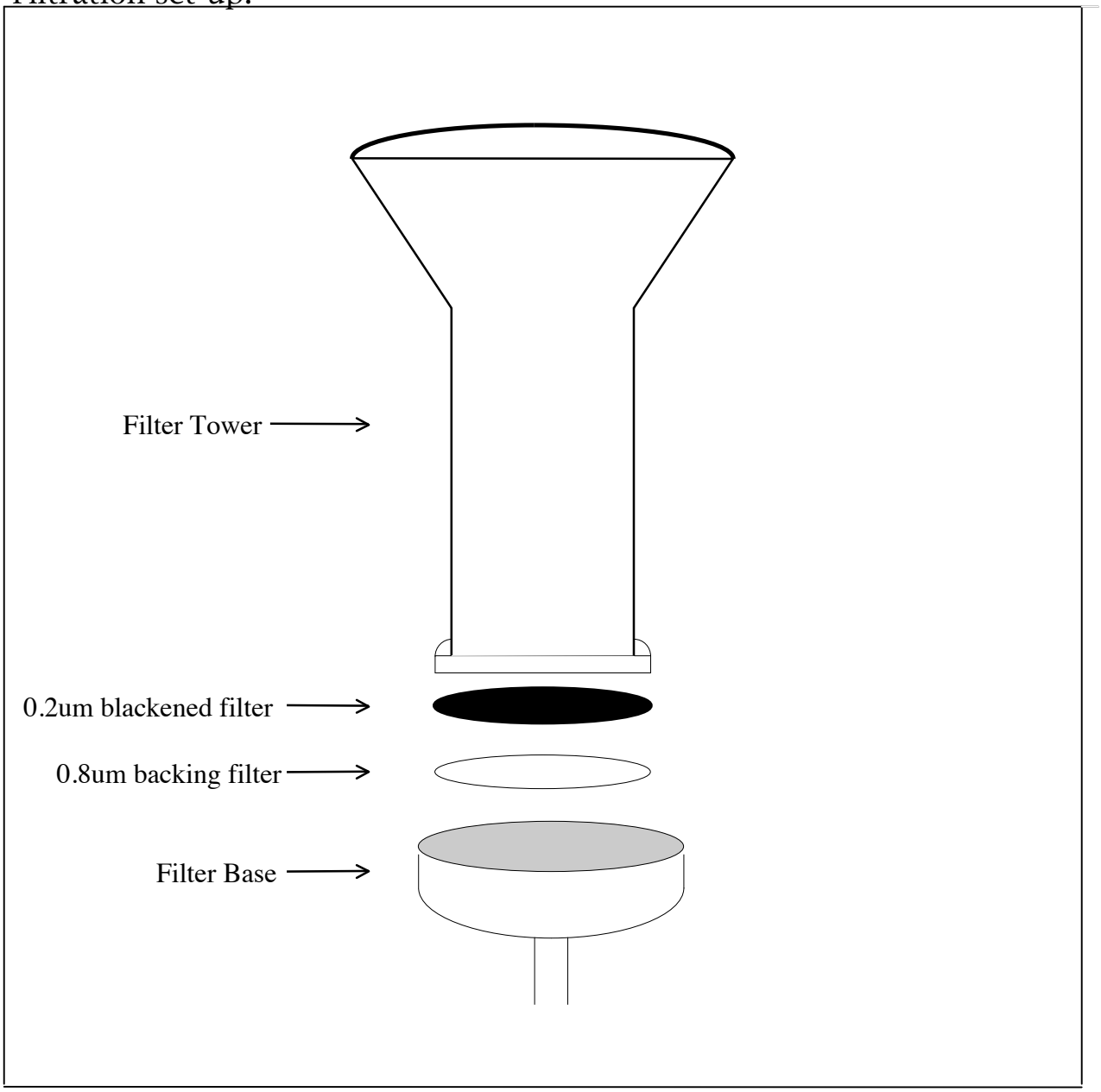
DAPI Staining of Bacteria --Carlson Lab --UCSB

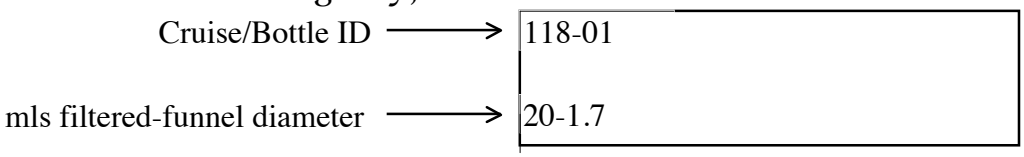
- **Irgalan Black staining of 25mm/0.2um PC filters:** Let filters stain for minimum 15 minutes in a petri dish. Filters cannot over stain.

Filtration set-up:



Start of filtration:

- Label slides in the following way;



- Take samples out of the fridge and shake them well.
- Use squirt bottle to wet filter base.

- Mount 0.8um backing filter followed by blackened 0.2um filter on all filter towers.

- Pipet in the volume of sample.

- Start pump (do not set above 150mmHg)
- Let filter until most of sample on all towers has gone through, leaving about half a mL.
- Stop the pump.
- Add 0.5ml DAPI solution to each sample and cover with foil or turn off lights.
- Let stain for 3 minutes.

- 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.

- After 3 minutes, 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 each filter on a separately labeled prepared 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 a zip-lock with dessicant pack and store in freezer.

Reagent and Preparation Protocols for DAPI Staining

- DAPI: Stock solution is [200ug/mL]. Working solution is [5ug/ml]. Store DAPI in freezer at all times and protect from light.

- Irgalan Black: 200mg Irgalan Black, 2ml Acetic Acid (conc.), 98ml 0.2um filtered 2% formaldehyde. Store in fridge.