Accelerating Voltage

Accelerating voltage is the difference in potential between the filament and the anode, and it can be varied between 5 KeV and 30 KeV on the S-500 and between 2 KeV and 30 KeV on the S-450. As the voltage is increased, the electrons travel with higher velocity and are more energetic. There are several consequences to an increased energy level of incident beam electrons.

There are a number of effects that occur when the accelerating voltage is changed. When considered alone, increasing the accelerating voltage results in a smaller beam spot which theoretically should improve resolution. This is not generally the case however as the diffusion of incident electrons sideways under the specimen surface is increased as accelerating voltage is increased. This sideways diffusion results in the generation of more secondary and backscattered electrons which effectively increases the diameter of the beam spot. As a result, fine surface detail is obscured by the larger beam spot, effectively decreasing resolution.

In addition to a reduced resolution, increasing accelerating voltages can result in other deleterious effects, particularly for biological specimens that typically have low density. These include, increase in edge effect, greater likelihood of charging, and increased possibility of specimen damage.

Edge effect is the enhanced brightness of object boundaries relative to their interior regions. When an electron beam penetrates the surface of a specimen, it spreads or diffuses laterally. For low density biological specimens, the shape of the “zone of diffusion” below the surface has the shape of a teardrop, or light bulb, which enlarges in diameter as accelerating voltage is increased. Depending on the angle of the incident beam of electrons, more or less secondary electrons are able to escape the surface. If the incident electron beam enters the specimen at a glancing angle, the secondary electron emission is greater than when the beam enters it perpendicular to the surface.

As might be expected, the potential of charging is increased as accelerating voltage is increased because more electrons are entering the specimen per unit time. If the specimen itself, or structures on the surface of the specimen, are not adequately grounded, electrons can build up and result in enhanced brightness of the entire specimen or the surface structures. An example in point is the bright appearance of surface hairs, or setae, commonly found on insects. Because these setae are thin and project a long distance from the surface, electrons are not easily removed. Increasing accelerating voltage magnifies this effect because more electrons have to be grounded. Therefore, in order to minimize charging, lower accelerating voltages are required.

Because biological specimens are often labile and easily damaged, the excessive beam current produced by higher accelerating voltages can easily produce surface damage. This damage can manifest itself as plastic deformation or cracking and splitting of the surface. As well, the surface can darken with increased exposure to the beam due to increased heating which can attract contaminants that settle on the scanned area.
Although reduced accelerating voltages are generally recommended for biological specimens due to the considerations given above, caution must be exercised because chromatic aberration can increase with lower velocity electrons. Chromatic aberration is a spreading or reduced coherence of the beam due to scattering as individual electrons strike gas molecules in the vacuum.

In general, accelerating voltages of between 5 KeV and 15 KeV are used with most biological specimens. If excessive charging occurs, accelerating voltages should be lowered.