Preparation protocols for Scanning Electron Microscopy of *Oxyrrhis marina*

I. Fixation with OsO_4, followed by air drying

fix 18ml *Oxyrrhis marina* culture by adding 2ml OsO_4 (stock solution: 2% (w/v) in distilled water; final concentration: 0.2%), incubate for 1h

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allow the cells to sediment onto a polyester filter (3nm pore size)

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wash the sample with ~ 5ml tap water, incubate for 10min each (repeat 3x)

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dehydrate the sample through a graded ethanol series (with ~ 4ml of 30, 50, 70, 80, 90, 100, and 100% EtOH; incubate for 10min each)

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allow to air-dry the filter and store it in a desiccator overnight

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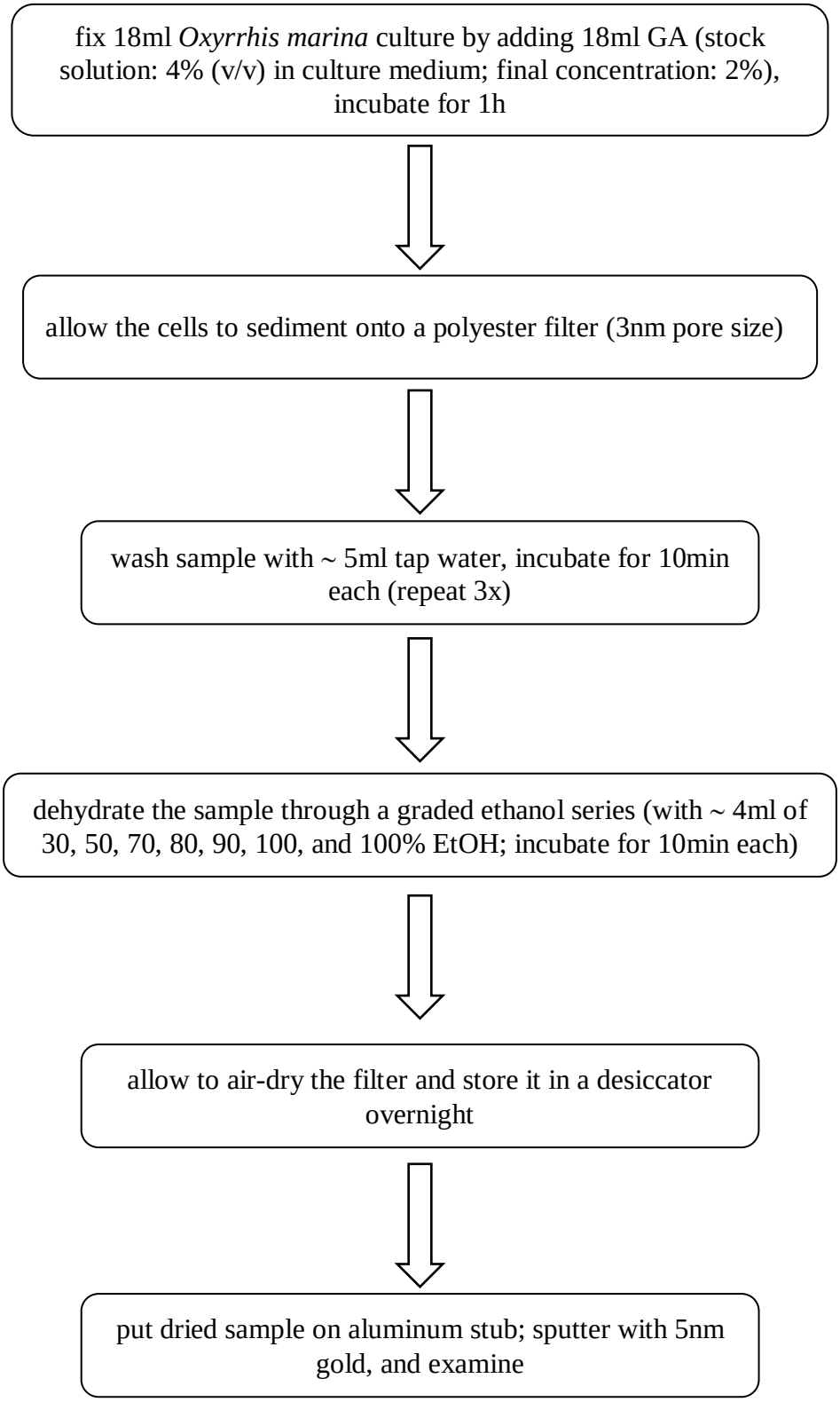
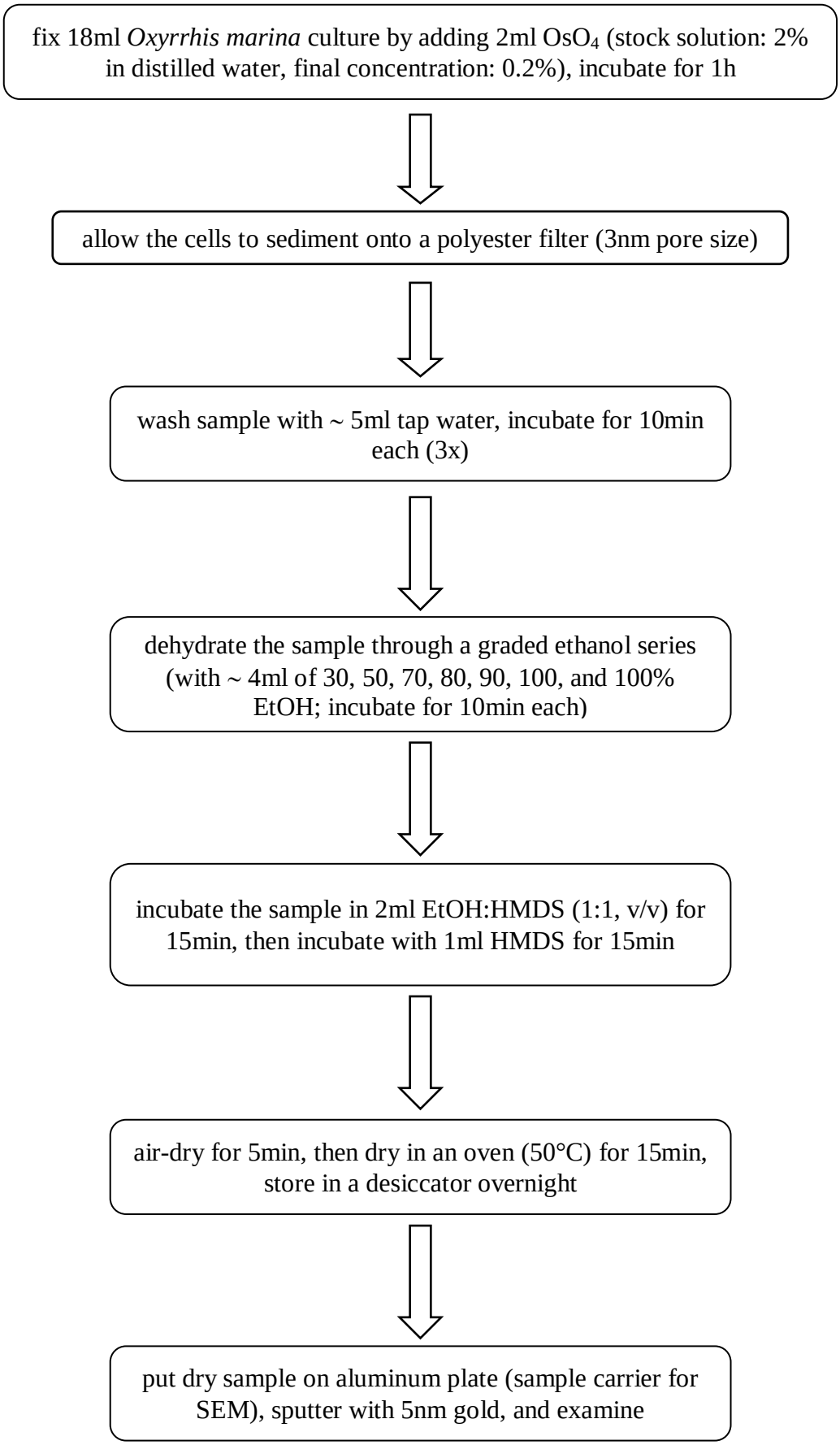
put dried filter on aluminum stub (sample carrier for SEM); sputter with 5nm gold, and examine

