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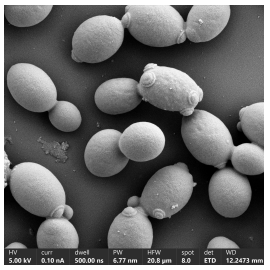
Yeast suspension for SEM (with HMDS)



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Protocol status: Working
We use this protocol and it's working

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Abstract

Suspension of yeast prepared for RT SEM imaging using HMDS drying

Materials

1) Stock solution of glutaraldehyde (could be from 10 to 25%)
2) Ethanol
3) Hexamethyldisilazane (HMDS)
4) OsO4 stock solution in water (not less than 1%)
5) 12mm metal SEM stubs
6) Sticky carbon adhesive tabs
7) Silver DAG
8) Round 10mm glass coverslips
9) Glow discharger
10) Sputter coater

Substrate preparation

1h 2m

1 Use glass coverslips that fit to your regular SEM metal stubs. If a stub is 12mm in diameter use 10mm round coverslips.

2 Glow discharge coverslips for about 1-2 min with standard settings (20-25mA). 1m

3 Briefly soak coverslips in Poly-L-lysine commercial solution (any), remove the solution excess by gentle blotting and leave them to dry in the open air for 30 min. 1m

Sample fixation

1h

4 Put coverslips on parafilm flat and apply about 30-50ul of yeast suspension on top of them taking care not to overspill on the parafilm. Wait for about 20-30 min, so yeast cells will drawn to the coverslip. 30m

5 Apply 2-5 ul of 10%-12% glutaraldehyde directly into yeast suspension and wait until the fixative will reach equilibrium concentration throughout. 30m

Osmium postfixation

1h 20m

6 Wash the coverslips with distilled water buffer 2 times for 5 min each. 10m

7 Put coverslips into appropriate glass container and apply 1%OsO4 solution in water. 1h

8 Wash coverslips with water 2 times for 5 min each. 10m

Dehydration

1h 15m

9 Incubate coverslips in 50% ethanol 15m

10 Incubate coverslips in 75% ethanol 15m

11 Incubate coverslips in 90% ethanol 15m

12 Incubate coverslips in 100% ethanol 2 times for 15 min each 30m

Drying 1h

13 Incubate coverslips in 50% HMDS in ethanol 30m

14 Incubate coverslips in 100% HMDS 15m

15 Incubate coverslips in 100% HMDS 15m

16 Remove most of HMDS from the containers with coverslips. Leave containers overnight opened in the fume cupboard.

17 Remove coverslips from the containers and glue them to SEM stubs with carbon sticky tape taking care about the side with attached cells.

Coating

18 To get better conductivity paint the conductive bridge between top of a coverslip and metal SEM stub with silver DAG. Wait until it is completely dry.

19 Use your sputter coater to create 7-9nm of metal coating with you favourite target (Au/Pd will do good).

20 Sample can be imaged with most room temperature SEMs using standard settings for moderately conductive samples.