An Investigation into the Use of Scanning Electron Microscopy in Textile Research

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Scanning Electron Microscopes (SEMs) have become a common research instrument in scientific research. When introduced, SEMs provided improved imaging when compared to the light microscopes available at the time. One scientific field that took advantage of this instrument was textile research. Over fifty years on, this research study investigates the extent of SEM use in textiles research and measures the reproducibility of this research.
Textiles production involves many processes taking raw materials such as oil (polyester or polyamide), reconstituted cellulose (viscose rayon); or from seed (cotton); plant stems (linen); animal hair (wool); originate from many sources, for example: plant material itself” (Whewell & Abrahart, 1999). Textiles have been used for thousands of years and can be made into fabric or cloth, and the resulting fabric: fibre/yarn spinning, weaving/knitting; finishing, colouration etc. These processes change the overall fabric properties and can alter the fibre surface topography at a microscopical level. Many processes are required to produce any textile--from raw fibres to produce finished products, Figure 1. Many processes are utilised to maintain a suitable quality textile fit for the intended purpose whether a disposable wipe or worsted suit. At the microscopic level there are many features of the fibres, yarns and fabrics that are both characteristic and are a measure of their performance and properties. However, there are two predominant characteristics of textiles that may affect the ability to image fibres in an electron microscope. The first is their behaviour as electrical insulators and the second is whether they contain absorbed water. An insulating textile under SEM operating conditions, can “charge up” creating a distorted image or can result in the physical movement of loose fibres. Further, fibres containing moisture may change shape under high vacuum conditions as it dries. Echlin has quoted surface tension pressures achieving up to 2000psi in the final stages of drying causing deformation in fragile specimens (Echlin, 2009).

There are several techniques that can be used to reduce charging such as: coating with a conductive metal; the use of variable pressure or environmental SEM; the use of a Back Scattered Electron (BSE) detector; using low voltage settings; conductive tape and conductive paints such as “silver dag.” In addition there is also an increased risk of beam damage depending on the nature of the substrate, for example, materials composed of low atomic number constituent atoms, such as carbon, are more susceptible (Greaves & Saville, 1995).

**Scanning Electron Microscopes**

SEMs produce images by focusing an electron beam onto the substrate material, raster scanning across the surface of a specimen, collecting the surface emitted electrons and displaying the surface topography on a screen. These original instruments had a tungsten filament; Everhart-Thornley detectors and only operated at a high vacuum. On their introduction, Whewell reported that the: “resolving power and depth of focus (five and three hundred times, respectively) than in the best light microscope” (Whewell, 1966). In the same report he described the new-found ability to view cuticular damage on wool fibres and surface smoothness on spinnert manufactured man-made fibres. However, features, such as medulla (fibre cells containing air pockets) in wools, observed by transmitted light microscopy, could not be observed using a traditional SEM.

Since the pioneering SEMs there is more choice on a modern SEM. Common variables of electron source, detector and chamber conditions are given in Table 1. From these alone, there are many acceptable configurations of modern SEM. The above observation does not include factors such as: installation of a focused ion beam column, more than one detector used at once or the variety of types of variable pressure SE detectors and BSE detectors.

**Traditional use of SEM in Textile research**

Many processes are required to produce any textile--from fibre/yarn spinning, weaving/knitting; finishing, colouration etc. These processes change the overall fabric properties and can alter the fibre surface topography at a microscopical level. Typical examples of SEM use in textile research are illustrated in Figure 2 (a) and (b), where a Hitachi S2600-N SEM, with a tungsten source and using Everhart-Thornley detector in high vacuum was used. The wool fibres and cotton fabric were
mounted on 12mm stubs with conductive tape. Coating with gold was achieved in an Emitech 550K sputter coater at 25mA for 6 minutes, with the coating thickness not determined. Operating parameters are provided in the micrographs. These conditions were also used for Figures 4 and 9 (b and c). Topographical features are clearly defined in Figure 2 the wool cuticle scales and their damage are identifiable in Figure 2 (a). While typical surface fibrillation is evident on the laundered cotton fibres, Figure 2 (b).

In addition, SEMs are used widely in textile research to observe typically: fibre damage; inter-fibre bonding points in nonwoven textiles; identification of specialty fibres through scale height and examining fibre cross-sections (Greaves & Saville, 1995) (Hearle, et al., 2006).

In the last two decades SEMs have changed considerably (Gwynn, 2010) allowing easier use, more specimen information to be gathered and a wider applicability within textile research.

