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Some Applications of Scanning Electron Microscopy to Archaeology

The scanning electron microscope (SEM) has been available commercially for ten years. It is now recognised as a valuable research tool, providing evidence complementary to both optical and transmission electron microscopy (TEM). Until recently, its use in archaeology was restricted to a few isolated investigations. A resumé (Brothwell) in 1969 of possible SEM uses in archaeology was of necessity very short (1). Interest has now become more widespread, and there are various archaeological research projects utilising the SEM, although most work is still in the very early stages.

Brief Description of principles and use of the SEM (2)

In 1876 Abbé showed that the resolution obtainable with an optical microscope was limited by the wavelength of light. Electrons have a much shorter wavelength and therefore a better resolving power is possible. As their path may be bent in an electric field, a microscope may be constructed:- In the SEM:

1. A heated filament emits an electron beam.

2. Diminishing lenses reduce the size of the beam.

3. Further lenses cause the beam to scan across the specimen in close parallel lines

4. As the electrons hit the specimen a number of interactions occur. When the SEM is being used in the usual emissive mode the most important of these is the emission of low energy secondary electrons from the atoms near the specimen surface.

5. A scintillation detector collects some of these secondary electrons and amplifies the resulting signal.

6. This signal is used to modulate the brightness of a spot on a cathode ray tube. This spot scans in synchronisation with the beam on the specimen.

Topographic information is obtained mainly because the parts of the specimen nearer to the detector appear brighter.

Main advantages of the SEM (in emissive mode)

1. Much better resolution than with optical microscopes (figures quoted vary, but something in the order of 2,000Å (200nm) for optical cf 200Å for SEM)

2. Magnification range (c20X - 20,000X for most routine work) links the optical and TEM ranges.

3. Very high depth of focus enables a clear, three-dimensional image of the specimen surface to be obtained directly.

4. Fairly large specimens (up to $1cm^3$) may be examined. This, combined with the ability to alter magnifications easily, means that a detailed study may be made of one area while its relationship to the entire sample is known.

5. The surfaces of objects are observed directly - thin sections need not be prepared (although section surfaces may be examined if required), and preparation is usually fairly easy.

6. The resulting signal is readily available for processing.

Specimen Preparation (3)

If the specimen will conduct electricity, then it need only be stuck onto a metal stub with a conducting glue (eg Silverdag). However, many specimens are non-conducting, therefore an electrostatic charge may build up on these in the SEM and cause image anomalies. This can be prevented by coating the specimen with a thin layer of a conducting substance such as carbon or a metal (eg gold-palladium), evaporated under vacuum. A fairly even coat may be obtained by rotating and tilting the specimen during coating. It is generally advisable to coat very thinly and recoat if charging occurs.

If the sample contains water, this will evaporate off under vacuum in the SEM column and this often causes deterioration of the visible structures. Therefore, there are various methods for drying samples prior to examination in the SEM. These are designed to cause as little distortion of structure as possible. Some of these methods will be mentioned as applied to the relevant materials.

Potential range of archaeological materials which may be examined in the SEM

The range of "archaeological materials" is enormous. It may be considered to include all artefacts manufactured by man, natural materials (including plants and animals) utilised by him, and all plants animals and minerals of the contemporary natural environment, the remains of which may be incorporated into deposits in and around archaeological sites.

There are very few categories of these "archaeological materials" for which it is impossible to find some aspect which might be investigated with the SEM. The mineral grains comprising the deposits themselves can be examined in the SEM (eg surface morphology of sand grains can give clues about their depositional history); artefacts may be usefully studied. (Some work has already been carried out on studies of pottery to try to establish firing temperatures[23] and origins) (22),(4) There is also a large potential field of study in ancient metallurgy.

However, the following discussion is restricted to materials of biological origin which may be found on archaeological sites. These are considered category by category.

WOOD

Much of the SEM work of the Ancient Monuments Laboratory to date has been on wood. This is therefore considered in some detail. It occurs widely

on archaeological sites and is preserved in a great variety of conditions. Identification of species of origin is usually required, in order to provide environmental and technological information.

