two options for freezing the store. **At End** is used to freeze the store when an acquisition cycle completes, whereas **Now** causes the image to freeze as soon as the button is selected.

3.7. Customising the User Interface

Careful thought and design has gone into producing a user interface which meets the requirements of *Stereoscan 400* users. However it is recognised that user requirements are so diverse that it is impossible to provide one interface which meets every requirement. Therefore several features are provided which allow the user to customise the interface to meet their individual needs. The following parts of the user interface can be customised:-

- The display look up table (LUT)
- The macros and function keys (F5 - F8)
- The status display
- The appearance and operation of the tool bar

3.7.1. The Display LUT Editor

The display LUT editor provides a mechanism for altering the mapping between the grey level in the image store and the way in which the pixel is displayed on the monitor. The LUT is really three LUTs in one, a mapping exists for each video colour (Red, Green and Blue). If these three mappings are the same then the image will appear as monochrome (grey) on the monitor.

There is one important rule to bear in mind when editing LUTs, which is:-

Each grey level must have one and only one LUT value.

It is however permissible for several grey levels to be mapped onto a single colour (or shade), in fact this is one of the more useful features of the display LUT.

The LUT Editor

The display LUT editor is selected from the **Edit** menu. Its own menu provides option for both grey and colour LUTs as well as facilities for loading and saving LUT files. Before considering the menu options in detail it is necessary to understand the concept of a LUT, as seen by the system.

A LUT is a collection of **points** which are connected by **arcs**. The simplest LUT has just two points (0,0) and (255,255) connected by a straight line. Various operations can be performed on points (they can be **added, moved** or **deleted**), other operations are performed on arcs (actually on the two points defining a arc) including **level** and **threshold**.

In the LUT editor, the middle and right mouse buttons have a special meaning. The middle button is used to select the colour being adjusted (when editing a colour

LUT). The right button is used to select between **move, add** and **level** modes, which determine the operation on the points.

Move
When in move mode, selecting a point in a LUT allows it to be moved. A point can be positioned anywhere on the vertical scale, but must lie between its neighbour points along the horizontal scale. It is always the point nearest to the cursor when the left mouse button is clicked which will move.

There is one further restriction required to ensure the LUT remains consistent, that is the two end points of the LUT can only be moved vertically.

Add
On a click of the left mouse button a point will be added at the cursor position. The LUT curve will be redrawn so that the point is included at its proper place. Note that point delete mode can only be selected from the **Points** menu appearing in the **Grey** and **Colour** options.

Level
In level mode, a click on an arc of the LUT will cause that arc to become horizontal. This is accomplished without moving the end points of the arcs either side of the selected arc, so it is sometimes necessary to introduce a new point at either end of the selected arc. Once the arc is horizontal it can be dragged upwards or downwards to the desired level.

A similar function exists for the vertical steps in the LUT curve, that is the **Threshold** function which can be selected from the **Points** menu. It allows a step in the LUT to be moved left or right.

Other Menu Features
The display of the LUT diagram can be scaled as 1 or 2 pixels per grey level in the horizontal scale. This is done with the **Scale** menu option.

The **Reset** menu option should be used with care. It will replace the existing LUT with the default linear grey LUT. Although **Reset** appears in several menus, its operation is always identical, with one exception - when it is selected from the **Colour->Points** menu, only the currently selected colour is reset. In all other cases the entire LUT is reset to linear.

When setting up a LUT it is sometimes useful to refer to the grey level of an area of the image, the **atPoint** function allows this. Selecting atPoint displays a line cursor on the LUT profile. Then when the mouse is clicked on a point of the image, the grey level at that point is reflected by the position of the line cursor.

There is one further feature which is often helpful in setting up LUTs, that is the **Grey Wedge** option on the **Grey** menu. If the store is frozen, this option can be selected to fill the store with a grey slope image (black on the left, to white on the right). This image clearly shows the effect of any changes to the display LUT in either grey or colour mode.

