DETECTION OF BACKSCATTERED ELECTRONS FOR BIOLOGICAL SPECIMENS STUDY

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Introduction:

Energy of backscattered electrons (BSEs) unlike energy of secondary electrons (SEs) is just little lower than primary electron beam energy. BSEs leave a specimen from a lower depth than SEs and they spread themselves forward to the space. They bring information especially about material character of a specimen expressed by atomic contrast. As a result of these properties BSE image is less exposed to charging artefacts of non-conductive specimens, smaller influence of contamination and lower overbrightness of edges. Under convenient operation conditions in SEM and at use of suitable preparation techniques, BSE image resolution can be comparable with SE image. BSE detection is an indispensable method for obtaining supplementary image information of material and biological research by SEM.

BSE detectors

BSE detectors must be positioned in the path of a chosen type of BSEs. Semiconductor detectors, channel plate detectors, plastic, phosphorus and single crystal scintillation detectors are used for BSE detection. If a very high resolution of BSE image is to be achieved, the YAG or YAP single crystal scintillation detector should be used. [1] Material contrast with the discrimination of the mean atomic number of 0.09 can be recorded if the detector is positioned close below the pole piece and BSE are collected in a “high take-off angle” (Fig. 1). If “low angle” BSEs are collected to the ring YAG detector, located around the specimen, topographical contrast of the noncoated biological specimens can be recorded. [2]
A special interest of the operation in SEM is the specimen observation at low accelerating voltages of the primary electron beam (around 1 kV). Because of many advantages of the low energy BSEs detection [3] , a new YAG – low voltage BSE detector was designed. [4] The detector is based on YAG scintillator on which high voltage of + 4 to 5 kV is applied. Low-energy BSEs and SEs are accelerated towards the scintillator, but the SEs are separated from the scintillator by a grid, which is supplied with negative low voltage. The result of the BSE imaging of the biological specimens is shown in Fig. 3.

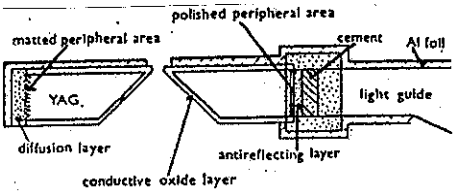

Fig. 1

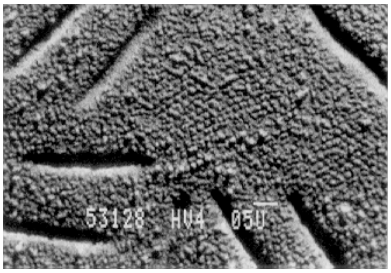

Fig. 2

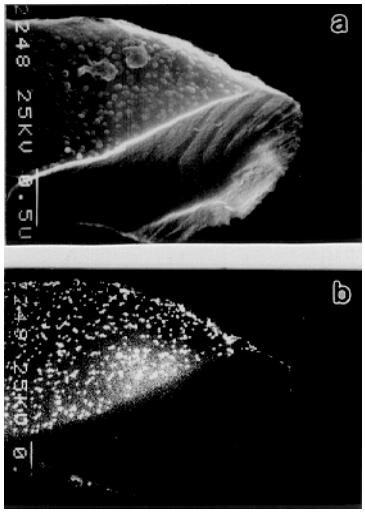

Fig. 3

BSE detection is an indispensable information method also at study of wet biological specimens in environmental scanning electron microscopy. BSEs move in gaseous environment of the specimen chamber without significant loss of energy and without bigger declination of their direction, in condition the distance between the specimen and detector is small. Charging of non-conductive biological specimens is suppressed. The detector has been constructed on the basis of YAG, which integrates three functions. Detection of BSEs, detection of SEs and pressure-limiting aperture. It enables working with specimens at pressure up to 1000 Pa.

Figures

Fig. 1. Planar YAG – BSE detector

Fig. 2. a,b Material contrast of the protein particles on a blood cell labelled by gold with the resolution of 5nm. Fig. a. – SE image, Fig. b. – BSE image.

Fig. 3. BSE image of a replica of the plasmatic fracture face of yeast cells recorded at 800 V accelerating voltage of the electron beam.

References

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