Use of automated image acquisition and stitching in scanning electron microscopy: Imaging of large scale areas of materials at high resolution

Jim Buckman
Institute of Petroleum Engineering, Centre for Environmental Scanning Electron Microscopy (CESEM), Heriot-Watt University, Edinburgh EH14 4AS, Scotland.

INTRODUCTION
Automated image acquisition for scanning electron microscope systems has a long history (see Tovey and Wang, [1]), and have become increasingly common over recent years. In general, these are concerned with capturing single images, or groups of associated images, for image analysis purposes [1, 2].

More recently, examples of automated image capture and stitching, designed to produce high-resolution large format images have been developed [3], with software offerings by Gatan (DigitalMontage), Zeiss (ATLAS) and FEI (MAPS). These systems typically have the capability of producing images that are several gigabytes or larger in size, and can often use a variety of detector types such as secondary electron (SE), backscattered electron (BSE) and cathodoluminescence (CL).

Although capable of acquiring and stitching images from a variety of sample types, image acquisition is simplest where the sample is flat. For samples that have pronounced surface topography, or an inclined surface, automated focusing solutions are required.

In addition, some packages allow superimposition of additional images from other sources such as optical microscopes, digital cameras or scanners (e.g. within ATLAS, and correlative workflow for MAPS).

Now that 64-bit computers, with fast processors, increased memory, and software that can fully utilize the 64-bit architecture (Windows 7 or newer), are more common (and being used by electron microscope manufacturers), constraints on image size and acquisition and image processing time have become less significant.

It was therefore decided to ascertain the suitability of automated image acquisition of backscattered electron images, from a polished thin section of sandstone, with the aim of assessing the suitability of such systems for rapid acquisition of stitched images for analysis and archiving purposes.

MATERIALS AND METHODS
SCANNING ELECTRON MICROSCOPY
A carbon-coated polished thin section of a feldspar-rich sandstone was examined using an FEI Quanta 650 field-emission gun scanning electron microscope, under high vacuum, at 20 kV, with a spot size of 4.5 and a working distance of 10 mm. Figure 1 shows an image of the section on the stage of the FEG-SEM, taken using the SEM navigation camera.

IMAGE ACQUISITION AND PROCESSING
The software MAPS from FEI was used to acquire large-format images of areas through the acquisition of a series of tiled BSE images. Individual images were taken with a 10% overlap. An overview image was first taken (Figure 1). This was then used to define the area of interest for investigation by MAPS. After selection of suitable parameters (see Table 1) tiled images were automatically acquired from the selected area. After acquisition, images were checked for alignment, and where necessary were manually adjusted, and then stitched and stored as an HD-viewer file.

RESULTS AND DISCUSSION
Backscattered electron images were successfully acquired over the selected area. Part of the scanned area is illustrated in Figure 2.

The results indicated that grain shape and size were clearly observed, as was also overall grain composition (quartz versus feldspar). Examination of individual tiles, also clearly illustrated the occurrence of features such as quartz and feldspar overgrowths (Figure 2b).

The combination of MAPS and field-emission SEM provides a stable platform, with zero drift, where image brightness remains constant over many days. High-resolution images can therefore be collected continuously over multiple days, through the acquisition of many thousands of individual micrographs (see Table 1).
The manual BSE examination of geological thin sections can often take many hours, and given time constraints, it is not always possible to cover all areas of interest. This technique allows whole slides to be imaged at a range of resolutions, during times when the microscope would normally be unavailable (e.g. evening and weekend). Through use of the HD-viewer, high-resolution images can be used as the basis for research, being used much in the way of a ‘virtual electron microscope’, and areas of interest can later be revisited if required. The images captured are also invaluable as an interactive teaching tool.

This technique is likely to be of use when looking at sandstones for porosity, diagenesis, mineralogical distribution and fracture analysis. This is equally applicable to carbonates and shale, and also has application to metamorphic and igneous geological materials. The ability to overlay images, using the MAPS-correlative flow, allows for direct correlation of images using different imaging methods (e.g. light microscopy and electron microscopy).

This technique can also be directly applied to other areas where polished surfaces regularly require examination at the micrometre or nanometre scale, such as building materials (mortars and cements), ceramics and metallography samples. The only requirement, other than a good flat polished surface, is that the base of the sample be parallel to the polished surface under examination.

This technique can also be used for archiving of samples, such as type specimens held in museum collections (e.g. foraminifera, diatoms, conodonts etc). In conjunction with low-vacuum microscopy, this would allow for a non-destructive rapid scanning technique of items, that can then potentially be accessed on-line.