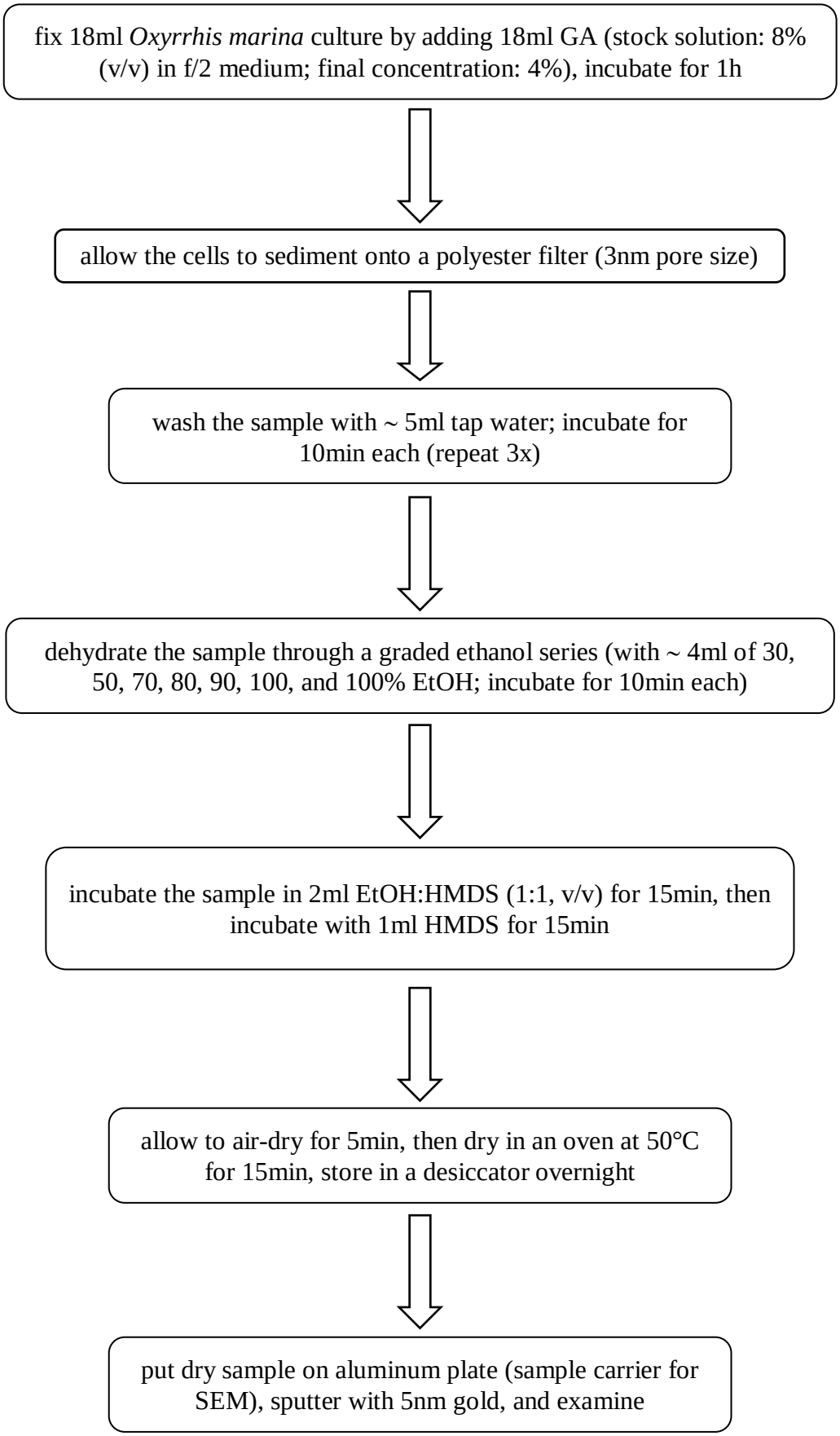
II. Fixation with GA, followed by air drying