Colour LUTs

Colour LUTs can be set up to achieve a variety of effects. It is possible to have a normal grey LUT, with a different colour set up for the low grey levels, to achieve a background effect for an image. A blue/red LUT for the low and high grey levels (described earlier in this chapter) may be useful for setting brightness levels, the operations to set it up are described below as an example.

A high/low limit LUT:-

1. Select Red colour (middle mouse button)
2. Select Add Point mode (right mouse button)
3. Add a point on the red line at the lowest grey level required (using grey wedge and atPoint if necessary)
4. Add a point at the highest grey level required
5. Repeat the above steps for green and blue
6. Select Level mode (right mouse button)
7. Select blue (if not already selected)
8. Select the low arc (between 0 and the low point)
9. Move the blue level to maximum (highest point)
10. Select the high arc
11. Move the blue level to minimum
12. Select Green
13. Select the low arc
14. Move the green level to minimum
15. Select the high arc
16. Move the green level to minimum
17. Select Red
18. Select the low arc
19. Move the red level to minimum
20. Select the high arc
21. Move the red level to maximum
22. Save the LUT as points or a LUT if required (see Saving and Loading LUTs)

Monochrome LUTs

Monochrome LUTs operate in two distinct ways. *Points* mode is similar to colour LUTs, *Formula* mode is an entirely different approach. In points mode the LUT consists of a series of points connected by straight lines, but in formula mode an equation is used to calculate the grey level at any given point. In this mode changes to the LUT are made by changing the coefficients of the LUT equation. Different equations are used for the different types of control required. The equation can be either **Brightness and Contrast** or **Gamma**. In addition to these the whole grey profile can be inverted with the **Invert** option (only available in formula mode), and plotted on the LUT display with the **Plot** option.

Brightness and Contrast

When **Brightness** or **Contrast** is selected a scroll bar appears at the bottom of the display LUT window. For brightness it controls the vertical position of the curve, for contrast the slope of the curve is adjusted. The effect of these controls is similar to the signal level brightness and contrast controls and can be used to adjust the overall appearance of the image. The LUT is determined by the straight line equation:-

$$\mathbf{y = ax + b}$$

where **a** is the contrast and **b** the brightness. Any point which is less than zero is set to black and any point exceeding 255 is set to white.

Gamma

The **Gamma** option allows the LUT to be defined as a curve. The gamma parameter can be adjusted either way from a straight line so that the LUT can be made to expand the low or high grey levels. This function can be used to enhance an image where a large amount of detail is contained within a few grey levels.

Points and Steps

In addition to the formula mode monochrome LUTs, it is possible to use a monochrome LUT in points mode (in the same way as colour LUTs). Points can be added, moved and deleted in the same way as for colour and the level and threshold functions are also available.

One further function is defined for monochrome LUTs, the **Steps** function. When steps is selected, the LUT is turned into a monotonic series of grey level steps. The height of each step (**Amplitude**), the position of the first step (**Offset**) and the number of steps (**Period**) can all be adjusted. It is also possible to subsequently modify the LUT using the point functions.

Saving and Loading LUTs

The are two ways of saving and loading LUT files. A LUT can be saved as a series of values (one for each grey level), or it can be saved as a series of points. Both types of LUT can subsequently be reloaded, but only a points file can be modified after it has been reloaded. Points and LUT files have different default extensions, .OLT for LUT and .DLU for points files.

Monochrome LUTs defined in formula mode can only be saved as LUT files.

3.7.2. *The Macro Editor*

A macro is a sequence of instructions which can be executed by the user to achieve a specific result. Macro execution is initiated in several ways:-

- From icons
- By panel buttons
- By function keys
- When restoring conditions

In addition it is also possible to edit, load and save macros and macro libraries. It is also possible to record a sequence of user instructions to generate a macro.

Each macro has a name which is displayed on the right of the toolbar while the macro is executing.