Much of this routine identification may be carried out satisfactorily by standard optical methods, although the SEM is useful in aiding separation of some difficult genera. The main use of the SEM in wood studies has proved to be in the study of poorly preserved or altered wood remains. Quite a lot of information may be incidentally obtained about how and why the form of the wood has been preserved. Various categories may be considered:-

1. Normal wood

Most wood from archaeological sites has been preserved in anaerobic waterlogged conditions. Wood consists mainly of a system of very long tubular cells (4) running parallel to the long axis of the tree trunk. These allow transport of food substances and water up and down the tree. The cells may be vessels (which only have remnants of cross walls), tracheids (which are closed at either end), or thick walled fibres. There is also a radial system of parenchyma cells (The rays). The cell wall consists of a middle lamella and a primary wall, and often a secondary wall.

The SEM has proved to be very useful for studying the decay of wood in various conditions. Bacteria and fungi can be observed in situ, with details of the associated cell wall breakdown (5).

2. Charcoal

This is an opaque material that must therefore be examined by reflected light (unless the sample is embedded and sectioned). This is satisfactory for routine examinations, but some high power details are difficult to recognise because of the poor resolution of the microscope and the very uneven surface of the charcoal. The SEM is therefore a valuable identification aid as it allows small details to be clearly seen, eg cross field pits which are very important in the identification of conifers.

Charred wood is often very well preserved, details such as pits being easily recognisable. although there is some shrinkage (mainly due to a reduction in overall cellular and cell wall dimensions and the apparent fusion of adjoining cell walls so that the middle lamella is no longer distinguishable) (6).

Contrary to most expectations ancient charcoals are very poor conductors and must usually be coated with a metal before examination in the SEM. It seems that the temperatures reached in the formation of the charcoals were insufficient to convert the carbon to a conducting form. Most charcoals may be air dried before examination with no apparent distortion of the structure.

Replaced Wood

Wood may be preserved relatively unchanged due to contact with a metal such as copper, which seems to inhibit biodegradation, or it may be "mineralised".

Mineralisation seems to occur commonly when wood is buried in contact with an iron object (eg a knife with a wooden handle). The resulting material superficially resembles wood, but is found to consist of iron corrosion products. It is often very fragile and cannot be sectioned for examination by transmitted light. Exposed surfaces or fractures tend to be very uneven, and difficult to observe at high power using reflected light. The SEM is therefore ideal for examination of this type of sample, because of the large depth of focus, and species identifications may often be made.

Examination of these samples in the SEM has incidentally yielded information about the mechanisms of wood replacement by iron corrosion products (7). The apparent "cells" are found to be widely spaced tubes, the walls of which are composed of iron compounds displaying a radiating crystalline structure. These observations suggest that a layer of iron corrosion products is deposited on the secondary cell wall soon after burial. Subsequently the cell wall decays away. The action of this mechanism is clearly illustrated by the presence of bi-convex discs between adjacent cell casts. These obviously represent pit-pair casts. Further deposition of iron may fill all of the remaining cavities.

Some examples from waterlogged conditions in association with iron have been examined. The wood is often well preserved, but with layers of iron deposited in the lumina. Sometimes, part of the cell wall has decayed away. The primary wall often seems to be lost first, and the resulting space may be filled with iron deposits.

Further study has shown that the preservation of the superficial form of wood by mineral deposits occurs frequently in archaeological samples. Additional examples have been observed of the replacement of wood by copper and lead corrosion products, and calcium carbonate. Copper and lead both tend to inhibit biodegradation of wood, therefore examples are observed of copper or lead deposits in the lumina of preserved wood. This is then seen as mineral "casts" in areas where the wood has partially or completely decayed away.

Similar "cast" structures have been observed in living wood which has silica deposits in the lumina, after chemical dissolution of the cell walls (8). Petrification of wood in fossil examples seems to occur by:-

a. Cell wall impregnation by silica.

b. Secondary deposition of silica in cell lumina and inter-cellular spaces.

No examples have yet been observed in the AM Lab SEM of silicified wood, although it is theoretically possible that silicification (at least in its early stages) might occur on archaeological sites.

Conservation

The SEM has also been used to study the effects of various treatments on wood, for example the degree of penetration by various resins (9).

Some SEM work is also in progress on the conservation of other materials, such as leather (10).

SEEDS

These are often preserved in waterlogged deposits, or are found charred. Species identification may provide environmental or dietary information. Preliminary identifications are based on the size and shape of the seed, but the surface topography of the epidermis (the outer layer of the testa) is often used as a further guide. The SEM is therefore very useful in the study of seeds, as this surface sculpturing can be easily seen in detail, and clear three dimensional record photographs obtained (11).