III. Fixation with OsO_4, followed by drying with HMDS

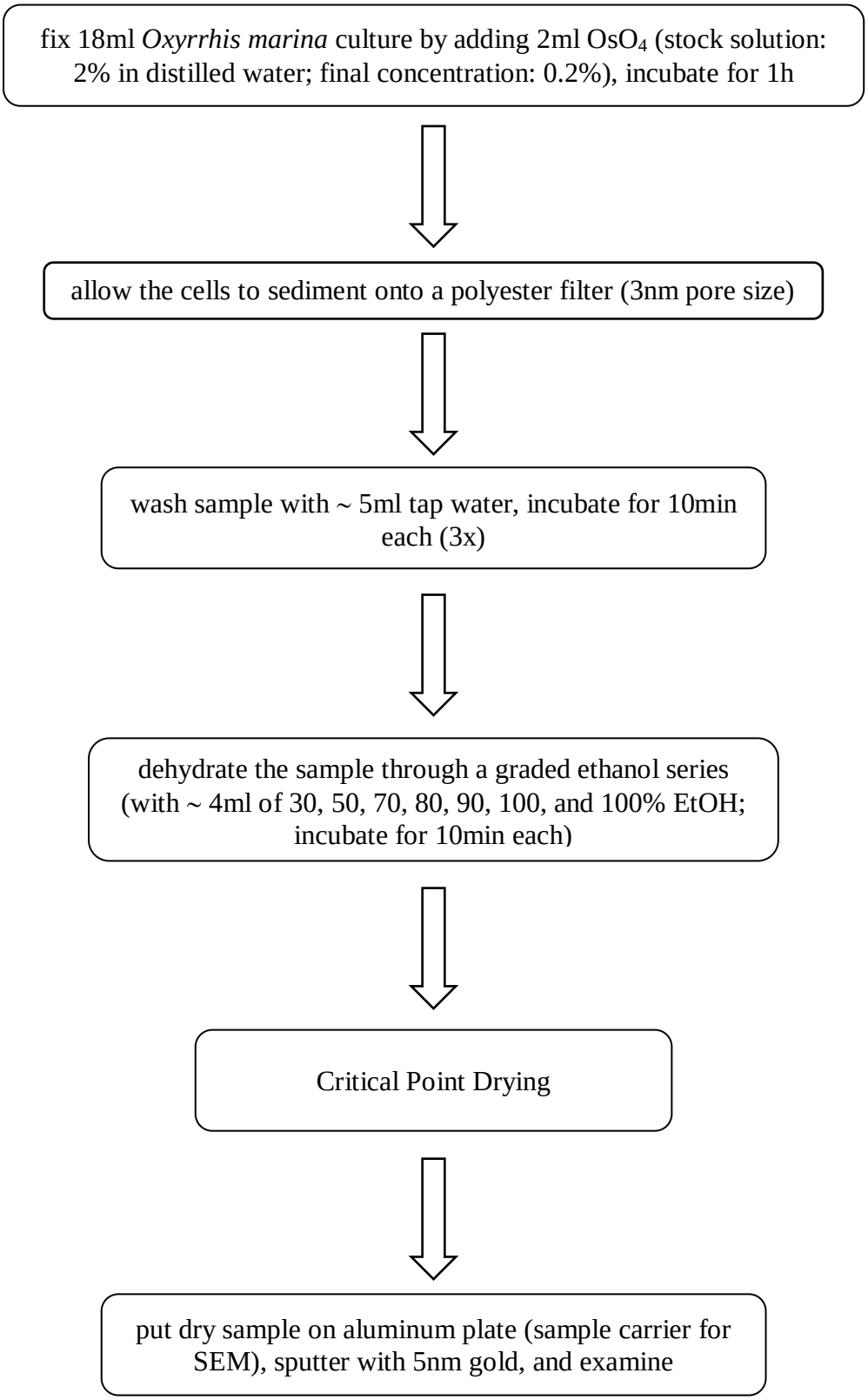


IV. Fixation with GA, followed by drying with HMDS

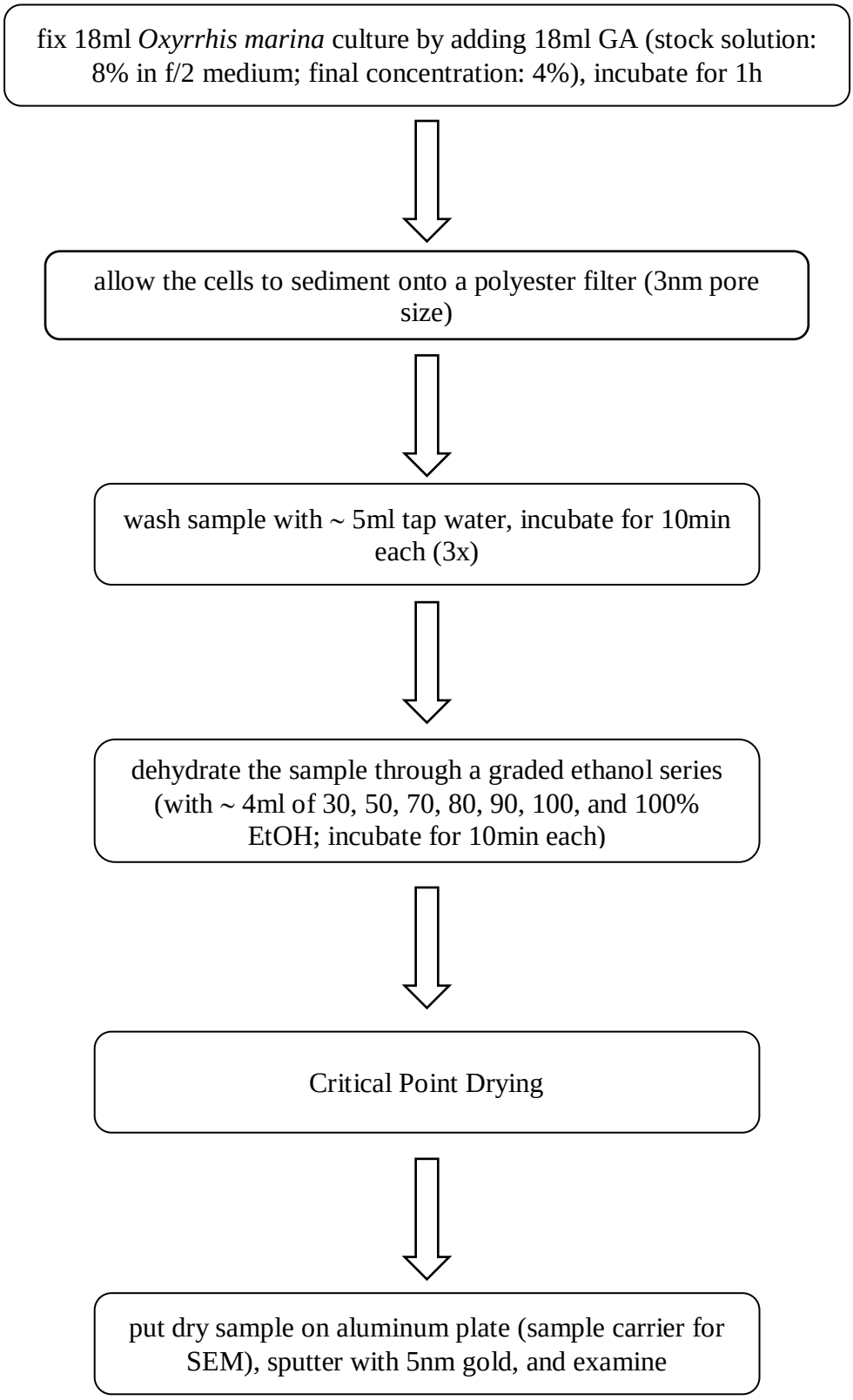


Note: The incubation of HMDS was also varied and shortened to 3min and 9min.

