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CALIBRATION OF THE BACKSCATTERED ELECTRON SIGNAL FOR BONE, BIOMATERIAL, AND IMPLANT RESEARCH

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INTRODUCTION: Backscattered electron (BSE) imaging has become a useful tool for implant and bone research. In addition to producing high quality images for stereological measurements, BSE methods now provide a means to quantify bone mineral content at a microscopic level. Digital BSE images of bone may be captured on-line, directly from the scanning electron microscope (SEM) and analyzed to produce a backscattered electron profile (BSEP) that shows the proportion of the image occupied by image elements (pixels) of each graylevel. The graylevels present in the image relate directly to the mineral content of the bone.

The key factor which limits the use of this technique for the quantitative determination of mineralization changes in bone has been the need for a method of calibrating the operating conditions (i.e. factors affecting the image graylevels) from working session to session and from lab to lab. If the BSE method is to be universally applicated in the assessment of bone mineral changes attributable to implants, weightlessness, drug therapies, and age, then a reproducible calibration method must be established. The objective of this work was to demonstrate a reproducible technique for calibrating the BSE signal using pure metals so that consistent operating conditions could be obtained.

MATERIALS AND METHODS: Calibration standards containing pure aluminum and pure magnesium wires were placed in the SEM with a polymethyl methacrylate-embedded human cancellous bone specimen. Aluminum and magnesium were chosen because their BSEPs fall within approximately the same BSE graylevel range as bone. As illustrated in Fig. 1, the following protocol was followed to show a calibration. The microscope was adjusted until the bone image showed good graylevel separation and the multiple phases of the bone were evident (Fig. 2). Images were then captured from both the bone (Fig. 2) and the standards (Fig. 3), and analyzed to provide "baseline" BSEPs. The filament was desaturated and switched off, and the microscope brightness and contrast controls were randomly adjusted to initiate the new operating conditions of another imaging session. The microscope was then turned on and readjusted back to baseline conditions, using the deviation between the BSEP of metal standards and its baseline curve as a guide for adjustment. Three imaging sessions ranging in length from 8 to 24 hours were performed to observe any fluctuations in the whole SEM-image analysis system and to establish the minimal time interval of stable operation.

RESULTS AND DISCUSSION: Average graylevel values of the BSEPs from five calibration trials were compared to those of the baseline BSEP, and data showed that the average graylevel of the bone image BSEPs could be reproduced within an accuracy of 1.2 percent.

For our equipment, it was determined that calibration should be checked at 20 minute intervals during SEM operation, and recalibration would be performed if BSEP changes of greater than 1.5 percent were observed. This work demonstrates that it is possible to calibrate SEM operating conditions (brightness and contrast) using pure materials. In addition, this scheme can be used to study mineral changes in individual trabeculae of cancellous bone, which previously had no accurate method for quantifying mineralization at a microscopic level. A real-time, automated calibration method is currently being devised.

Figure 1 - The steps involved in determining baseline BSEPs (Steps 1-5) and performing a trial calibration (Steps 6-14).

Figure 2 - BSE image of bone used for determining effectiveness of calibration (x200).

Figure 3 - BSE image of aluminum (AL) and magnesium (Mg) wire standards (x200).

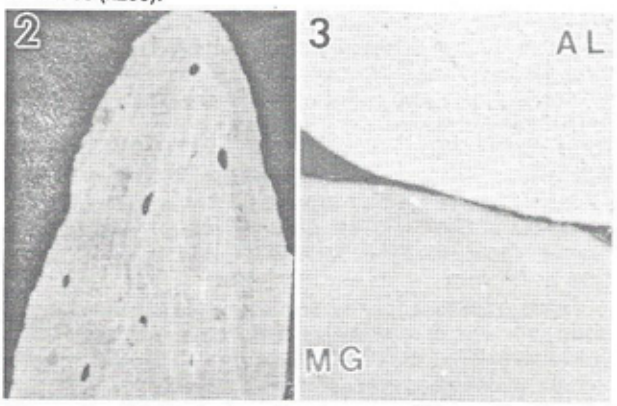


Figure 4 - BSEPs for baseline image and five calibration trials (Al/Mg standard).

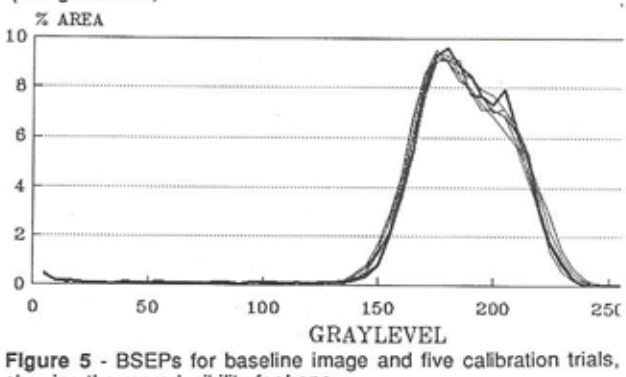
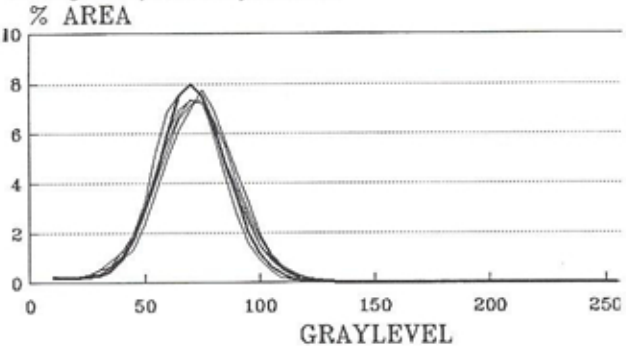


Figure 5 - BSEPs for baseline image and five calibration trials, showing the reproducibility for bone.



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