Detailed studies on known populations are necessary to define the variation of appearance within one species. This data may then be applied to enable the identification of unknown seeds from archaeological sites. A few projects of this type are now being undertaken, (eg Dr Stant, Jodrell Lab), but a lack of this data is restricting archaeological work at present.

Most mature modern seeds are already dry and therefore do not suffer from direct observation in the SEM (after surface coating with a metal). However, many seeds from archaeological deposits are poorly preserved and these should probably be dried carefully (for example by freeze drying or critical point drying) to prevent collapse and distortion.

POLLEN

Pollen is often found in large quantities in acid or waterlogged buried soil, peats, etc. Identification yields valuable environmental evidence. As pollen grains are so small, many types cannot be identified with any certainty by optical methods. The use of the SEM allows detailed examination of the pollen grain walls (exines), and therefore seems to promise better identifications, often to species level.

It is difficult to prevent some clumping together of grains when a large quantity of pollen is evaporated onto an SEM stub. Precise counting of the different species present in an archaeological sample might therefore present problems. This, and the high cost of SEM time, means that the SEM is probably not very suitable for routine quantitative work.

However, it can be of great value for making more specific identifications of selected pollen types. For example, Pilcher (12) has been able to distinguish *Myrica gale* (bog myrtle) from *Corylus avellana* (hazel), found in peat samples, by differences in surface sculpturing observed at high magnifications (more than 3,000X). He has also studied peat samples from Co. Tyrone (N. Ireland) which indicated a forest clearance phase followed by regeneration including much rosaceous pollen. Using the SEM, this was identified as *Sorbus* (probably *aria*, white beam).

Pollen grains are often air dried or dried from ethanol. They can be dried directly onto a metal stub, or stuck on with double sided 'sellotape'.

However, critical point drying or freeze drying may be more suitable for delicate archaeological specimens. The surface must be coated with a thin metal film before examination in the SEM.

OTHER BOTANICAL

Many other types of botanical material are found, particularly in waterlogged conditions, eg leaves, bud scales, grasses, mosses, brackens. The SEM is of some use in the examination of most of these materials. It is very suitable for studying surface details such as the shape of epidermal cells, stoma and trichomes (hairs). This is particularly true of charred remains, which are very delicate and opaque, and therefore difficult to examine in detail by conventional light microscopy.

Charred remains may normally be air dried without deterioration, but waterlogged materials tend to be very delicate, and retain their shape mainly because of the large quantities of water contained in the tissues. Freeze drying or critical point drying (13) may be carried out, but it seems likely that even these techniques may damage very delicate remains. Sometimes, it might therefore be preferable to examine the specimen while it is frozen (therefore it need not be dried), or else a surface replica might be made.

Replication techniques are also useful when a large object which will not fit into the SEM cannot be sampled. Replicas can be very faithful reproductions of the original surface, although they are often not very satisfactory if the specimen is highly convoluted or hairy.

There are many methods for preparing replicas(13). It generally involves:-

1. Making a negative impression of the original surface.

2. Making a cast of this to produce an exact replica of the original.

3. The first impression is then removed.

A standard replication method is used in the AM Lab for making entire replicas of archaeological objects. This method was applied to some very poorly preserved waterlogged leaves and the replica examined in the SEM. This was fairly acceptable, considering the poor state of the original. Individual epidermal cells could be recognised.

The method is:-

1. First impression made with silicon rubber (eg Dow corning 3110 RTV encapsulant). This sets in about 24 hours, and is then peeled away from the specimen).

2. This impression is lightly brushed with graphite and put into a bath containing acidified copper sulphate.

3. A small current is passed and a copper deposit built up on the impression.

4. The upper layer is backed (eg with a resin), and the impression peeled away from the front.

A precise replica in copper is therefore obtained of the original surface of the object. This need not be coated before examination in the SEM. The results obtained by this method have not yet been compared with others.

FIBRES

Textiles and fibres are discovered in a wide range of conditions on archaeological sites, as already described for wood. Textiles are examined for details of the weave, etc, and individual fibres for identification of plant or animal of origin.