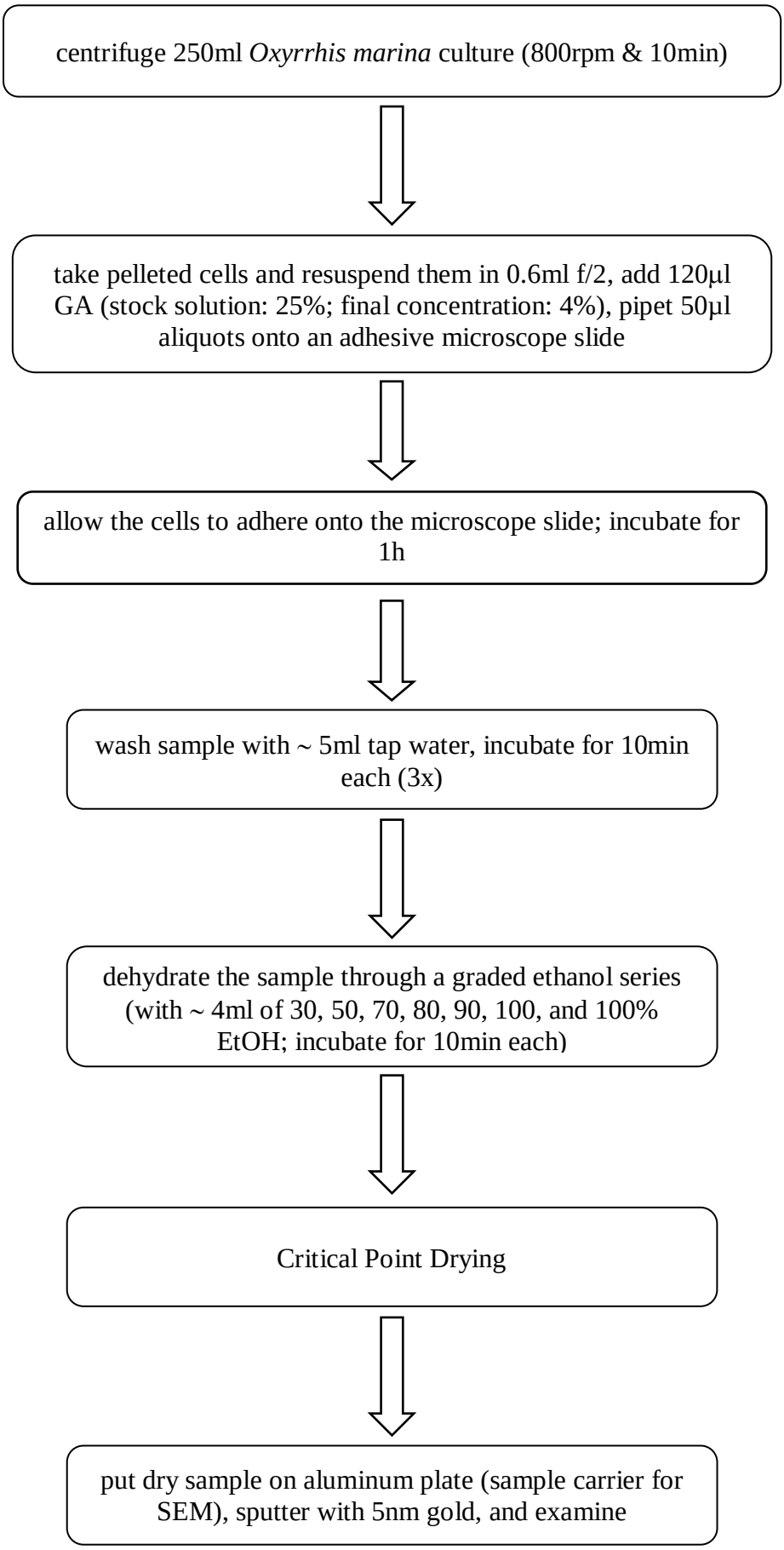
V. Fixation with OsO_4, followed by Critical Point Drying (CPD)



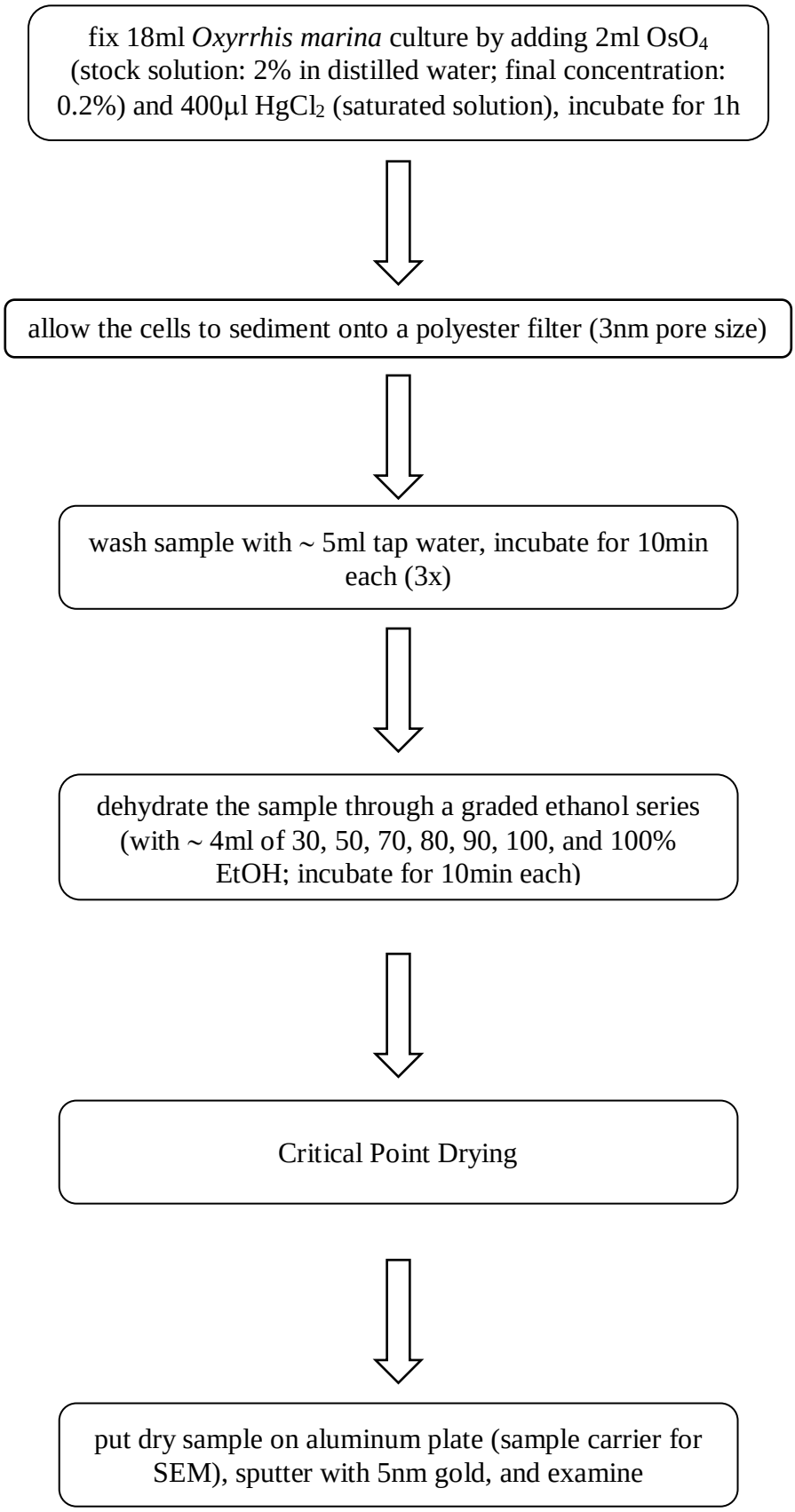
VI. Fixation with GA, followed by Critical Point Drying (CPD)