Macro execution may be terminated by pressing the **Esc** on the keyboard or by clicking on the macro name in the toolbar. Execution can be suspended by use of the keyboard **Pause** key. A paused Macro is indicated by the **!** character before the macro name in the toolbar. The macro may be resumed by a second selection of the **Pause** key.

Instructions

The following types of instruction can be used within macros:-

Calls
A call instruction executes another installed macro. The maximum nesting level of calls is 5.

Comments
A comment provides the user with the ability to annotate a macro and performs no action when executed.

Commands
A command executes a specific SEM command. If the command is disabled, the macro will not step on to the next instruction.

Set Digital State
Sets the specified digital parameter into the defined state. It will not step on to the next instruction if the parameter is currently disabled.

Set Analogue Value
Sets the specified analogue parameter to the defined value. If the value is outside the parameter's allowed range, then the parameter will be set to the nearest limit. It will not step on to the next instruction if the parameter is disabled.

Set Co-ordinate Value
This instruction performs the same action as *set analogue value* except that it sets the value for a pair of parameters making up a co-ordinate pair (x and y).

Stage Goto
This instruction moves the stage to the defined X, Y and Z co-ordinates. It does not step on to the next instruction if stage movement is currently disabled.

Wait For
The wait for instruction waits until the specified state is achieved. An optional timeout parameter may be specified (in seconds) which limits the amount of time the instruction will wait. If no timeout parameter is specified (not zero seconds, but no characters) then the instruction will wait for the state indefinitely.

Delay
This instruction waits for the specified number of seconds before continuing.

Pause
The pause instruction acts in the same way as the pause key. It causes the execution of the Macro to be suspended.

Confirm
This instruction check that the instrument configuration matches the defined configuration. If the configuration is different, the user is given the option of continuing the execution or aborting the macro.

Message
The defined text is displayed in a dialog box until the user clicks on the **OK** button. Execution of the macro then continues.

Show/Hide Panel
The show and hide panel instructions are used to display and remove panels to and from the screen.

Load Display LUT
The load display LUT instruction loads the display LUT from the specified .OLT file. When this instruction is inserted into a macro, the current display LUT is used to generate a file and the user is prompted for a filename.

Function
Performs one of the set of specially defined actions if the appropriate licences are installed.

Control Structures

There are two types of control structure provided:-

While
If then

While Constructs
The While construct allows a series of instructions to be repeatedly executed while the specified condition is true.It takes the form:-

While <condition> (alternatively While Not <condition>)
other statements
End While

While Statement
The While statement is part of the While construct. If the condition defined is true then the next instruction in sequence is executed, otherwise execution continues after the end While statement.

While Not Statement
The While Not statement is part of the While construct. If the condition defined is false then the next instruction in sequence is executed, otherwise execution continues after the end While statement.

End While Statement
The End While statement is part of the While construct. When this function is executed it resets the statement counter to the corresponding While or While Not statement. Each While or While Not statement must have one and only one End While statement.

If Constructs

The If construct allows selection of the appropriate set of instructions based on a condition. It takes the form:-

If <condition> (alternatively If Not <condition> then)
other statements
optional else
other statements for else clause
End if

If <condition> then
The If statement is part of the if construct. If the condition is true execution continues at the statement after the If. If the condition is false execution continues after the Else statement if one exists, or after the End If if there is no Else clause.

If Not <condition> then
The If Not statement is part of the if construct. If the condition is false execution continues at the statement after the If. If the condition is true execution continues after the Else statement if one exists, or after the End If if there is no Else clause.

Else statement
The Else statement is an optional part of the if construct and is used to indicate the start of the Else Clause.

End If statement
The End If statement is part of the if construct and identifies the end of the conditional sequence. Each If or If Not statement must have one and only one End If statement.

Editor Facilities

The macro editor is selected from the **Tools->Macro** menu option. The name of the macro is displayed in the window caption together with the mode. The macro instructions are displayed in the window in the sequence in which they will be executed. The menu offers a range of options which are described below:

Reset
The reset instruction clears the macro window and name (setting the name to *ANON*) and inserts a comment defining the time and date in the macro window.

