Pollen Preparation Procedure, short version
A summary the Pollen SOP, LacCore SOP series.
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Important Notes
Lab coats, chemical goggles and gloves must be worn at all times. Many of these chemicals can be very dangerous and this document is not intended to train you on safety. Additional safety information can be found in the Art of Pollen. It is assumed that you have read the Art of Pollen and centrifuge SOP before starting for details on set up, record keeping, safety, equipment use and tailoring this process for sample composition.

Equipment/Consumables
A list of necessary equipment can be found in the Art of Pollen SOP.

Procedure
1) Fill and start the hot water bath (90+ deg. C).
2) Ideally samples were submitted in screw cap 15 mL centrifuge tubes, add ~6 mL high purity deionized water (dH_20). If not, transfer a batch of 20 samples to 15 mL plastic centrifuge tubes using dH_2O, there should be a minimum of 6 mL dH_20 in each tube before continuing.
3) Add the required amount of spike. The spike should be on the stir plate for one hour before use and should remain stirring as it is added to the samples. For a typical lacustrine sediment sample 1cc in size, 0.5-1.0 mL of spike is required. Add a few drops of tert-butyl alcohol (TBA) and stir with a wooden stir stick. Centrifuge the samples at 2400 rcf for a minimum of 3 minutes then decant the supernatant off of the pellet.
4) Add ~6 mL of 10% potassium hydroxide to each centrifuge tube. Stir with a wooden stir stick and boil for 10-30 minutes, stirring halfway. Add dH_2O, add few drops of TBA, centrifuge and decant. Add ~6 mL of deionized water, add a few drops of TBA, stir, centrifuge and decant; repeat this rinse procedure until supernatant is transparent.
5) Sieve with 160 μm screens, retain <160 μm portion and sieve through a 7μm sieve. Retain the >7μm portion and transfer it back to a 15 mL centrifuge tube. Add a few drops of TBA, stir, centrifuge and decant.
6) Add ~6 mL of 10% hydrochloric acid (HCl) to each centrifuge tube. Stir with a wooden stir stick and boil for 10-20 minutes, stirring halfway. Add dH_2O, add a few drops of TBA, stir, centrifuge and decant. Add ~6 mL of deionized water, add a few drops of TBA, stir, centrifuge and decant; repeat rinse twice.
7) Add ~6 mL of hydrofluoric acid to each centrifuge tube. High health HAZARD. Additional personal protective gear required, see Art of Pollen for details. Stir with a wooden stir stick and boil for 20 minutes, stirring halfway. Add a few mL of 95% ethanol (EtOH), stir, centrifuge and decant.
8) Add ~6 mL of 10% HCl to each centrifuge tube. Stir with a wooden stir stick and boil for 3 minutes. Add dH_20, add a few drops of TBA, stir, centrifuge and decant. Add ~6 mL of deionized water, add a few drops of TBA, stir, centrifuge and decant; repeat rinse twice.
9) Add ~6 mL glacial acetic acid, stir, centrifuge and decant; repeat this step one more time.
10) Add ~6 mL 9:1 mix acetic anhydride : sulfuric acid, boil 2 minutes. Add 1 mL glacial acetic acid, stir, centrifuge and decant. Timing is very important; do not boil for more than 2 minutes.
11) Add ~6 mL glacial acetic acid, stir, centrifuge and decant.
12) Rinse 3 times with DI (don't forget TBA).
13) Add ~6 mL 95% EtOH, stir, centrifuge and decant.
14) Add ~6 mL 100% EtOH, stir, centrifuge and decant.
15) Add ~6 mL TBA, stir, centrifuge and decant.
16) Transfer samples to 1 dram vials with TBA, centrifuge and decant. Add enough silicone oil or glycerol to cover the sample, stir. Add vial labels. Allow TBA to evaporate for up to 24 hours; cap the vials.