It is possible to set up an image acquisition at extremely high-resolution (small tiles, slow scan time, and high pixel resolution) over large areas (in the case of the current microscope, 25,000 mm²). Such parameters will return extremely high estimated run times (see Table 1). Where run time exceeds two days, there are likely to be problems with maintaining ion getter pump pressures, leading to vacuum problems and unstable imaging conditions. In addition, run times that exceed two days (a weekend) are likely to be impractical in terms of machine time required, having too great an impact on other users.

Additional time is required for samples with topography, as auto-focus routines are required to correct focus on every tile collected (personal observation). Similarly, flat but inclined surfaces require refocusing based on a sample height formula calculated.
from three reference points. In both cases, these will substantially add to acquisition times, potentially beyond practical use. Auto-focus may also in some cases lead to spurious results, especially where there is a high order of surface topographic variation. Auto-contrast is also available, but similar problems exist as with auto-focus. In the case illustrated here, auto-focus and auto-contrast were not required.

Where few features are visible per tile, a high degree of manual stitching can be required. Manual stitching can be minimized by careful selection of an appropriate magnification, to ensure that most individual fields of view are likely to have identifiable features to aid in the automated stitching process. Similar problems in stitching the tiles together can also occur where a simple repeated pattern occurs (personal observation), leading to problems in automated stitching. The latter can become troublesome in images that contain in excess of several thousand individual tiles.

It is also important to firmly secure the slide to the stage, as slide movement during image acquisition tends to lead to automated stitching problems (Figure 3).

**CONCLUSIONS**

Automated image acquisition and stitching are useful tools for collecting high-resolution images over large-scale areas. Until relatively recently, such techniques were not a practical proposition. This technique is particularly suited to the analysis of flat polished surfaces, such as polished thin sections. However, where extreme high resolution is called for (e.g., shale thin sections), or samples are particularly large (>1,200 mm²), it becomes necessary to be more selective in choosing areas to be analyzed.

This technique is also suitable for acquiring low-resolution larger scale images, for example taking images of important small museum samples, particularly those attached to reference slides, such as foraminifera.

Images acquired in this way can be used for archiving purposes and form a solid basis for substantial research projects, acting as a 'virtual electron microscope'.

**REFERENCES**


**TABLE 1**

Typical scan parameters and acquisition times while using MAPS.

* Indicates parameters used during image acquisition presented here.

<table>
<thead>
<tr>
<th>Tile width (µm)</th>
<th>Number of tiles</th>
<th>Grid</th>
<th>Acquisition scan time (micro seconds)</th>
<th>Tile resolution (pixels)</th>
<th>Approximate acquisition time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,000</td>
<td>364</td>
<td>13x28</td>
<td>10</td>
<td>768x512</td>
<td>40 mins</td>
</tr>
<tr>
<td>400</td>
<td>6,420</td>
<td>54x119</td>
<td>10</td>
<td>768x512</td>
<td>11 hours</td>
</tr>
<tr>
<td>86</td>
<td>3,196</td>
<td>68x47</td>
<td>20</td>
<td>768x512</td>
<td>11 hours</td>
</tr>
<tr>
<td>259*</td>
<td>5,247*</td>
<td>53x99*</td>
<td>10*</td>
<td>768x512*</td>
<td>12 hours*</td>
</tr>
<tr>
<td>400</td>
<td>11,808</td>
<td>72x164</td>
<td>20</td>
<td>768x512</td>
<td>1 day</td>
</tr>
<tr>
<td>259</td>
<td>15,485</td>
<td>95x163</td>
<td>10</td>
<td>768x512</td>
<td>1 day</td>
</tr>
<tr>
<td>200</td>
<td>26,001</td>
<td>107x243</td>
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<td>768x512</td>
<td>2 days</td>
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<td>107,484</td>
<td>212x507</td>
<td>10</td>
<td>768x512</td>
<td>7 days</td>
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</table>

**ABSTRACT**

Automated image acquisition of many scanning electron micrographs, followed by automated stitching to form a single large image, can be used to collect high-resolution images over large areas. Potential uses include the imaging of whole polished thin sections or blocks of geological samples, mortars and building materials, ceramics and metallurgy. The constructed high-resolution images can be used as the basis for the extraction of data for research, and potentially for teaching purposes. This paper describes the use of MAPS, an automated image acquisition and stitching program, and illustrates its use with a polished thin section of a feldspathic sandstone.

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**CORRESPONDING AUTHOR DETAILS**

Dr Jim O. Buckman, Institute of Petroleum Engineering, Centre for Environmental Scanning Electron Microscopy (CESEM), Heriot-Watt University, Riccarton, Edinburgh, EH14 4AS, Scotland.

Email: Jim.Buckman@pet.hw.ac.uk


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