Procedure of SEM sample preparation in EM lab. (morphology)

Fix sample in Fixation Buffer* for 4hr in room temp. or overnight in 4°C buffer and rinses 3X 15 min in room temp. before proceed to following steps

Buffer rinses	3X 15 min in room temp.
30% EtOH	10 min
50% EtOH	10 min
70% EtOH	10 min or overnoight
85% EtOH	20 min
95% EtOH	20 min
100% EtOH	20 min
100% EtOH	20 min or overnight
100% Acetone	20 min[$]
100% Acetone	20 min

Critical point drying or freeze drying
Sample on stubs
Coating Au
Observation

Note:
*Fixation Buffers (choose one):
(1) **Gluteraldehyde buffer**
2.5% GA + 4% PFA/0.1M phosphate or cacodylate buffer
The buffer should be pH to 7.2, store at 4°C (good for one year)

(2) **Osmium tetroxide buffer**
1% OsO_4/0.1M phosphate or cacodylate buffer 4hr in room temp.
Make fresh - 1-2 days in advance to disolve
Use FUME HOOD! and use gloves all the time
This buffer can be used after buffer (1)

[$]Do not proceed to acetone step if you are not going to the drying procedure right away, as the materials will be fragile if left in acetone for too long.

GA = gluteraldehyde
PFA = paraformaldehyde
OsO_4 = osmium tetroxide