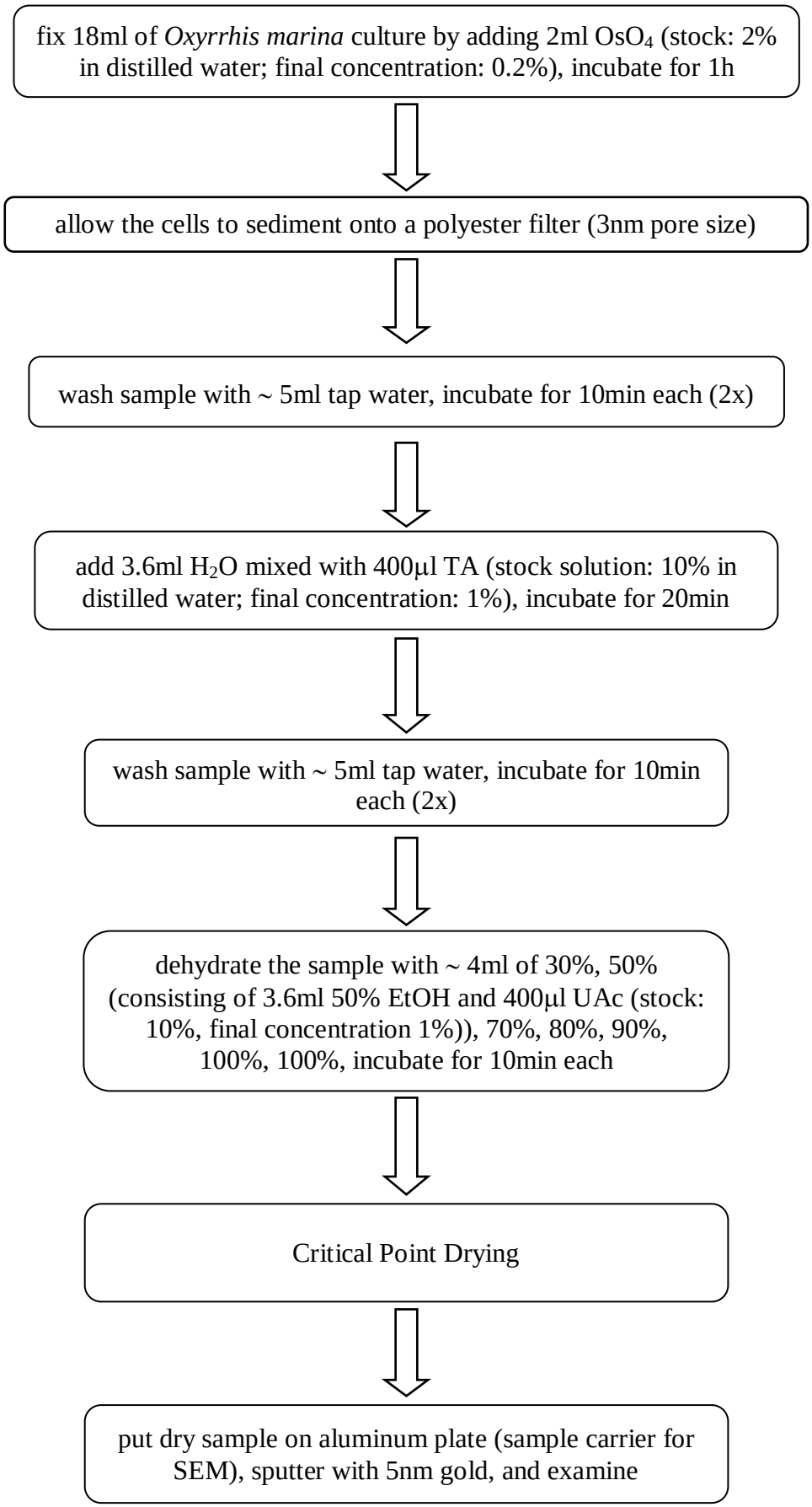
VII. Fixation with GA on adhesive microscope slides, followed by Critical Point Drying (CPD)



