Particulate Si Determination

At Sea:

1. Filter sample through a 0.6um polycarbonate membrane filter, record volume filtered.
2. Fold filter into quarters, place in a plastic petri dish and dry at 65^{o}C (any hotter will melt the dish). Once dry (in ~3 days) store at room temperature.
3. For every box of filters used, make 3 filter blanks – take a clean filter, fold in quarters, place in plastic petri dish, tape and label BLANK. These blanks will go through the same procedure as the samples.

In the Lab:

4. Rinse a set of PMP tubes with Nanopure 3x. Note on PMP usage log which tubes were used. Place dry filter in the bottom of a 15 ml conical polymethylpentene (PMP) tube keeping filter as open as possible. If necessary, use long forceps to open filter, exposing the surface for NaOH digestion. Rinse forceps well with Nanopure between samples.
5. Make 3 tube blanks – empty PMP tubes. Treat blanks as you do all other vials with filters.
6. Cover filter with 4ml of 0.2N NaOH. Cap and vortex (make sure cap has a small hole in top). Be sure filter remains submerged. Wrap each cap with a small square of aluminum foil to keep condensation drips out of sample and to keep caps from coming off.
7. Place in water bath at **95^{o}C** for :
   - RoMP samples = 1 hr
   - BATS samples = 2hr
   - JGOFS = 40 min
   - Plumes and Blooms = 30 min
   - LTER = 40 min
8. Cool in ice water bath immediately. Remove foil while cooling. This should take about 3-5 minutes, then samples should be cool enough for acid addition.
9. Add 1.0 ml of 1 N HCl and vortex after each addition. This neutralizes the NaOH and along with the cooling stops the digestion. Work quickly to minimize the time difference between the first and last sample.
10. With a clean spatula or long forceps, gently crunch filter into bottom of tube.
11. Centrifuge for 10 min at setting 6 to drive the lithogenic Si particles to the bottom of the tube.
12. Withdraw 3ml of the 5ml in the PMP tube and place in 30ml PP (polypropylene) bottle – be sure to take the sample from the top of the liquid, don't push the pipette tip down into the filter. Add 18ml of Nanopure to the PP bottle for a total of 21ml.
13. Withdraw the top 1ml of the remaining 2ml in the PMP tube and place it in a microcentrifuge tube for later determination of BSi / LSi crossover (the amount of LSi actually digested during the BSi digestion). The remaining 1ml of sample + scrunched filter in the PMP tube will be used for lithogenic determination.
14. From the PMP bottle containing 21ml of sample, transfer 10ml to another 30ml PP bottle for DSi analysis. Your dilution factor for this 10ml sample will be (5/3)*(21/10)=3.5. If you need to dilute the sample, take an aliquot less than 10ml and make up to 10ml with Nanopure. Reflect the change in your dilution factor: i.e. if you only use 5ml of 21ml then (5/3)*(21/5)=7.
15. To rinse the LSi sample, add 12ml Nano to the remaining 1ml of sample + filter in the PMP tube. Cap tube (cap without holes), vortex and centrifuge for 10 min at setting 6 on clinical centrifuge. If filter does NOT spin to the bottom of tube, push it down with clean poker and spin again for 10 minutes (remember we are trying to rid ourselves of any remaining dissolved silica from the BSi digestion not the particulate LSi). Aspirate to 1ml (BE SURE to rinse slurper tip before you use it!!)
16. Repeat rinse/aspirate step. Two rinses are necessary.

17. Uncap PMP tubes, cover with large clean Petri dishes (to allow drying while keeping dust out of tubes), and place in drying oven at 65°C until dry (takes approximately 48hours, after that the filters may burn). Once dry, these filters can be stored at room temperature until you have time to continue – cap the PMP tubes with caps without holes and cover entire rack with plastic wrap if you're going to let them sit around.

