Characterisation of ESEM conditions for specimen hydration control

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Abstract. The concept of “calibrated ESEM” – the ability to determine and create the exact conditions within the ESEM required for specimen stability and/or accurate in-situ hydration/dehydration – is an attractive idea. It has the potential to allow true natural state imaging, enhanced analysis and a whole range of new and novel applications. The present work reports on the use of in-situ temperature and humidity sensors to accurately measure and characterise the conditions within an ESEM.

1. Introduction
The environmental scanning electron microscope (ESEM) has enabled major developments in the examination of soft, moist and/or electrically insulating materials, permitting them to be viewed without much of the preparation and pre-treatment associated with conventional SEM (CSEM) [1]. In addition, in-situ experiments may be carried out, such as mechanical deformation, corrosion studies, and the observation of dynamic processes including wetting, swelling, hydration/dehydration and colloidal aggregation.

ESEM is largely hailed as revolutionary because it has the potential to allow samples to be viewed in their natural unaltered state. However, although differing from those associated with conventional SEM, there is still the potential for artefacts or unwanted specimen changes to be created during ESEM analysis. Of particular importance to wet or hydrated specimens is the control of temperature and pressure within the microscope chamber. For stabilisation of such specimens, the appropriate saturated vapour pressure must be maintained which prevents dehydration; or in the case of dynamic in-situ experiments too, precise and accurate control of the state of hydration may be essential for a comprehensive study to be performed. In both instances it must be varied in a controlled manner.

2. The need for “calibrated ESEM”
For the full potential of ESEM to be exploited, the state of hydration of a specimen must be carefully controlled so that it remains sufficiently similar to that prior to entry to the ESEM environment. For particularly sensitive substances such as liquids or biological specimens, this will require highly precise and accurate control of the conditions in the microscope chamber [2]. In addition, many authors have reported difficulties with water layers forming over the sample and reducing contrast [3]. With precise control of conditions, the formation of this water layer could be avoided. For dynamic in-situ experiments too, precise and accurate control of the state of hydration may be essential for a comprehensive study to be performed. In both instances it may also be of fundamental importance to
know the exact conditions (e.g. temperature, pressure and relative humidity) which the sample is experiencing.

The concept of “calibrated ESEM” is envisaged as the ability to determine and create the exact conditions within the ESEM required for specimen stability and/or accurate in-situ hydration/dehydration. Such control would not only allow more effective imaging of samples, but would vastly improve the quality of dynamic analysis. It could even open up the ESEM to a whole new range of applications. For instance, standard testing that requires specified conditions (e.g. moisture vapour transmission of textiles [4]) could be performed in-situ, enabling simultaneous measurement and analysis of the process on a micro/nanometre scale.

3. Towards calibrated ESEM
The path towards calibrated ESEM is twofold. On the one hand, greater understanding of specimens is required so that the necessary conditions for stability – most notably for hydrated samples, the specific saturated vapour pressure – may be determined. While this is vital information for the microscopist, and such analysis has received greater attention in recent years [5], much of the input for this area must come from the specialist in the particular field with a detailed knowledge of the specimen characteristics.

The second aspect is to obtain a greater knowledge of the ESEM instrument and the environment it creates for the sample. In this aspect, there is still much unknown; relatively, ESEM is still in its infancy when compared to other more established forms of electron microscopy such as conventional SEM and TEM.

The effect of the electron beam on an uncoated native sample in the ESEM and its potential for the introduction of artefacts has been noted and the processes studied in some detail [6]. Consideration of the pumpdown from ambient to observation conditions has also received theoretical and qualitative attention, leading to the almost ubiquitous purge-flood technique [7]. Quantitative monitoring and analysis of the environment in the ESEM on the other hand, has received comparatively little coverage in the published literature.

As potentially the most vital aspect of “calibrated ESEM,” this may well be the area where significant gains can be made. Aspects of interest include differences in temperature between sample and Peltier stage, electron beam heating effects, temperature, pressure and humidity gradients, and of course knowledge of the exact conditions experienced by the sample itself.

