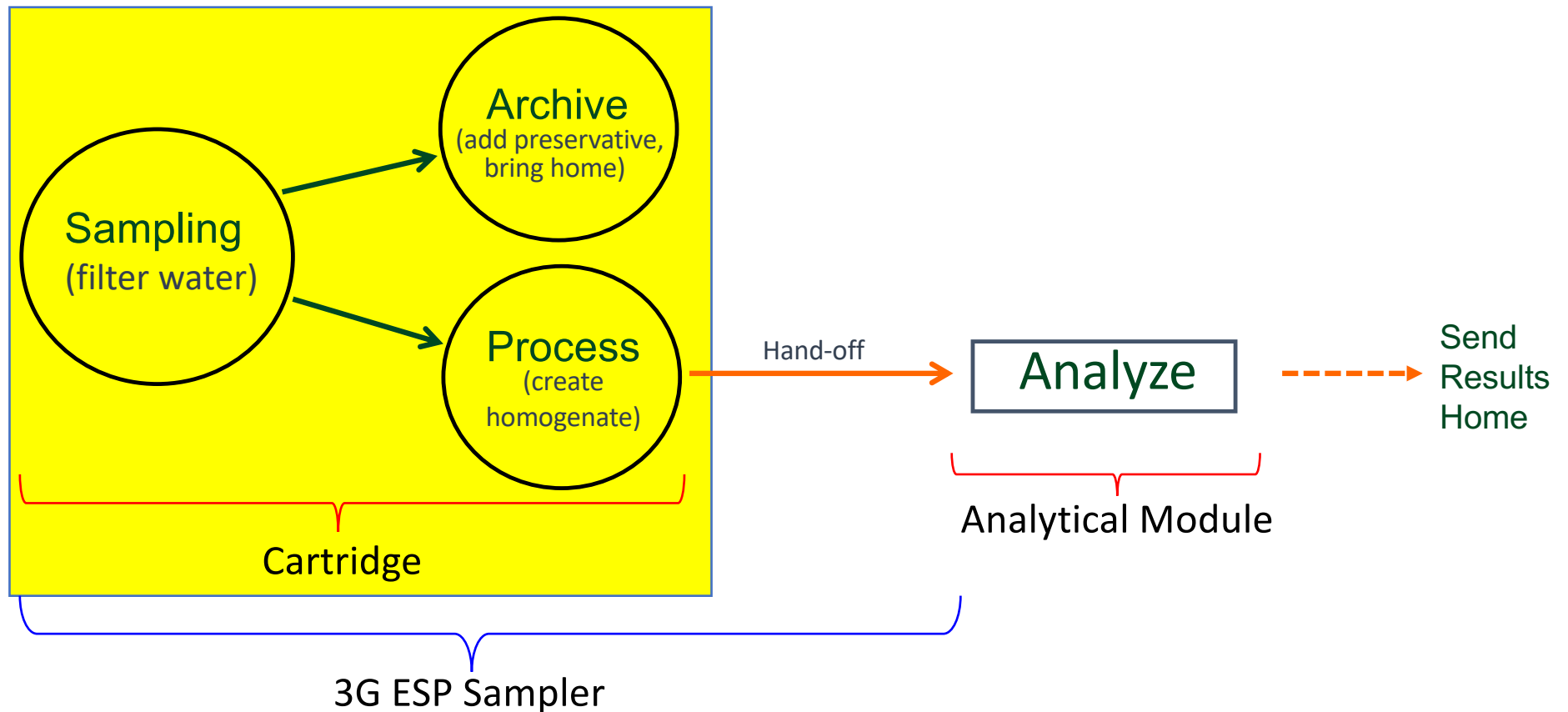


3G ESP

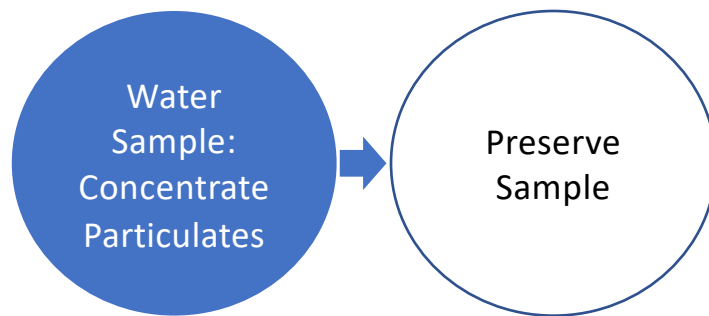
General Overview of Cartridges Archive and Lyse-n-Go