**Research Method**

In this study, all the research papers published in the Journal of the Textile Institute and the Textile Research Journal during 2015 and 2016 were studied, as was 1996 but only as a historical perspective for comparison. Details of SEM use within each paper were placed into an Excel spreadsheet.

The data collected was: the journal, it's year and volume number; if it contains work using SEM; title; if “SEM”, or similar, was used in the keywords; authors name; institution of principle author; field of study; make and model of instrument; number of images; if ESEM/VP and its settings were used; Acceleration Voltage (kV); working distance; beam; type of electron source; detector used; other imaging techniques used; magnification and distance bar size. Not all fields collected were found to be suitable for analysis.

Data was collected from both the written methodology and the published micrographs. If a disparity arose between the methodology and the micrograph it was assumed that the micrograph would contain the correct information because this is displayed autonomously. For analysis, “COUNTIF” statements were used to count the information given when applicable. Where information was not supplied, not given (N/G) was entered into the database.

**Results and Discussion**

**Research using SEM**

It was found that 31.1% (218 out of 702) of all the papers evaluated within this study utilised an SEM. In 1996 it was 11.7% (23 out of 175), implying an increase in SEM usage. This rise may have occurred because SEMs are more available, or they have become more versatile and are producing more varied and useful information or more research, requiring SEM for results verification, was being performed. A further factor, that may again influence the data going forward, is that some researchers in the field of textiles, however, may not publish in these traditional textile-type journals because of the lower impact factor values in these journals.

From Figure 3 it is apparent that the largest area of textile research published using SEM is finishing. Finishing can be applied after yarn and fabric formation (Figure 1.), researchers in this area are adding chemicals to or affecting the surface of a textile via physical means (e.g. temperature or washing). SEM is suitable to observe these surface changes and hence reflects its increased use.

The second largest subject for SEM use is that of nano-fibres. In recent years researchers have been interested in providing sub-micron diameter fibres to address a variety of engineering solutions (Liu, et al., 2018), created by electrospinning. The technique involves extruding a thin fibre ejected from a syringe using an electric current and has become a relatively simple and inexpensive laboratory technique capable of routinely producing nanofibre webs. While the individual filaments are not visible to the naked eye, a modern tungsten source SEM, from the author’s experience, can easily image these fibres with a diameter down to 200 nanometres. Figure 4 demonstrates the difference in size between a spider web (approx. 450nm diameter) with conventional fibres; a (ribbon shaped) cotton fibre (approx. 10µm width) and a micro fibre (approx. 10µm diameter). The solid particles are dust.

Figure 2. (a) Photomicrograph of a wool fibre and (b) a cotton fibre (M. Brookes).
Figure 4. A sample of dust containing spider's web with a cotton fibre and a micro-fibre (M. Brookes).

From Figure 3 investigations of fibre and fabric properties, combined, utilize SEM in about 22% of all the papers in total. The properties being studied are affected by microscopic topographical features and SEM is ideal at identifying such alterations.

One anomalous area was textile dyeing, the fifth largest area of research using SEM, imparting colour onto a textile, at fibre, yarn, fabric or final product processing stages. Despite traditional SEM micrographs not displaying in colour, SEM is perfectly capable of identifying damage from the colouration processes, observe deposits on fibres (such as oligomers) and identify the active atoms, by EDS/EDX and BSE. Hence the SEM technique is a very useful research tool in this area.

Keywords
In implementing any study’s literature survey, the filtering of abstracts and keywords to determine the relevance of any paper is a vital “focusing” process. While it was found that 2.3% of the papers assessed using the SEM technique put “SEM”, or a similar phrase in the keywords, it does appear that many researchers are omitting keywords that highlight the instrumental techniques being utilised. It is possible that authors now view instrumental analysis as routine and omit keywords, on that basis, or journals provide guidance that the technique is only specified if the paper concerns alterations or improvements to the method. Accordingly, in the future, if a problem were identified with a validation technique, finding affected papers using keywords would prove difficult.

Manufacturer
The instrument manufacturer and model tend to be a standard piece of information provided in research papers. Figure 5 identifies all the major manufacturers of SEMs are used in textile research papers published in 2015 and 2016. In this study, following company acquisition or mergers, now defunct company names have been assigned to the current company brand, e.g. Thermo Fisher encompasses, Philips, FEI and Phenom. Theoretically a researcher can always go to the manufacturer or the internet to find the specifications of the instrument a previous researcher has used. However, this is not always the case, for example, where manufacturers have deleted older model information on websites. Each model tends to be slightly different in some way, for example, BSE detector can have 1, 3, 4 or 5 segments. From Figure 5 the first two companies to introduce SEMs, Cambridge Instruments (now Zeiss) and JEOL, have maintained use over the last
20 years. Contemporary textile researchers appear to have a greater selection of SEM manufacturers available.

