#### Supplement 1

## **SBFSEM** step-by-step protocol

by Sebastian Büsse, Thomas Hörnschemeyer & Christian Fischer

All steps described in this protocol are realised using a rotary disc/plate and a fume cupboard. Always follow the recommended safety measures of the respective chemical. The precise concentrations and compositions of each chemical used can be found within the 'Material & Methods' section.

#### **Used Chemicals with hazard statements**

#### Glutaraldehyde









- H302+H332-H314-H317-H334-H335-H410

Sodium cacodylate (rinsing buffer)







- H301+H331-H351-H410

Osmium tetroxide (OsO<sub>4</sub>)







- H300-H310-H314-H330-H334

Potassium hexacyanoferrate – H412

Thiocarbohydrazide (TCH)



- H300+H330-H311

Uranyl acetate







- H330+H300-H373-H411

Lead citrate







- H302+H332-H360Df-H373-H410

Ethanol (EtOH)





- H225-H319

Acetone





- H225-H319-H336

Durcupan



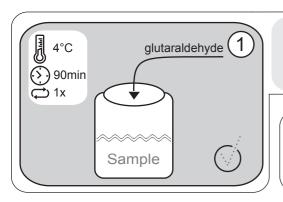




- H302-H315-H317-H319-H360Df-H400-H411

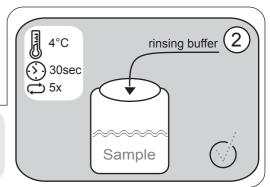
### SBFSEM step-by-step protocol

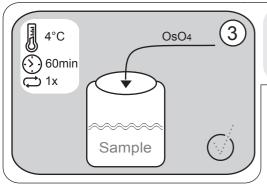
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Prefixation for 90 min using glutaraldehyde at 4°C

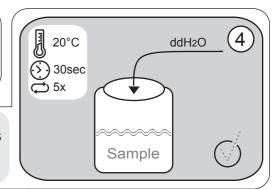
Rinsing the sample for 5 times using a cacodylate buffer (rinsing buffer)

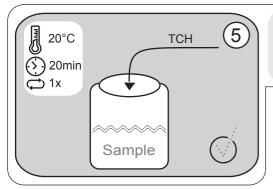




Staining with osmium tetroxide & potassium hexacyanoferrate for 60 min at 4°C

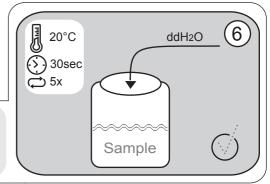
Rinsing the sample for 5 times using double-distilled water

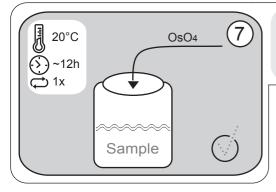




Sample fixation with thiocarbohydrazide for 20 min at 20°C

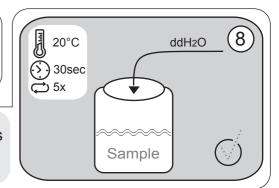
Rinsing the sample for 5 times using double-distilled water

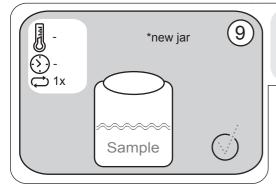




Staining with osmium tetroxide over night at 20°C (keep dark)

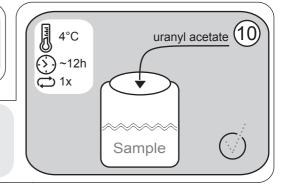
Rinsing the sample for 5 times using double-distilled water

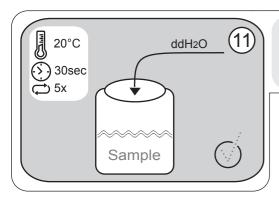




Change the sample jar to a new one

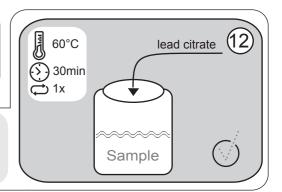
Staining with uranyl acetate over night at 4°C

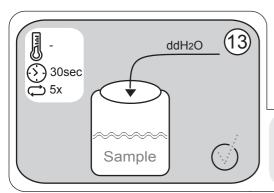




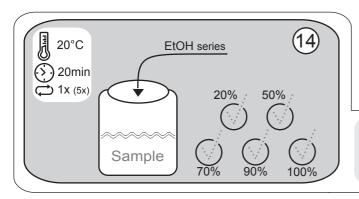
Rinsing the sample for 5 times using double-distilled water

Staining with lead citrate for 30 min at 60°C

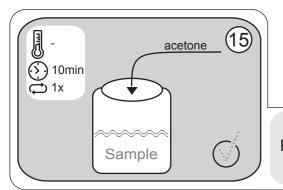




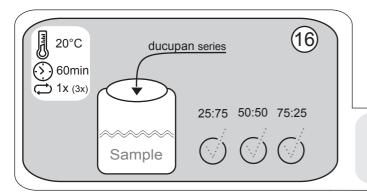
Rinsing the sample for 5 times using double-distilled water



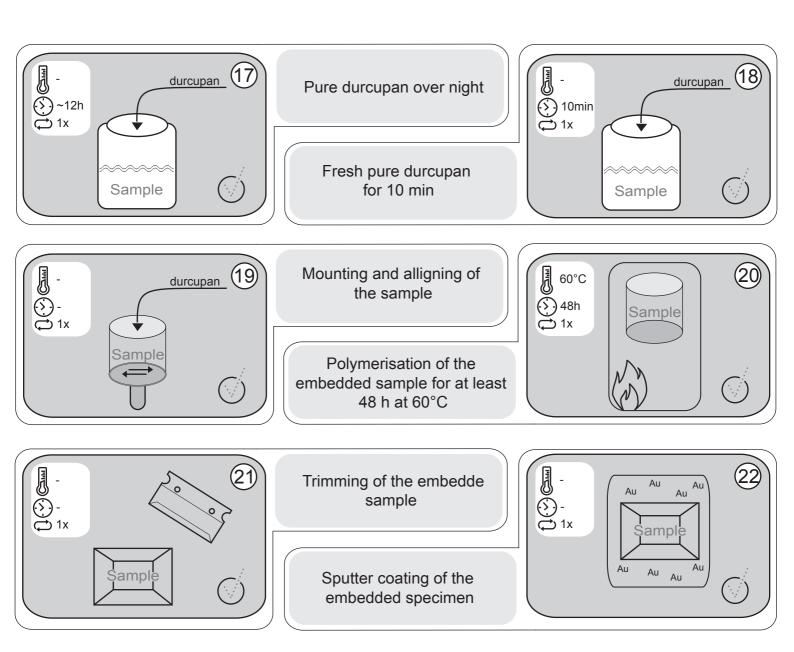
Series of graded ethanol concentrations from 20% to 50% to 70% to 90% to 99,99% for 20 min each



Rinsing for 10 min in acetone



Series of graded durcupan concentrations from 25:75 to 50:50 to 75:50 for 60 min each at 20°C



# ... ready for SBFSEM!