New
This option performs the same action as reset, except that the user is prompted for the name of the new macro.

Rename
Allow the user to change the name of a macro.

Insert
Initiates the dialog which adds an instruction of the specified type into the macro after the current instruction. The type of instruction is selected from a list box, then according to the type of instruction, the user is presented with further options to specify the instruction.

Edit

Some but not all instructions may be edited. Selecting edit allows the text of an instruction to be changed. If an instruction is not editable, delete it and insert an new one.

Delete

The delete functions deletes the currently highlighted instruction.

Load/Save

A macro may be saved in a file with a .MAC extension by the save option. The user is able to specify the filename for the file. Any .MAC files can also be loaded into the macro editor for further alteration or for execution with the load option.

Save as Text

This option allows a macro file to be written in text format. The default .TXT extension is used and the resulting file can be read by the notepad editor. It is not possible to load a text macro file.

Fetch/Install/Remove

The fetch, install and remove options all relate to the subject of macro libraries which are described below.

Execute/Stop/Continue

The **Execute** command causes the current macro to be executed from the beginning. Execution can be interrupted at any point with the **Stop** function. It is then possible to edit the macro before selecting **Continue** which will resume execution of the macro from the currently highlighted instruction.

Record

The record option allows a sequence of user interface operations to be converted into a macro. All operations between selecting **Record** and the subsequent selection of **Stop** are recorded as a macro.

To reduce redundant instructions only the final value of analogue parameters is inserted as an instruction. There is no sense of time delay in the recorded sequence, each instruction follows directly after its predecessor. To introduce time delays, ***Wait For*** *and* ***Delay*** *instructions must be inserted with the editor.*

Libraries

A library is a collection of macros. The *Stereoscan 430* comes with a standard library which contains the macros required by the user interface for standard calls from menus and panels.

All macro libraries must contain the standard macros as a subset.

Once a macro is in a library it can be assigned to a toolbar icon (see Section 3.7.4 *The Toolbar Editor*) or called from one of the user defined function keys.

The assignment to function keys is made entirely by the macro name. For example, macro **F5** is assigned to function key 5, while macro **Shift F6** is assigned to the shifted function key 6.

The following operations may be performed on macro libraries:-

Install
A macro can be installed into a library, which means that it will appear in the list of macros which can be selected. If a macro of the same name already exists in the library, the user is asked whether the old macro should be overwritten.

Fetch
Fetching a macro from a library, loads it into the edit window where it can be executed or edited.

Remove
Removing a macro from a library deletes that macro from the library.

Save/Load
Macro libraries may be saved as files as well as individual macro files. Libraries have the .MLF extension. Note that the standard library calls should not be altered as this could cause unexpected side effects when loading macro libraries. The **Load->Standard** option is provided to allow an easy route back to the default macro library should it be required.

Example

This macro will enable the operator to select operating conditions suitable for adjusting the final aperture, by selecting function key F5.

Click on words in normal type, enter text and values in bold type on the keyboard:-

Tools, Macro, Macro, New, **F5**, OK
Insert, Instructions, Analogue, **M**, Mag, OK, **2000**, OK
Insert, Instructions, Command, **R**, Reduced, OK
Insert, Instructions, Analogue, **R,** Red. Raster posn X, OK, **384**, OK
Insert, Instructions, Analogue, **R**, Red. Raster posn Y, OK, **256** OK
Insert, Instructions, Analogue, **R**, Red. Raster height, OK, **256**, OK.
Insert, Instructions, Analogue, **R**, Red. Raster width, OK **256**, OK

Insert, Instructions, Command, **P**, Pixel average 4, OK
Insert, Instructions, Command, **F**, Frame average
Insert, Instructions, Digital, **F**, Focus wobble, OK, On, OK
Insert, Instructions, Digital, **W**, Wobble rate, OK, Fast, OK
Insert, Instructions, Analogue, **C3W**, C3 Wobble Amplitude, **50**
Install, Yes

Press function key F5 to see it working.