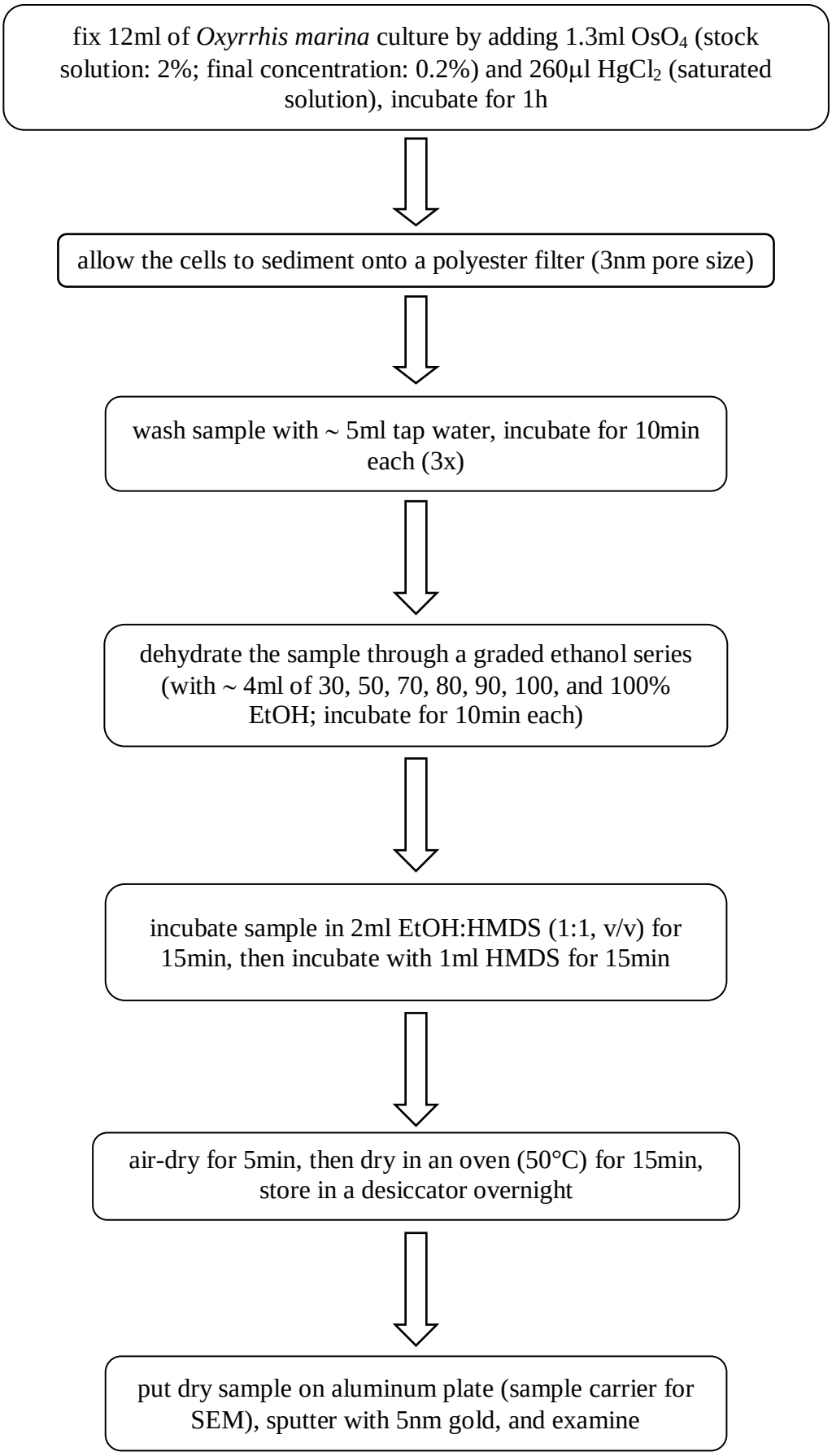
VIII. Fixation with OsO_4 and $HgCl_2$ (Mercury (II) chloride), followed by Critical Point Drying (CPD)



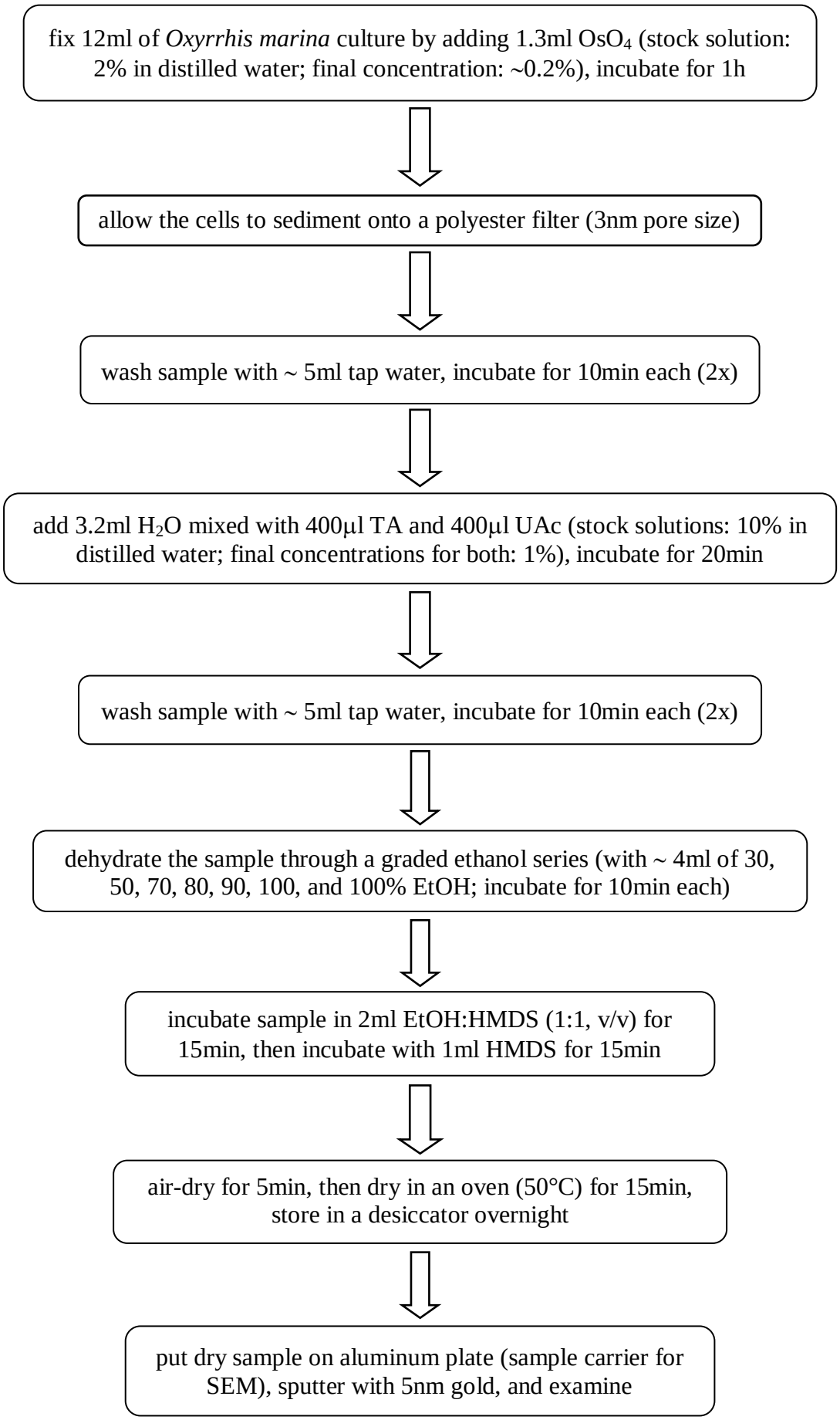
IX. Fixation with OsO_4, Tannic acid (TA), and Uranyl acetate (UAc), followed by Critical Point Drying (CPD)



