DAPI Staining of Bacteria
Updated March 2017 – Rachel Parsons

This experiment is to enumerate bacteria using a microscope. To do this you need to get the bacteria onto a flat surface. A filter provides this surface. We use a 0.2µM filter that allows the water and viruses to pass through the membrane but retains the bacteria and larger plankton onto the filter so they can be counted.

Equipment

Filtration rig – usually 4-5 place
Filter bases
Filter Towers
Vacuum Flask
Pump
Backing Filters – Supor 0.8µM 25mm
Filters – PC 0.2µM 25mm
Pipet – 5-10ml
Pipet Tips 5-10ml
Pipet 1ml
Pipet tips 1ml
Forcepts
Slides
Cover slips
High Viscosity Oil
Ultra fine sharpie
50ml falcon tubes
0.2µM filtered formalin
Glass waste container
Fixative Waste container – Haz Mat labeled.
Epiflurescent Microscope with UV light filter cubes.

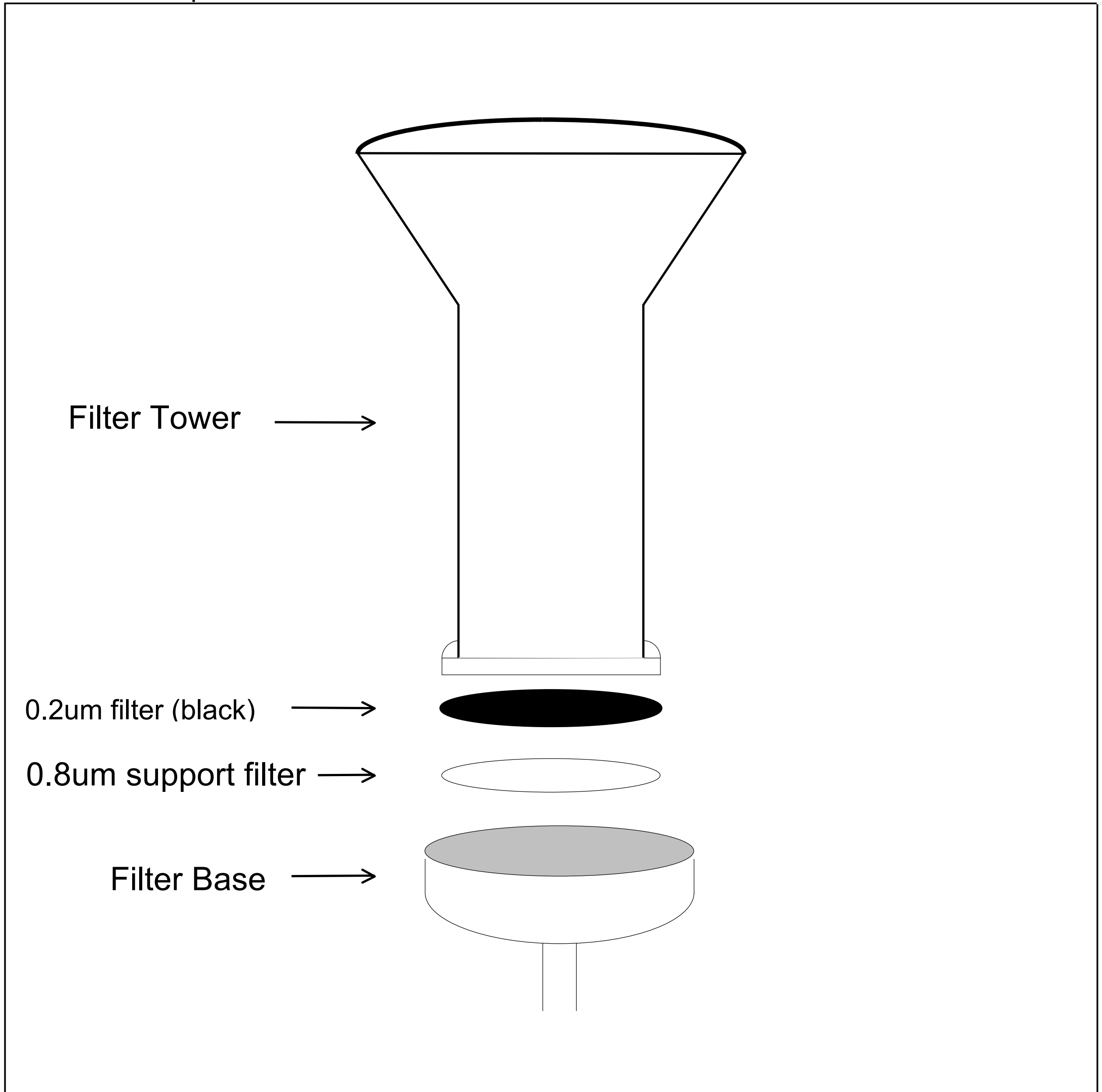
Reagents

DAPI 5µg/ml
Stock is 1ml of 200µg/ml. Dilute into 40mls 0.2µM filtered QW using 50ml falcon tube.

Irgalan Black for staining the fliters.
200mg of Irgalan Black dye into 2% Acetic Acid (2mls Acetic Acid into 98mls of 0.2µM filtered QW)

Q Water to rinse the filters – 0.2µM filtered

Filtration set-up:



Start of filtration:

- Label slides in the following way;

Sample ID (Cruise)	→	B183
Depth(m)		0m 10m
		A B
mls filtered/tower diameter	→	10mls 10mls

- Take samples out of the fridge and shake them well. Allow them to come to room temperature. The samples have been killed with formalin (2%) to stop bacterial replication.

- Mount 0.8um backing filter (GN4 Metricel) followed by blackened 0.2um PC filter on all 4 filter towers. Leave all valves open.

- Pipet 10 mls (BATS surface) or 3-5mL (Bermuda Inshore) of sample into the filter tower using the DEAD pipets in the fume hood.

- Start pump (vacuum on 5-8 with all valves closed).

- Let filter until all sample on all towers has gone through.

- While samples are filtering prepare slides for mounting by putting a drop of immersion oil on the slide and spreading it across the slide with a cover slip.

- Stop filtration by stopping the pump using the foot control.

- Add approximately 0.5-1ml DAPI solution to each sample using the DEAD pipet found in the fume hood. DAPI is a stain that binds to DNA and fluoresces under UV light so the cells can be seen under the microscope.

- Let stain for 3 minutes (use the timer provided).

- Turn on pump to draw down DAPI.

- As soon as DAPI goes through, take the filter off the tower while the pump and vacuum are still on. Mount two filters per slide, add a drop of immersion oil on the filter, and cover with a cover slip.

- Store prepared filters in slide-box at room-temp. until finished preparing the samples.

- Put slide box in DEAD fridge until you examine them under the microscope.