Now to save this macro:-

Click on Macro->Save, then delete the default name and enter WOBBLE.MAC. You can then FETCH the file from the macro library or load it from the file and EXECUTE it if required.

Saving and Loading Conditions

A further use for macros is in saving and loading instrument conditions. The **Restore** macro has already been described (Chapter 1 *Getting Started* Section 1.4 *Common Operating Routines*). It is initiated from the run up icon and restores the previous operating conditions. Those conditions are saved when the system is closed down by the macro generator.

The macro generator builds a macro which when executed restores the previous condition of the instrument. It can be selected at any time from the **File->Save State** menu option and can be made to generate a macro to restore any combination of the following:-

- Configuration
- Setup
- Gun Conditions
- Application Conditions
- Display LUT

The user is prompted for a LUT file name (if that option is chosen) and a name for the macro.

*The **Standard** option is provided to set the instrument to a default state should the restore macro be saved as some undesirable state.*

Any instrument conditions macro may be loaded directly in and executed with the **Load State** option. However the **Preview State** option has the advantage that the conditions can be examined before the macro is executed. The preview menu also allows the conditions to be executed (**OK**), edited (**Edit**) or changed for another set (**Fetch**).

*Any macro of instrument conditions that is created by the **File->Save State** menu option is saved with the file extension .APP and not .MAC so that it is easier for the operator to separate the two types of macro.*

3.7.3. *The Status Display*

The status display was introduced in Chapter 1. There is a little more to it than meets the eye. It is of course a window for displaying instrument parameters which can be chosen using the **Select** menu option. But it also provides certain control facilities which will be described in this section. The basic operation of the display will be covered first.

Selecting a Set of Parameters

The status display can be cleared at any time using the **Reset** menu option. The **Select** option is used to add or remove individual items from the display. when select is chosen, a select box will appear containing a list of all the possible system parameters which may be displayed. Highlighting a value means that it will appear in the display when **OK** is selected.

The position in the list can be controlled using the scroll bar or by typing the first one or two letters of the required parameter in the text entry box. When all the required parameters are selected, the OK button will cause the status display to be reconfigured to reflect the new selections.

Some of the parameters in the display may appear *greyed out* which means that in the current operating mode, that parameter cannot be altered by the user.

Saving and Loading Status Display Files

It is likely that the user will require different parameters in the status display for different activities. To avoid the need to constantly reconfigure the display, a file save and load facility has been provided. Selecting **File->Save** from the menu will cause a dialog box to appear allowing the user to specify the filename before the file is saved. All status display files are saved with the .SSL extension. **File->Load** works in the usual way (overwriting the current status display configuration).

Adjusting Parameters from the Status Display

The way in which a parameter can be adjusted from the status display varies according to the parameter type. Parameters may be read only, analogue, binary digital (boolean) or multi value digital. The methods of adjusting them are summarised below:-

	Left Button	Middle Button	Right Button	Left Double Click
Analogue	Assign to Left Mouse Parameter	Assign to Right Mouse Parameter	Preset Values	Dialog Value Entry
Binary Digital	Toggle	-	-	-
Multi Value Digital	Select Box	-	-	-
Keyboard Only	Dialog Value Entry	Dialog Value Entry	Dialog Value Entry	-
Read Only	-	-	-	-

The actions listed above are defined as follows:-

Assign to Mouse
Control of the parameter is assigned to the mouse (and navigation box if present) for adjustment.

Dialog Value Entry
A dialog box appears which allows the new value for the parameter to be entered.

Preset Values
A select box appears which displays all the previously defined preset values for that parameter. A new preset value can be defined as a label for the current parameter value, or a previously defined value can be used. The preset values are labels which can be used to set an individual parameter to a fixed value.

Toggle
A binary digital parameter has only two states (Off/On, Yes/No, Fast/Slow, etc). Selecting such a parameter simply changes it to its alternative state.

