Sample preconditioning before scanning electron microscopy (SEM)

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ABSTRACT

Bacterial samples need to be preconditioned before visualized by scanning electron microscope (SEM). This protocol describes preconditioning methods of bacteria before SEM.
**Bacterial samples preconditioning**

1. Obtain a small quantity of bacteria to be tested from a culture.

2. Centrifuge the samples at 5000 rpm, 4°C, 00:10:00. Resuspend the pellet using 1X phosphate buffered saline (PBS). Repeat this step for three times. Keep the supernatant after the third centrifuge and preserve the samples at 4 °C.

**SEM sample preconditioning**

3. Wash silica wafers with ddH$_2$O and acetone for three times.

4. Add samples onto the silica wafer using a pipette tip.

5. Incubate the silica wafers in fixation solution (2.5% glutaraldehyde in 1X PBS) at 4 °C overnight.

6. Incubate the samples in ethanol solution with an increasing concentration (40%, 70%, 96%, 100%) for 01:00:00 each concentration.

7. Dry the samples at room temperature for 01:00:00.
Use a precision etching coating system, sputtering the samples with Au-Pd alloy.

**Note**

If samples contain gold, sputter the samples with chromium.

The samples are ready to be visualized using SEM.