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## **PRINCIPLE AND CAPACITIES OF SCANNING ELECTRON MICROSCOPE**

The types of signals produced by a SEM include secondary electron (SE), back-scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence) (CL), specimen current and transmitted electrons.

Secondary electron detectors are standard equipment in all SEMs, but it is rare that a single machine would have detectors for all possible signals. The signals result from interactions of the electron beam with atoms at or near the surface of the sample. In the most common or standard detection mode, secondary electron imaging or SEI, the SEM can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. This is exemplified by the micrograph of pollen shown above. A wide range of magnification is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times the magnification limit of the best light microscopes.

Back-scattered electrons (BSE) are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays, because the intensity of the BSE signal is strongly related to atomic number (Z) of the specimen. BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can colloidal gold immune-labels of 5 or 10 nm diameter, which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens.

Characteristics X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher-energy electron to fill the shell and release energy. These characteristics X-rays are used to identify the composition and measure the abundance of elements in the sample.

## **SAMPLE PREPARATION**

All sample must be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. Several models of SEM can examine any part of a 6-inch (15 cm) semiconductor wafer, and some can tilt an object of that size to 45°.

Samples are coated with platinum coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation. SEM instruments place the specimen in a relative high-pressure chamber where the working distance is short and the electron optical column is differentially pumped to keep vacuum adequately low at the electron gun. The high-pressure region around the sample in the ESEM neutralizes charge and provides an amplification of the secondary electron signal. Low-voltage SEM is typically conducted in an FEG-SEM because the field emission guns (FEG) is capable of producing high primary electron brightness and small spot size even at low accelerating potentials.

Embedding in a resin with further polishing to a mirror-like finish can be used for both biological and materials specimens when imaging in backscattered electrons or when doing quantitative X-rays microanalysis.



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