Bioree

Protocol for visualisation of stained samples by scanning electron microscope (ESEM|EP)

Instructions for setting up and using scanning electron microscope in low vacuum mode (EP – mode, ESEM) for visualisation of cell structures stained by BioREE-Ln kit		**Doc.№** BRLN002 v1.0
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Introduction: Scanning Electron Microscope was not originally considered an instrument for visualization of internal structure of a biological sample. Its applicability was limited to acquiring information on the form of its surface after certain preparation. However, due to the technology of elective accumulation of elements of lanthanum group in individual structures of a cell becoming available, it's now possible to use SEM for visualization of cell ultrastructure. Particularly useful for such analysis are the types of SEM that support low vacuum modes and don't require the spattering of electroconductive film on the sample. Setting up the SEM for examination of biological samples stained by BioREE reagents (or using similar methods) requires consideration of some specifics. Following the suggested parameters from this protocol will improve your chances of acquiring high quality images of stained samples on scanning electron microscope.

Subject of research: The protocol helps to acquire contrasting images of tissue and structure elements of cells cultivated on plastic of all types, silicate glass, including coated, on carbonic and polymer scaffolds, excluding those based on phosphates.

Attention! Successfully acquiring good images with no artefacts directly depends on correct sample preparation described in protocol **BRLN001**.

Attention! Be reminded that staining of cell structures by elements of lanthanum group (Ln-staining) is supravital. Acquired results depend on the condition of cells, activity and pattern of cell metabolism as of the time of staining.

Procedures:

1. Put the sample on the microscope stage. Optimal size of the sample for examination (together with the substrate) must not exceed 8 mm by the shortest measuring. We recommend fragmenting large samples of cultural plastic beforehand. This requirement is related to the process of charge redistribution on the surface of plane objects.
2. Make sure that the microscope is in the EP mode. We recommend setting the pressure of residual atmosphere of microscope camera in the range of 55-90 Pa.
3. Place the microscope stage in the camera and reduce the pressure to the recommended level. If the sample is large and heavily watered (and if the design of the microscope allows it) carry out the vacuuming in several cycles combining periods of exhausting to transitory pressure values with camera ventilation. This will prevent sample overstraining and residual salts crystallization on its surface.
4. Turn on high voltage and set the current. Choosing the acceleration voltage depends on desired effective thickness of the layer which structures you need to visualize after lanthanoid staining.
5. The visualization must be carried out using the detector of back-scattered electrons (BSE) in high contrast mode. In order to get the sufficient signal-to-noise ratio on the detector try to select the beam current empirically (for example, for Zeiss EVO LS10 microscope it is set in the range of 440-570 pA.

6. Use large beam aperture. The best visualization quality can be achieved choosing short flange distance (WD < 6 mm). However in case of examination of bulk samples try to choose the flange distance so that projecting parts of the sample won't damage the microscope when moving the stage.

Table of approximate values of accelerating voltage for some objects

Object of examination		Accelerating voltage, kV
Animal cells attached to substrate. Visualization depth corresponds to full volume of the cell.		
Individual "spreading" animal cells	on plastic	20-23
	on glass	20-21
Individual "spherical" animal cells	on plastic	22-24
	on glass	20-23
Animal cells attached to substrate. Visualization depth corresponds to surface of the cell membrane.		
All types of animal cells on plastic or glass with adhesive coating		10-15
All types of animal cells on uncoated silicate glass		7-11
Other objects for visualization after staining with elements of lanthanum group according to protocol BRLN001		
All types of animal cells deposited from suspension onto substrate (carbon, plastic or glass with adhesive coating)		23-27
All types of plant cells with thin, artificially thinned or partially fermented cell wall		23-30
All types of animal cells that during the process of cultivation formed a monolayer or multilayered structures, tissue samples (visualization of structures closest to cell surface)		20-27
Bacteria, protozoa, yeasts etc.		20-22