4. Use of in-situ sensors
In this work, dual purpose digital temperature and humidity sensors (Sensirion SHT75, Sensirion, Staefa, Switzerland) were positioned within the chamber of a Philips XL30 ESEM for the purpose of monitoring and recording these parameters during pumpdown and imaging. One sensor was attached directly to the specimen stub, while three further sensors were positioned at progressively greater (variable) distances within the chamber.

Figure 1(a) shows an example of temperature and humidity profiles during a four-cycle purge-flood pumpdown for the sensor attached to the sample stub, as well as a sensor positioned close to the edge of the chamber. Prior to inserting the specimen, the microscope chamber had been flooded to high humidity. The sensors were used to monitor the conditions during various pumpdown regimes, with and without purge-flood cycling, and during subsequent imaging. Qualitative assessment was also made by observing sections of celery, whose open cellular structure made it easy to gauge the success of a pumpdown sequence, and/or to see any dehydration/beam damage during imaging.

With no purge-flood cycling during the pumpdown the sensors showed prolonged periods of low humidity and samples were frequently irreversibly damaged, as illustrated in figure 1(b). The optimum pumpdown sequence was found to consist of eight purge-flood cycles, as recommended by Cameron and Donald [7]. This preserved the cellular network structure of the sample, as shown in figure 1(c).
5. Analysis

Although the use of purge-flood cycling during the pumpdown is a well established method for specimen preservation, the use of the sensors has enabled this to be further validated in practice, and has given insight into how it may be more precisely controlled. It is, for instance, clear in figure 1 that there is still a large drop in relative humidity during the initial pumpdown (stage 4). This could be avoided by additive hydration methods such as water drop placement on the Peltier stage adjacent to the sample, and the improvement could be assessed quantitatively using the sensors. While this is again a standard technique, use of the sensors allows its success to be validated and may take on greater importance for more delicate samples than the celery examined here.

Use of the sensors in this work has also shown that the temperature and humidity values may drift and fluctuate on both a short time scale during the course of a single analysis session, and also on a longer timescale over days and weeks. Similar findings have been made by Zimmermann et al. regarding pressure instability [8]. In their work, they ensured calibration of the relative humidity by using an additional in-situ pressure gauge, a thermistor on the Peltier stage and by observing the deliquescence point of salt particles [9]. Similar methods were employed by the authors of this paper but were found to be extremely time consuming and not feasible when analysis of the salt particles is not the main aim of the investigation. It is essential therefore to have in-situ sensors during every use of the ESEM in order to monitor the exact conditions experienced by the sample.

6. Discussion

If ESEM is to develop to become a more precise and sophisticated environment then accurate characterisation of conditions is vital. In addition, this needs to be a routine part of the analysis and the machine itself rather than the addition of make-shift sensors or novel routines. As increased information about the conditions in the ESEM is gained, appropriate changes to operating procedures,
imaging parameters and even the design of the ESEM instrument may be implemented. It may well be the case that much of the additional sensing/analytical apparatus currently being investigated (by the authors of this paper and others) may become standard parts of an ESEM instrument.

Furthermore, it may also become possible to develop the machine so that a larger range of conditions can be permitted. Since the time of the work in this paper, this concept has been demonstrated in practice with the development of an improved cryo-ESEM system [10]. However, for many biological specimens, a true natural state would be at body temperature, ~37ºC and imaging at this temperature is still to be achieved (although recent work is coming close [11]). With the increasing understanding that may be brought by characterisation of the current ESEM environment, of which the work here is only the beginning, this type of “calibrated ESEM” may not be so far away. The authors of this paper are presently involved in work optimising ESEM conditions for the observation of human dentine, and hope to report new findings elsewhere in the near future.

7. Conclusions
The need for precise knowledge and control of conditions in the environmental scanning electron microscope has been highlighted for both static and dynamic analysis. Greater control of specimen stability and dynamic hydration may be achieved through improved characterisation of the environment within the ESEM. Work to this aim has been initiated through the use of additional in-situ temperature and relative humidity sensors. Further work is in progress.

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