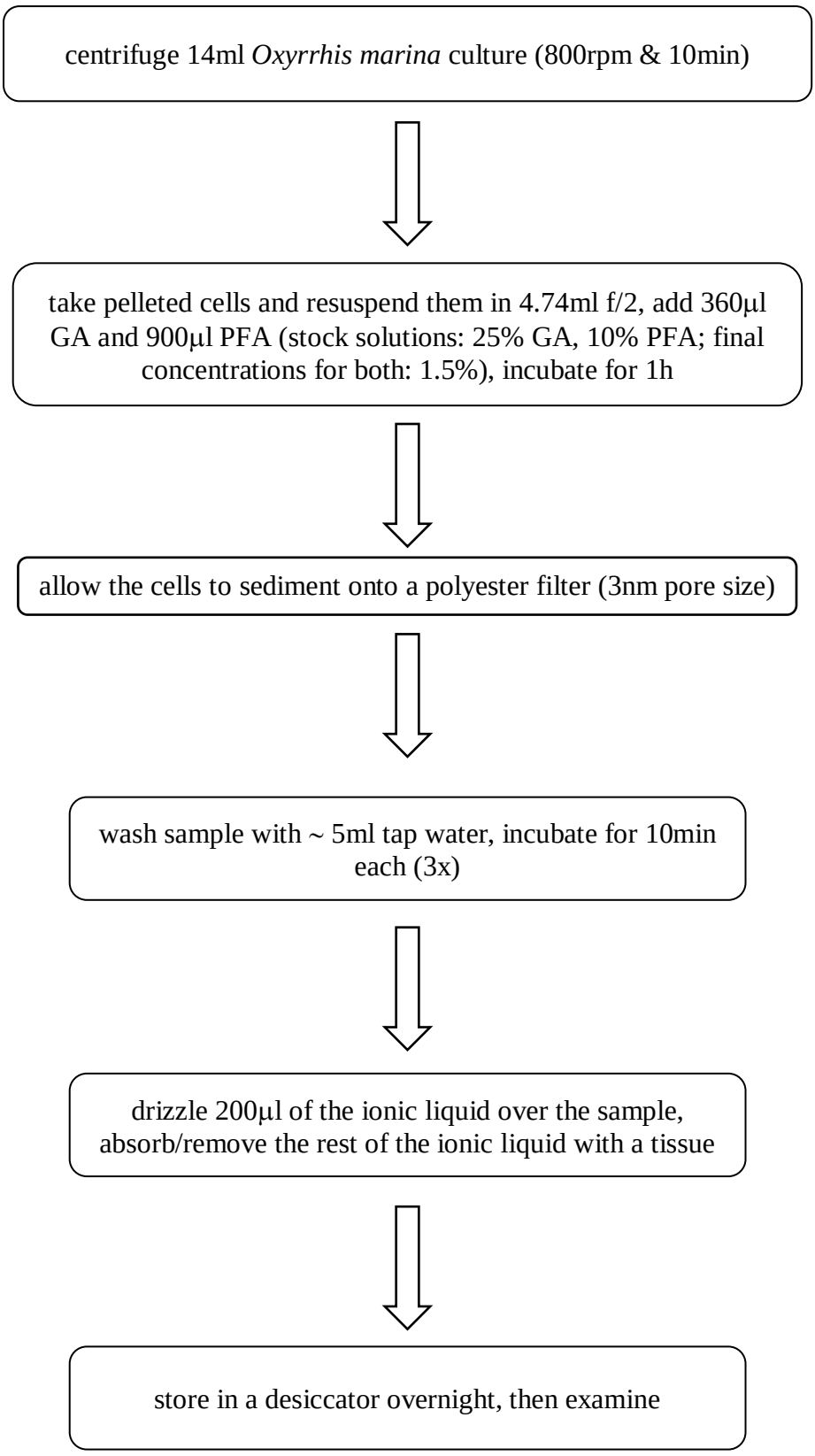
X. Fixation with OsO_4 and $HgCl_2$, followed by HMDS drying



XI. Fixation with OsO_4, TA, and UAc, followed by HMDS drying



XII. Fixation with GA and PFA (Paraformaldehyde), drying with the ionic liquid 1-Ethyl-3-methyl-imidazolium-bis-(trifluormethylsulfonyl)-imidat



XIII. Fixation with OsO_4, followed by cryo-fixation and freeze-drying