Sample Fixation Protocol for Pico/Nanoplankton Flow Cytometry

Preparation of Paraformaldehyde Solution

Materials
Paraformaldehyde powder
1N NaOH
stirring/hot plate
Chemical fume hood
pH meter

Mix 900 mL DI water and 100 g paraformaldehyde powder. Set up on a stirring/hot plate under hood. Heat to approximately 60 ^{0}C. Do not boil! Stir for approx 1hr.
Turn off heat. Add 100 μL 1N NaOH to "clear" solution.
Cool to room temperature.
Add 100 mL phosphate buffer solution or filtered seawater.
Filter through GF/F filter to remove precipitate.
Test pH. Should be 7.4 - 8.0 (approx. equal to seawater). If necessary, add more NaOH.
This yields a 10% solution (approximately).

Sample Fixation Procedure

Materials
cryovials with O-rings (eg., Wheaton #985747 Cryule vials)
liquid N2
aluminum canes
GF/F filters and filtration apparatus
pipettors

1. Add 50 μL of 10% paraformaldehyde to a labeled cryovial.
2. Add 1 mL sample to cryovial, cap, and mix. This yields a 0.5% final conc.
3. Allow fixation for 1 – 8 h in refrigerator (4^{0}C).
4. Place vials in labeled aluminum canes and put directly into liquid N2.
5. Store samples in liquid N2 – can be transferred to –80°C freezer.

Notes:
1. If sample has large particles (detritus, zooplankton) pre-screen through 100 – 150 um mesh nytex.
2. Cryovials can be pre-loaded with paraform., then add sample, etc.
3. Method is derived from Vaulot et al. 1989 (Vaulot, D., Courties, C. & Partensky, F. 1989. A simple method to preserve oceanic phytoplankton for flow cytometric analyses. *Cytometry* 10:629-635).