Select Box
A select box appears with all the possible values for the parameter in question. A value is selected from the list and the parameter is set to it.

Whenever a double click is performed the relevant left or middle single click action is performed before the double click action.

3.7.4. The Toolbar Editor

The final option available for modifying the user interface is the toolbar editor. It allows the actions and appearance of the icons in the toolbar to be modified. Icons may be added, deleted or edited using the toolbar editor which is selected from the **Toolbar** option of the **Edit** menu. Different toolbars can be loaded and saved and a new toolbar can be installed from the editor.

Toolbars

A toolbar is a sequence of icons with their associated bitmaps and actions. A popup menu controls the changes to the icons in the editor. Some operations apply to the whole list, others apply to individual icons. The former are considered in this section.

Reset
The reset command deletes the current icon list.

Fetch
This command loads the current toolbar into the editor, overwriting any icons in the editor at the time.

Load/Save
A toolbar can be saved as a file (with an .ITB extension). A toolbar file can be loaded into the editor overwriting its current contents.

Install
The install option copies the icon list from the editor into the toolbar area. The current icon list is then lost. The current toolbar is saved when the system is shutdown.

Should the distribution toolbar be inadvertently lost, it can be restored by selecting ***Novice*** *or* ***Expert*** *mode (as appropriate) from the* ***File*** *menu.*

Icon Order
The order of the icons in the toolbar can be changed simply by dragging an icon to another position, this will swap the icon with the one currently occupying that position. Identical icons may appear several times in the toolbar to ensure that any given icon is always visible.

Icons

Individual icons in the toolbar can be defined to appear and behave in certain ways. There are two types of icons, *Buttons* and *Toggles.* A button executes an action when it is selected, whereas a toggle changes state (for example, image *inverted* to *not inverted* or vice versa). New icons of either type may be created and also deleted (by selecting an icon then **delete** on the popup menu).

A red border is used to indicate the currently selected icon. The following properties of the selected icon may be edited:-

Size
There are two sizes of icon available large (64 x 64 pixels) and small (36 x 36 pixels). Selecting the smaller icons allows more icons to be displayed.

Bitmaps
The bitmap associated with any given icon may be altered using the **Next** and **Prev** options on the popup menu. Repeated selections will cycle through the set of installed bitmaps for that type of icon.

Actions
Each icon has an action associated with a selection with the left mouse button and one associated with the middle or right mouse buttons. The actions can be selected using the **Edit** option on the popup menu. The choices available for an icon depend on its type, but will be from the following list:-

- Command
  Executes the low level command
- Panel
  Displays the panel.
- Macro
  Executes the defined macro
- Mouse
  Assigns the selected parameters to mouse control
- Function
  Executes the high level function
- State
  Toggles the state of the binary parameter

Icon Example

This example shows how to add an icon to the toolbar which will select and deselect the lowest available magnification.

1. Select **Edit->Toolbar**.
2. Select right mouse button while over Toolbar editor panel (to bring up popup menu).
3. Select **Fetch**.
4. Select **New Toggle**.
5. Select **Next** (until desired bit map is selected).
6. Select **Edit** (on popup menu).
7. If required, enter a Default name for the icon.
8. Select **State** (for the left button action).
9. Select **Low Mag** from the list of available parameters (or any preferred parameter), and **O.K**.
10. Select **Panel** (for the right button action).

11. Select **Noise Reduction** from the list of available panels (or any preferred panel), and **O.K.**
12. Select **O.K.** on the edit panel.
13. Select **Install**, and **Off**.

Selecting the new icon with the left mouse button will cause the image to be switched between the present magnification and the lowest currently available magnification.

