Sample preparation for scanning electron microscopy

General schedule for animal cells or biofilm on biomaterials

	Chemical	Temperature	Time	Repetitions
Primary fixation	2.5% glutaraldehyde in pH7.2 0.1M PBS	room or 0-4°C	Overnight 4°C, or 30 mins RT	1
Wash	pH7.2, 0.1M PBS (1x)	room or 0-4°C	5-10 minutes	3-5
Secondary fixation	2% osmium tetroxide in distilled water	room or 0-4°C	20 mins	1
Wash	pH7.2, 0.1M PBS	room or 0-4°C	5-10 minutes	3-5
Dehydration	25% ethanol 50% ethanol 70-75% ethanol (may stop o/n) 90-95% ethanol 100% ethanol	room or 0-4°C	5 minutes 5 minutes 5 minutes 5 minutes 5-10 minutes	1 1 1 2 2
Transfer to HMDS	50% ethanol 50% HDMS 100% HDMS		10 min 10 min	1-2 2
Place specimens in fume hood, covering with glass lid, air dry overnight. In high humid season, place in desiccator to dry. Dry specimen completely under vacuum for 20 mins to 1 hour				
Mount on SEM specimen stub with silver or graphite paste, double side carbon tape				
Coat with carbon or slightly Au/Pd				
Store stubs in desiccators				

Note: 1. dehydration procedure must go as indicated time without stop except 70-75% EtOH, which the specimens can be left over night at 4°C. Other steps have to be done without stopping.
2. In this modified protocol, critical point dry is replaced by HMDS (hexamethyldisilazane), but the mammal cell morphology will be compromised. It is OK for microbiology study.
3. for better fixation, 0.05 M cacodylate buffer is suggested with 2.5% glutaraldehyde
4. CO2 critical point dry is the golden standard.

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