It was also observed that some manufacturers appear to change their models regularly, hence a brand-new model in a research project may not be relevant by the time the paper has been published. Some models allow significant flexibility in their operational mode, for example, some models of the Zeiss EVO, allow for both tungsten and LaB₆ filaments. Knowing the make and model of an SEM is now not enough to perform an identical study to those of a previous researcher. From this study, it was not possible to determine which manufacturers produced “better” SEMs for textile research as some laboratories produced more papers with one SEM and some manufacturers might have a better sales team.

Electron Source

Figure 6 shows that there are nearly the same number of SEMs using field emission (80 papers) sources as tungsten (76 papers) and in some papers both methods are used. As mentioned previously, modern tungsten source SEMs are capable of imaging fibres down to about 200nm, negating the need for a Field Emission (FE) source SEM in most uses. None of the papers studied appeared to show a knowledge in the difference between cold and hot FE sources and, hence it was difficult to separate in this study.

From Figure 6 there has been an increase in the use of FE sources. Tungsten sources appear to have been widely used in 1996 and the lack of reporting of electron sources in 1996 may be that researchers expected all SEM to have tungsten filaments.

FE sources have a theoretically higher resolution than tungsten machines owing to their higher brightness, more parallel electrons in their beams due to a smaller virtual source and smaller energy spread (Egerton, 2005). Therefore, researchers may want to publish using these machines to imply that they have superior facilities. In some cases, the bulk of the analysis may have been performed on a tungsten source SEM with an FE source SEM, utilised to provide images for the finished paper, hence skewing the results for this study. It was also found in some of the papers that images from more than one SEM were published but only one instrument was mentioned in the experimental method section.

Specimen Preparation

One of the traditional strengths of the SEM techniques was the perceived ease of specimen preparation. However, with the introduction of Variable Pressure/Environmental Scanning Electron Microscope (VP/ESEM) techniques and table/bench top SEMs the need for coating specimens is now greatly reduced, if not negated.

From Figure 7 it is apparent that the most common method of coating specimens is that of gold coating, though, in a few cases researchers are using more than one metal for coating. Nevertheless, the most startling aspect of Figure 7 was the number of SEM papers that do not describe preparation of SEM specimens at all, 62.8%.

Use of Variable Pressure Imaging Techniques

For the textile researcher the evolution of VP/ESEM systems has theoretically provided a powerful tool for imaging. The gas reduces charging and can maintain water within the structure without the need to dehydrate the specimen, during coating or imaging.

Interestingly on analysing Figure 8 (a) and (b), while it was apparent in 126 (57.8%) papers VP conditions
Operational Parameters

Acceleration Voltage (kV)

One of the key operational settings of the SEM is the acceleration voltage (kV). This setting is important to the surface image as it controls the penetration depth of the beam into the sample (Chescoe & Goodhew, 1990). Textile materials are predominantly carbonaceous in nature and Stokes (Stokes, 2008) has published calculated penetration depths for carbon, using the Monte-Carlo method. Figure 9(a) was created by this author incorporating all the kV settings of a Hitachi S-2600N SEM, using a Monte-Carlo software package provided by...
Stephen Chapman at Protrain. A negative value was assigned to demonstrate penetration. Results were found to be comparable, though slightly higher, with Stokes’s results. Figure 9 (a) shows that higher levels of kV are capable of fully penetrating fibres of diameters below 10µm. Therefore, images with electrons penetrating through the fibres reduce identifiable surface features and fibres become more transparent particularly at cross-over points. Therefore, textile researchers need to be aware of kV settings when imaging fibres with a diameter less than 10µm.

Figures 9 (b) and (c) illustrate images of the same specimen, slightly different positions, with different kV settings. From Figure 9 (b) it can be seen that while the higher accelerating voltage appears to provide a sharper image, the fibres look flatter and, at cross-over points, background fibres are visible. Figure 9(c) might not appear as visually sharp, but more surface features are visible, providing better fibre information. Modern techniques, such as electron beam deceleration stages, are helping to alleviate the problems identified, above, for low kV imaging (Bell & Erdman, 2013).