3.8. Trouble-Shooting

(A)	**Lack of Sharpness**	(C)	**Poor Final Image Quality**
	CAUSES		CAUSES
	Astigmatism not correct		Incorrect LEVEL & CONTRAST settings
	Misaligned final aperture		Incorrect acceleration voltage
	Poor focusing		Incorrect probe current
	Sample charge - up or vibration		Inadequate specimen preparation
	Magnetic fields (frame sample or external)		
	Poor depth of focus		
	Too much noise		
	Specimen contaminated or of low potential reduction		
(B)	**Image Noise**	(D)	**Image Distortion**
	CAUSES		CAUSES
	Probe current too low		Specimen charge-up
	Sample not facing electron collector		Specimen magnetism
	Faulty scintillator and/or photomultiplier		Specimen vibration
	Too much CONTRAST, incorrect LEVEL		Specimen damage
	Dark regions of sample being observed		External magnetic fields
			External vibration

Table "Summary of Image Defects"

(A)	**Operator Error**	
	DEFECT	CAUSE
(1)	Low contrast, lack of video signal (if probe current too low), possible specimen damage (if too high)	Incorrect probe current
(2)	Lack of image sharpness, image shift when focusing	Incorrect final aperture alignment
(3)	Less image sharpness in one direction, poor resolution	Insufficient astigmatism correction
(4)	Noisy image, beam deflection on charging sample, specimen damage	Wrong scanning period
(5)	Poor image quality	Wrong brightness level selected
(6)	Poor image quality	Wrong contrast level selected
(7)	Influence on penetration and charging	Wrong accelerating voltage
(B)	**External Influence**	
	(DEFECT)	(CAUSE)
(1)	Image distortion, jagged edges in image	Magnetic fields
(2)	Jagged edge in image	Vibration
(C)	**Sample Type and Penetration**	
	DEFECT	CAUSE
(1)	Reflected and secondary electron images brighten with increasing atomic number	Atomic number effect
(2)	Image shift and distortion, abnormal contrast and unstable image	Charge-up
(3)	Deformation and cracking of specimen, coating peeling	Overheating (kV or probe current too high)
(4)	Charge-up or sample damage	Incorrect or inadequate sample coating
(5)	Surface coating leading to poor image quality	Contamination
(6)	Sample deformation, damage and charge-up	Incorrect sample preparation (especially soft tissues etc)

Table "Image Defect Causes"

PARAMETER	EFFECT	OPTIMUM CONDITIONS	COMMENTS
Mounting on to specimen stub	Charging, Vibration	Securely attached	Use quick - drying adhesive (preferably conducting)
Conductivity and grounding	Charging	Good conduction to earth	Use conducting paint (Dag)
Sensitivity to beam damage	Specimen distortion	Use:- Low beam current and/or low kV, short frame period. Focus away from area of interest	Optimum conditions may result in poor SNR
Coating	Conductivity	No charging	Charging seen as: local intensity changes; astigmatism; image distortion; dark micrographs
Coating	S.E. Signal	20nm metal coating	If too thick can mask detail
Coating	B.S.E. Signal	Carbon coating	If too thick will absorb signal
Coating	C.L. Signal	Carbon, aluminium coating	If too thick will absorb signal
Coating	X-Ray Signal	Carbon, aluminium coating	Avoid interference with element(s) of interest
Coating	Specimen Current	C - Material contrast Au - Topography	Use inverted S.C. image for best topography
Coating	Resolution	Gold/Palladium	Grain size of coating may be resolution limit
Mechanical Stability	Resolution: Cracks in coating	Vibration amplitude less than require resolution	Reduce kV, and beam current
Eucentric Position	Specimen tilting, Stereo pairs	No translation motion during tilting	Use Eucentric goniometer stage for best results
Specimen Tilting	Topography, signal to noise ratio	About 45° for SE1 detector. Zero for BSD	Found by expt. Depends on sample
Specimen Tilting	Stereo Viewing	Filt difference 5-10°	Use low diff for large height differences
Working Distance	Resolution	Short	Beware of final lens damage
Working Distance	Depth of focus	Long	Limit = Loss of resolution
Working Distance	Sample - BSD Distance	Long - Best for Topog and Crystal Orientation Short - Best for Z-Contrast	

Table "Specimen Parameters"