The SEM has already proved to be of great use in the study of modern fibres. Many workers are using the SEM increasingly for routine observation and illustration of surface details such as damage caused by various treatments (14) (15). The scale patterns of animal fibres are clearly observed in the SEM. This is useful for identification purposes. Cross sections may be cut or freeze fractured and also examined in the SEM. However, rolled impressions of fibres are useful for establishing overall scale patterns, and these may be examined in the light microscope.

The SEM is particularly useful for examining poorly preserved remains, such as "iron replaced" and carbonised textiles. Both of these types are opaque, and must be examined in reflected light, when it is difficult to see high power details because of the very uneven surfaces. The SEM enables the entire sample to be quickly scanned, and small areas displaying recognisable structures studied in detail.

Mineralised and charred fibres may apparently be air dried without damage, but waterlogged samples require more careful drying. Samples must be metal-coated.

BONE

Human and animal bone occurs on most sites, although the state of preservation is extremely variable. Species identification is usually required, along with evidence for age and sex, and any bone pathology.

Bone is a complex three-dimensional structure and is therefore very suitable for profitable examination in the SEM. A fairly large specimen can be scanned and small details examined in relationship to the rest of the structure. The large depth of field is very useful for looking at uneven bone surfaces and fractures.

Work on modern material has shown that resorbing, resting, and forming surfaces can be recognised in the SEM (16). This would seem to indicate a potential use on archaeological material for studies of pathology (where gross bone formation and destruction may occur).

Recent SEM work has altered the concept of lamellar bone as layers of parallel orientation with abrupt changes between layers. It has demonstrated that collagen bundles are not discrete but that fibres change from bundle to bundle (17).

Foetal bone has been found to have a distinctive appearance in the SEM (18).

It would therefore seem that the SEM is likely to be useful in studies of bone from archaeological sites. Both low magnification observation of gross morphological differences in bone from different sources, and high magnification details of pathology, etc, may be useful. However, these possibilities do not yet seem to have been fully explored.

SNAILS

The mineral shells of molluscs frequently occur on archaeological sites. Identification and quantification of species can provide environmental or dietary data.

The SEM has been applied to studies on living molluscs; (19) this has mainly been concerned with ultrastructural details of the shell and soft parts. This does not seem to be of any immediate use in species identification, although it is difficult to predict future developments.

The main value of the SEM in archaeological mollusc work at the moment seems to be in fairly low magnification studies of structures already observed with reflected light, to provide clear three-dimensional images.

INSECTS

The chitinous parts of insects (mainly beetles) are frequently preserved in waterlogged conditions. Mineralised "fossils" may also occur, for example, soft bodied larvae etc are sometimes replaced by calcium carbonate. Species identification is required to provide environmental and cultural evidence.

SEM micrographs are often used as illustrations in papers on modern insects, although they are not frequently used as a major research tool. The SEM has been used to study the fine structure of modern beetle's elytra. Details of the very fine surface hairs (microtrichia) could not be observed optically (20).

Many insect remains may be identified by low magnification examination, therefore the SEM need not be used, although it is useful for general illustrations. However, certain species are difficult to separate, and the SEM may be useful for high magnification study of small details such as reproductive organ structure which aid identification. The SEM has been used as an identification guide in work on Pleistocene insects (21).

SLIDES (stored in Rm 531)

1. Diagram showing cell wall structure.

2. (SEM 8(14))Oak Charcoal CS 700 X.

3. Iron replaced wood on Fe object, Rudston Well.

4. (SEM 3(8)) Iron repl'd wood, Mucking CS 700X.

5. (P22) Iron repl'd wood. Vessel with crystalline wall. 'Kidney ore' appearance 2000X.

6. Diagram showing progressive stages in Fe replment.

7. Fe repl'd wood (SEM 4 (8)) Mucking, 1,000 X(?) Showing pit-pair casts and fungal hyphae (coated).

8. (P16) Fe repl'd wood, Mucking. Vessel showing crystalline 'wall' deposits and coated fungal hyphae 2000X.

9. (SEM 4 (13)) Fe repl'd wood, Mucking. Pit pair casts between internal casts of vessel and tracheid cells, ls, 2000X.

10. (SEM 9 (13)) Rudston Well, Fe repl'd wood CS, Structures blocked by Secondary Fe deposits 200 X.