ONCE THE PMP TUBES FOR LSi ARE IN THE OVEN AND THE BSi SAMPLES ARE READY TO ENTER THE DSi PART OF THE ANALYSIS YOU CAN STOP FOR THE DAY. THE BSi SAMPLES SHOULD BE PROCESSED WITHIN 48 HOURS

18. When dry, remove filters from oven and let cool. Wear gloves when working with HF, and you may want to work in the hood but it's not necessary. Completely cover each filter with 0.2ml of 2.5M HF.
BE SURE FILTER IS DRY AND COOL, DO NOT ADD HF TO WARM FILTERS

19. Crunch the filter down into the bottom of the PMP tube with the HF poker (teflon or plastic stirring rod or spatula), remove all air bubbles and completely submerge the filter ball under the HF. Rinse the poker well with Nanopure between samples. The filter will have to be removed from the tube later so you'll want to try and flip it over or lift it slightly off the bottom to make that removal easier!!.
DO NOT USE GLASS OR METAL STIR RODS / FORCEPS / SPATULAS WITH HF

20. Cap tightly with caps without holes. Be sure to treat the tube and filter blanks with 0.2ml HF and the HF poker as well. Let samples and blanks sit covered with HF for **48 hours**.

21. A couple of hours before the 48hour waiting period is over, the saturated boric acid solution (~1M) needs to be filtered. The H_3BO_3 **MUST** be freshly filtered prior to use. A total volume of 14.8ml per sample is required, plus 110ml for the standard curve plus enough to make dilutions if necessary. The saturated boric acid should be filtered through a 0.6um 47mm PC membrane filter and collected in a clean bottle. Filter enough boric acid for all samples, standard curves and dilutions you might need – it doesn't hurt to filter too much.

22. Vortex the PMP tube to release the HF inside the crunched filter. Set the dispensette on the filtered boric acid to 7.4ml. Dispense 7.4ml of filtered boric acid into the PMP tube, vortex to resuspend filter and transfer the boric acid, HF and scrunched filter to a 30ml PP bottle. Dispense a second 7.4ml aliquot of boric acid into PMP tube, vortex to rinse the tube and transfer volume to same 30ml bottle. The total volume in the bottle is 15ml – 14.8ml of filtered boric acid and 0.2ml of 2.5M HF.

23. Withdraw 10ml of the 15ml in the PP bottle and transfer to a clean 30ml PP bottle for the DSi reaction. The dilution factor here is (15/10)=1.5.

24. Withdraw 1ml of the remaining 5ml in the PP bottle and place it in a microcentrifuge tube for later determination of BSi / LSi crossover (the amount of LSi actually digested during the BSi digestion).

25. The standard curve is prepared in a 0.2ml**:**14.8ml ratio of 2.5M HF**:**filtered saturated boric acid. Mix 3ml 2.5M HF and 222ml filtered boric acid in a beaker, transfer 10ml of this solution to each of the standard curve bottles. **DO NOT USE NANOPURE FOR THE LSi STANDARD CURVE**. Use this solution for dilutions as well. If you'll have many dilutions, make a larger volume.

26. Follow same protocol as for Dissolved Si Analysis - 4 ml of the Acid/Moly reagent, wait 10 minutes to form silicomolybdic acid, add 6 ml reducing reagent.

27. Tube blanks correct for signal generated by the effect of the NaOH & HF digestions on the tube. Filter blanks correct for signal generated by the effect of the digestions on the filter. When the signal from the tube blank gets too high (<90%T for 1cm cell, <80% for 10cm cell) the tubes need to be discarded – usually they can only be used for HF approximately 10 times. Since the filter blank incorporates the tube blank, the signal from the filter blank is subtracted from the signal on all samples.

28. Calculate the LSi concentration in the original seawater sample (LSi). Correct the value based on the number of nanopure rinses in steps 12/13. The calculation is as follows:

$$LSi_{umol/filter\ corrected} = LSi_{umol/filter} - ((1/5)*(1/13)*(1/13)*BSi_{umol/filter}) = LSi - (0.00118*BSi_{umol/filter})$$