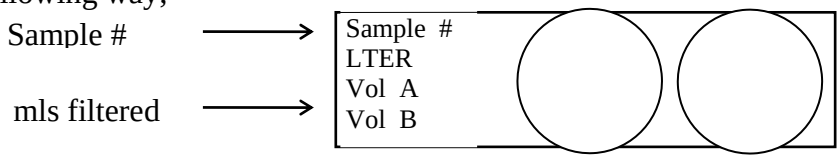
WATER COLUMN TIME SERIES PROTOCOL
DAPI Slide Preparation for Bacteria
(Collected 2005-2010 only)
Moorea LTER

Get Together
- Irgalan Black stained 0.2 um, 25 mm polycarbonate filters (buy them stained)
- *DAPI-solution*: . DAPI: Stock solution is 1mg DAPI in 100ml milliQ water (makes 10ug/ml soon.). Store DAPI in Freezer at all times.
- two filter flasks, bases, and towers
- 0.2 µm acrodiscs and a 3 to 5 ml syringe
- hand vacuum pump
- 0.8 µm backing filters
- slides
- immersion oil
- forceps
- hexamine buffered and 0.2 um filtered formalin
- squirt bottle with milliQ water
- 1 ml pipette and tips

Procedure:

1. Add filtered formalin to the samples to a concentration of 1%. Store no more than 24 hours.
2. Label slides in the following way;



3. Take samples out of the fridge and shake them well.
4. Use squirt bottle to wet filter base.
5. Mount 0.8um backing filter followed by stained 0.2um filter on both filter towers.
6. Pipette sample into the first tower-usually 2 ml.
7. Pump the hand pump and filter until about ½ ml of sample remains.
8. Add approximately 0.5ml DAPI solution to each sample using 3ml syringe attached to an Acrodisc filter. Be sure to store the DAPI-filled syringe in a drawer so it is kept in the dark except when used.
9. Cover the tower with a foil “mask” to reduce light and let sit for 3 minutes.
10. Add a replicate sample to the second tower and repeat the process.
11. While samples are staining prepare slides for mounting of filter by putting a drop of immersion oil on the slide and spreading it across the slide with a cover slip. (Slides can be cleaned with acetone to make oil spread more evenly)
12. At the end of 3 minutes draw down the DAPI.

13. As soon as DAPI goes through the filter, remove the filter from the tower while the pump and vacuum are still on. Mount both replicate filters on a labeled prepared slide, add a drop of immersion oil on top of each filter, and cover with a cover slip.
14. Store prepared filters in the dark in a drawer at room-temp until finished processing the samples.
15. Put the slides in a slide box in a zip-lock and store in the freezer
16. Return them to UCSB on ice and store in a freezer

Counting

1. Using an epiflourescence microscope with UV excitation and a magnification of 1000X, count 10 fields of view. Optimally there will be 30-60 cells per field.
2. Calculate cell concentration as follows:
   F is equivalent to the number of fields of view that fit into a filtration area.
   F = Area of filtration/ area of counting grid

   $$\text{Cells/ ml} = \frac{(\text{average cell count/ field of view}) * F}{(\text{mls filtered}) * (\text{fields counted})}$$