SEM Sample Prep

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dx.doi.org/10.17504/protocols.io.w9ufh6w

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ABSTRACT

The SEM can be used to directly image biofilms on solid surfaces such as polished rock chips or collected crustal samples. Seawater or fluid bacteria can be imaged after filtering the sample onto a 0.2 μM filter.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

Reagents:

- 4% Paraformaldehyde in 1x PBS (VWR 102091-888)
- Hexamethyldisilane (HMDS) (VWR TCH0638)
- Ethanol, 100% molecular biology grade
- MilliQ water, sterile
- 1x PBS, autoclaved, filtered.

Supplies:

· Desiccator with fresh dessicant
· 1.5ml eppitubes, sterile
· Forceps
· Lighter or Bunsen burner
· Plastic syringes, sterile
· 0.2um-mesh syringe filters, sterile
· Small petri dishes, sterile
· Scissors
· Sharpie marker
· 12.5 mm aluminum pin stubs - Electron Microscopy Sciences #75210 ($17/pack 50)
· Aluminum-nickel conductive tape – Electron Microscopy Sciences #77813 ($28)
· Double-sided carbon adhesive disc, 12mm – Electron Microscopy Sciences #77825-12 ($17/pack 100)
· Stub holder box – Electron Microscopy Sciences #76500 ($4/box)

SAFETY WARNINGS

Hexamethyldisilane (HMDS) is volatile and flammable, do not open the bottle outside of the hood. It is stored in the flammable's cabinet in A010. Wear gloves while handling.

Paraformaldehyde (PFA) is toxic and should only be opened inside a BSC or Fume Hood. Wear gloves while handling.

Fixation of Sample

1 Using sterile forceps, submerge sample in an appropriate amount of 4°C 4% PFA in PBS in a 1.5 ml eppitube

2 Incubate in a 4°C refrigerator for 1 - 3 hours.

3 Rinse briefly in 1xPBS

4 Dispose of PFA waste in the G2 waste container in the Biological Safety Cabinet

5 Proceed to HMDS dehydration

HMDS Dehydration

6 Make a dilution series of ethanol in milliq water at the following concentrations; 50%, 60%, 70%, 80%, 90% and 100% ethanol by diluting 100% ethanol in milliq water.Filter all dilutions with a syringe and 0.2uM filter

7 Fill an appropriate amount of eppitubes or petri dishes with enough of each ethanol solution to submerge the samples

8 Incubate samples in each dilution for 10 minutes, transfer samples between dilutions with sterile forceps. Let ethanol waste evaporate in the hood or dump down the sink while running the faucet.

9 Fill another set of eppitubes or petri dishes with 100% HMDS and incubate samples for 10 minutes.

10 Remove samples and place them in a sterile petri dish to air-dry for 48 hours in the fume hood. After this period, they will be ready for gold-sputtering and SEM analysis.

11 Dispose of HMDS waste in Q22 waste container in the flammable's cabinet under the Fume hood in A211

Mounting

12 Wear gloves while mounting.

13 Label the underside of the aluminum stub with the sample number in sharpie marker.

14 Peel the carbon disc (two-sided tape) away from the backing and secure to the aluminum stub.

15 Cut a small strip of conductive tape about 10-12mm long and cut in half lengthwise with a sharp pair of scissors. Peel off the sticky backing and attach to two opposite edges of the two-sided tape. Press the tape firmly down with forceps to ensure good contact. (Skip until step 6 if you are mounting filters).

16 Using sterilized forceps, pick up the sample (rock chip etc.) and gently press onto the two-sided carbon tape.

17 If you are mounting filtered samples, affix the 0.2 μM filter to the two-sided tape, then repeat step 4.

18 Store mounted samples in the stub holder, preferably in a dessicator or a closed container with a dessicant pack. Keep the filter remains and store likewise.