Figure 10 (a) illustrates the accelerating voltages used in the studied research papers. Most papers provide more than one micrograph and use more than one kV setting, with each setting registered separately, allowing one paper to be counted more than once. From Figure 10 (a) the largest number of papers used experimental conditions of between 10 and 14.9kV. What these results don’t demonstrate is whether the kV settings are correctly being chosen for the material and required image as demonstrated by Figure 9. Optimum settings for textile surface observation vary, depending on the information required in the study, the instrument, the detector and the specimen. In 1966 Whewell (Whewell, 1966) advocated settings of 2kV. From Figure 10 Whewell’s setting appears to be justified with an electron penetration in the region of 100nm at 2kV, without coating. However, some instruments will be unable to achieve a suitable image at this voltage. The author, initiating any specimen investigation, will start at 3kV and alter accordingly.

**Working Distance**

The working distance in a SEM system is the distance of the focused specimen from the final lens (Chescoe & Goodhew, 1990). Figure 10 (b) indicates the most used working distance tends to be between 5 and 14.9mm, suggesting SEM operators prefer to use close and medium distances, balancing between higher and lower settings. Higher settings maximise the field of depth and area observed, while lower settings improve resolution. In-lens use was not greatly observed in the studies reviewed. It is possible that there are very few of these instruments available to researchers or the high resolution achievable by these instruments was not required.

**Beam Size**

Beam size is the size of the beam as it impinges on the specimen and is loosely analogous to the pixel size on a digital camera. Only 9.6% of papers provided information on the beam size used, hence reflecting that there are not enough papers quoting beam size to form suitable conclusions. In addition, none of the papers studied mentioned aperture size.

**Magnification Used and Scale Bars Used in the Research**

Magnification is linked to what the SEM is being required to observe. The magnification of the image is calculated as the change from the original dimensions scanned to that being displayed. Therefore, it changes with display medium.
From Figure 10 (c) it was shown that the most used magnifications were those between 1,000X to under 10kX, with the distribution close to a normal distribution. The results were similar to those observed in Figure 10 (d). Scale bars were provided in more papers than the magnification and changes proportionally with the display media. Both Figures 10 (c) and (d) showed that some researchers were using the SEM for samples that could be imaged with light microscopes or cameras. This may be because it is easier to produce images with scale bars, required by journals, on an SEM than a light microscope.

**Detectors Used**

SEMs are being used to create information, each type of detector provides a slightly different type of information. It was found that SE, BSE and EDS/EDX detectors were the only detectors used in the papers studied, Figure 11 (a), although some use of in-lens and low vacuum secondary detectors was noted. Everhart-Thornley, in-lens and low vacuum were all classified within the category of SE detectors. From Figure 11 (a) it was shown that the most used detector was a SE detector with 74% of the papers using this type of detector. The next most widespread use is that of EDS/EDX (13%) with the BSE detector the least used with 9%. However, when compared to Figure 7, there appears to be some ambiguity. With most SE detectors requiring coating of gold, or similar, the least used detector, BSE, is predominately done when using a BSE detector; the least used detector. Carbon coating tends to be used for EDS/EDX, the second largest detector used, but from Figure 7 there appears to be very little carbon coating reported. Indeed, several researchers coated the specimens with gold but removed gold from the EDS/EDX results. The most likely reason for this ambiguity is due to the widespread lack of reporting of specimen preparation.

Benchtop SEMs have recently become more available and were reported in this research. These instruments, unlike traditional SEM, use a BSE detector as its primary detector (Wilkinson, et al., 2011). The benefit being that BSE detectors can operate in variable pressure conditions without the need of coating. BSE detectors can observe chemical differences because signal strengths change with atomic number unlike SE signals (Echlin, 2009). In the papers observed, researchers using these instruments do not appear to have understood these fundamental differences, reporting as if using a traditional SEM.

**Additional Imaging Devices used in Papers Using SEM**

SEM provides useful information for many research studies but their images may not full illustrate the required information for a research paper and other techniques may need to be utilised. From Figure 11 (b) it can be observed that cameras are the most used additional imaging technique when an SEM is used. Camera images can indicate the equipment used and, with light microscopy, present coloured materials in colour.

Modern Scanning Transmission Electron Microscope (STEM) sensors now make it possible to do transmission electron microscopy analysis using an SEM, allowing SEM and STEM imaging/analysis of an identical spot. From Figure 11 (b), less than 10% of the papers using SEM analysis would have benefitted from this technique.

