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🌐 Scanning Electron Microscopy - Focused Ion Beam Biological Sample Preparation

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The combination of the scanning electron microscopy (SEM) with a focused ion beam (FIB), represents a pioneering and interesting tool to allow the investigation of the relationship occurring at the interface between cells and biomaterials. Herein, we provide a suitable protocol for cell-biomaterial sample preparation before imaging by SEM-FIB.

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FIXATION

40m

- 1 Remove culturing medium from the samples.
- 2 Gently wash the samples twice in Phosphate Buffer Saline.
- 3 Cover the surface of the samples with **Gluthraldehyde 2.5%** in **Na-Cacodylate 0.1M** buffer. ^{30m}
- 4 Remove the Glutaraldehyde 2.5% in Na-cacodylate 0.1M buffer from the samples and cover ^{5m} the surface of the samples with **Na-Cacodylate 0.1M** buffer.
- 5 Remove the Na-cacodylate 0.1M buffer from the samples.

DEHYDRATION

1h

- 6 De-hydrate the samples in **Ethanol at increasing concentrations (35%, 55%, 70%, 90%, 95%)**. Each alcohol stands for 10 minutes at room temperature. ^{50m}
- 7 Store the samples in EtOH 95% at 4°C until further processing.
- 8 Cover the samples with **Ethanol 99%**. 10m
- 9 Remove The Ethanol 99% from the samples and freeze dry the samples with **Liquid Carbon Dioxide**.

SPUTTER COATING

30s

- 10 Sputter coat the samples for 30 seconds with Gold using a coating device 30s

SAMPLE OBSERVATION

- 11 Perform SEM-FIB analysis of the samples. We suggest to perform the SEM analysis at 5keV and to cross-section the samples with a Gallium ion beam accelerated at 30kV.