11. (SEM 10 (8)) Fe repl'd wood, Rudston Well. Tracheid with Fe corrosion products blocking space initially occupied by cell wall 2000x.

12. (SEM 10 (1)) Ptly Fe repl'd wood Rudston Well. Exposed secondary cell wall of a vessel with slightly protruding pit casts and internal iron corrosion deposits 2,000 X.

13. (SEM 11(5)) Ptly Fe repl'd wood. Rudston Well. Fibrous secondary cell wall of tracheids with internal Fe deposits and Fe in place of primary cell wall (?).

14. 6X Refl'd light. Cu impregnated Grass/wood on artefact.

15. Cu repl'd wood (p 164) Pit casts, etc on vessels 2,000 X.

16. (P143) Brough Cu repl'd wood. RLS with secondary deposits, so no spaces = cell walls 200 X.

17. Refl'd light micrograph Mucking oak wood from close contact with lead 6X showing some pb deposits in lumina.

18. (P135) Wood from next to Pb (as (17)) CS (showing remnants of primary (and part secondary) cell wall preserved) 2,000 X

19. (P136) Wood (as (18)) TLS of wide ray with preserved primary cell wall and cell cast of lumina, with simple pit casts. 2,000 X.

20. Refl'd light. $CaCo_3$ repl'd wood. Southwark 25x Roman writing tablet.

21. (P162) $CaCo_3$ repl'd wood (as (20)) Tracheid casts and pit pair casts (softwood - probably silver fir (Abies alba).

22. Reflected light photo. Seeds from medieval cesspit deposit.

23. (SEM 14 (4)) Ickham Roman seed - ident. as Caryophyllacae, probly Silene sps 70 X. [By T.A. Arthur]

24. (15(9)) Silene vulgaris Modern seed 70 X.

25. (15(16)) Silene alba (modern seed) 70 X.

26. (SEM 15 (13)) Silene coeli-rosa (modern seed) 70 X.

27. (SEM 15(6)) Silene maritima (modern seed) 70 X.

28. (SEM 15(2)) Silene dioica (modern seed) 70 X.

29. (SEM 14(3)) Roman unknown; seed cast epidermal cell detail. Sinuous anticlinal walls) Tuberculate. Side view near back 700 X.

30. (SEM 15 915)) Silene coeli-rosa (modern) Same part of seed as (29) Lgr epidermal cells: Entire surface slopes with flattened top to tubercles 700 X.

31. (SEM 15(5)) Silene dioica (modern) More similar to (29) therefore unknown identified as S.dioica.

32. Refl'd light. Poorly preserved leaf (medieval, Farningham) 6 X.

33. Refl'd light Copper replica of leaf 6 X.

34. (P126) Copper leaf replica (No 33) Epidermal cells near region of small vein 2,000 X.

35. (P165) Modern Soay sheep wool fibre 700 X.

36. Reflected light. Iron replaced textile on Fe brooch (Iron Age).

37. (P174) Iron repl'd textile ((Mucking, Saxon) Bunch of 'fibres' in CS-fibres decayed away, leaving coating of Fe corrn. products behind 2,000 X.

38. (P169) Iron repl'd textile (same as (37)) with original fibres still preserved. ? Some scale pattern of wool 700 X.

39. Reflected light. Carbonised textile, Bolton. 25 X.

40. (P159) Same as (39). All of weave visible, but fibres coalesced. 70X.

41. (P156) Same as (40) few fibres and wool scale pattern preserved. 2,000 X.

42. (SEM 16(12)) Pisidium amnicum External texture of shell 70 X.

43. (16(9)) Pisidium casertanum External texture of shell 70 X.

NB These are freshwater bivalves (pea mussels) from a Roman site in Southwark (ident, by rel., sizes, shape of umbone, cardinal teeth, texture of shell etc, by P Spencer).

44. (16(6)) Pisidium amnicum lateral tooth on hingeplate viewed from inside 200X.

45. (16(1)) Pisidium casertanum lateral tooth from inside.

46. Refl'd light, insect remains from medieval cess pit deposit, Denny Abbey.

47. (P37) Short nosed weevil 70 X.

48. (P No No.) Detail of head showing setae and hairs (probably sensory). Varying shapes of setae in different sps may be a guide to identification, partic. with poorly preserved or incomplete specimens where the usual identification features are less use.

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