**Reproducibility of Results**

When collating the data in this study, the most striking observation was the number of gaps in the information provided. In Figures 5, 6, 7, 8, 9, and 10, one of the larger columns is that of “not given”. Such is this lack of information that it could hamper the ability to reproduce the SEM analyses in future studies. In a recent article, Baker (Baker, 2016) estimated the non-reproducibility of results was over 70%. However, reproducibility of textile testing, described by Saville (Saville, 2007) is a statistical test of the given results which would be impossible to apply to images. Therefore, from the above data collected, we can determine the number of papers where the SEM component could not be unequivocally repeated/reproduced. It was assumed that the number of papers where the test method cannot be repeated potentially suggests that those results were not reproducible. This view was taken by Samuel et al (Samuel, et al., 2017) who took the approach of creating a system chronicling all experimental parameters.

From a microscopist’s perspective, the information required to repeat a SEM analyses can be reduced to three separate items: a suitable description of the SEM used, a suitable description of its settings and a suitable description of the specimen preparation before testing. Like a tripol, all three sections are required to maintain the integrity of the experimental description. To measure the reproducibility of the papers in this study, a simple test was applied by observing 3 parameters: one describing the instrument (manufacturer, make, ability to operate at variable pressure and electron source), one stating an operational setting (kV, working distance, beam, use of VP-SEM) and one stating the specimen preparation (coating). If one, or more, of these three parameters was missing then the paper was classed as not reproducible.

Choosing a single parameter to best describe the SEM operational settings is not a perfect solution...
and is flawed. Not all SEMs have variable pressure, the beam setting is manufacturer and model dependent, the working distance can vary with focussing, and the kV varies. Many researchers often wrongly assume that identifying the SEM manufacturer and model also provides additional information to describe the instrument such as VP/ESEM capabilities or electron source. However, the use of a single parameter provides a simple objective test that can be applied to a suitable database or spreadsheet.

Table 2 demonstrates the variance of parameter selection. The model and make descriptors give identical results being given together. The importance of choosing suitable parameters when using this test was demonstrated, as results alter from 67% to 96.8%.

Using the parameters of manufacturer, kV and coating, 67.0% of papers were not reproducible. This figure corresponds with Baker’s figure of over 70% previously mentioned, although Baker’s results were obtained using a questionnaire sent to scientists and was, inherently, more subjective. Baker had asked researchers whether they had tried to replicate other’s work and if it was successful. The higher value of 96.8% not reproducible was closer to the figures reported by Samuel et al. (10% reproducible). A likely difference between the two figures is that Samuel et al was requiring a complete report of their instrumental opportunity to gain maximum data. Researchers do not appear to be making the most of their instrumental opportunity to gain maximum information when utilising the modern SEMs available to them.

A further observation is that, it is the journals who publish the papers and assign the referees. Some of the papers demonstrated basic errors, such as: conflicting information between the written experimental method and the micrographs; images from more than one machine evident with only one or a wrong SEM mentioned; charging of specimens; stating unobtainable conditions and unreadable information bars on the micrographs. These issues are understandable and forgivable, at the proof reading stage, but should be identified and corrected in the refereeing process. Often referees volunteer their time and fit these tasks between other commitments and are not necessarily experts at SEM but on primary topics in the paper. While journals could provide checklists to the referees this may become too impractical when more than one analytical technique is being used. It is also possible to have more than one referee, as it does authors, who assess different aspects to the paper, though this, could considerably slow the refereeing process. Other solutions could include, increasing word limits on papers to allow more detailed method descriptions and publishers employing full-time proof readers or referees.

Some SEMs, like digital cameras, provide an additional data file containing settings and variables for each image, the Hitachi S-2600N is one example. Publishing these files in appendices or embedded in on-line journal formats could assist in increasing reproducibility. The main problem would be for researchers, publishers and SEM manufacturers harmonizing on the required information format in these files.

Conclusions

The use of the SEM technique is important in textile research and is increasing in its application. Usually the use of SEM is very “traditional” and many researchers do not appear to be making the most of their instrumental opportunity to gain maximum information when utilising the modern SEMs available to them. Very few of the researchers put SEM in their key words, making it difficult for future readers to gain an overview of the impact and applicability of this type of research.

Use of a three-information-test to assess reproducibility of the test method descriptions, given in these papers, has proven simple to implement and generated useful results, provided suitable test parameters have been chosen. This technique found that 67% of papers using SEM were not reproducible, which is a similar result to earlier research produced by a different research methodology The largest contributing factor was a lack of reporting on specimen preparation for SEM analysis.

This research has not highlighted any problems with the scientific aspect of the research but with the reporting of the work and the ability to replicate it by third parties from these descriptions.

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