MBARI, Monterey Bay Aquarium Research Institute

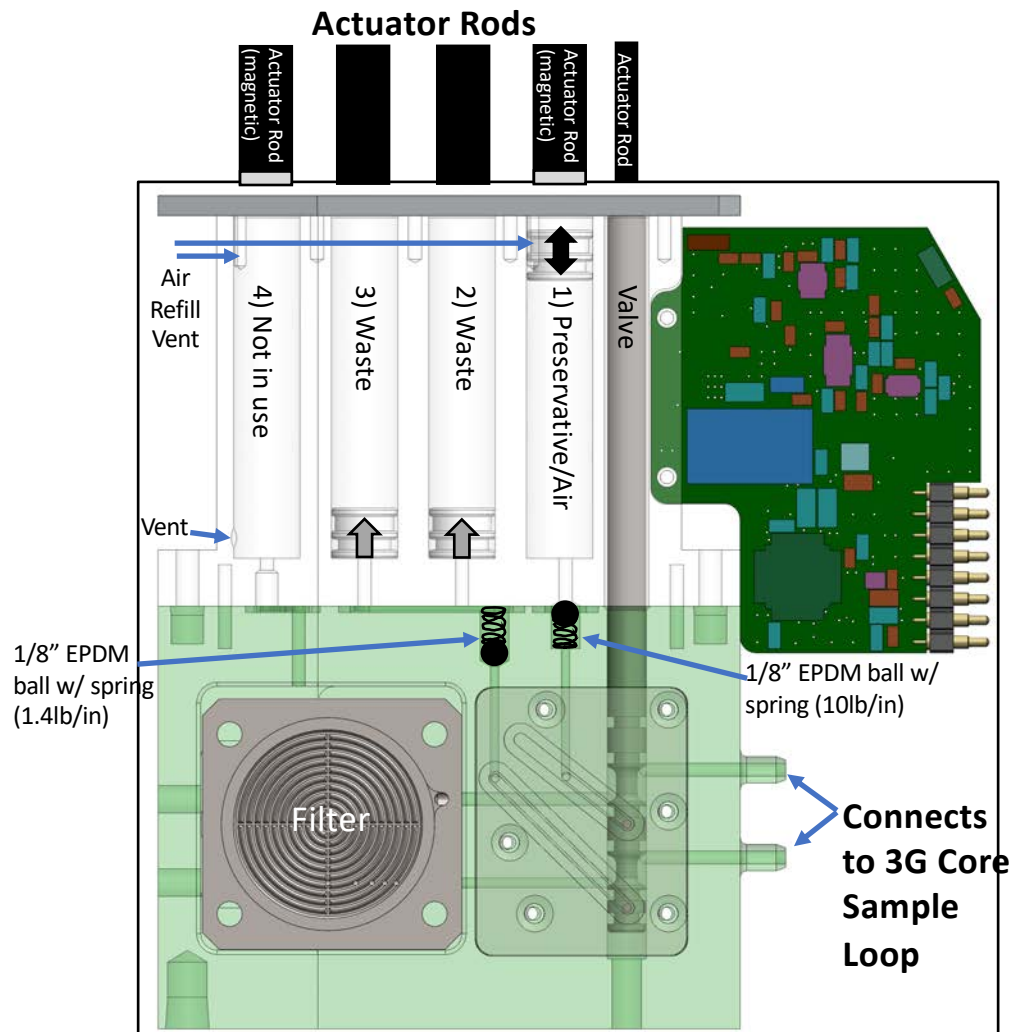
3G ESP Overview



Archive Process



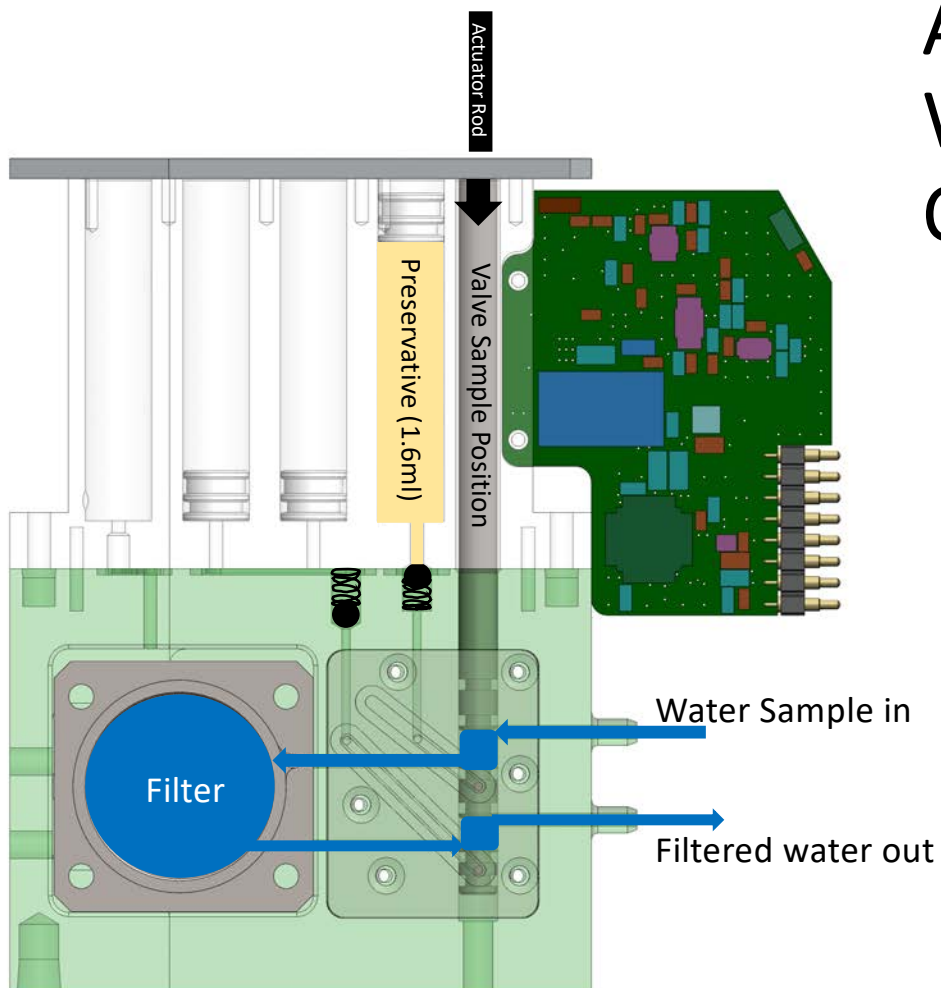
- Requirements
 - Pressure range 0-500 psi
 - Temperature 3C – 30C
 - Multiple 25 mm filter porosities/types
 - 30 μm – 0.1 μm
 - Chemicals: seawater, freshwater, 10% bleach, 10% HCl, silicone oil
 - Deliver fixative across/through whole filter (No bubbles)



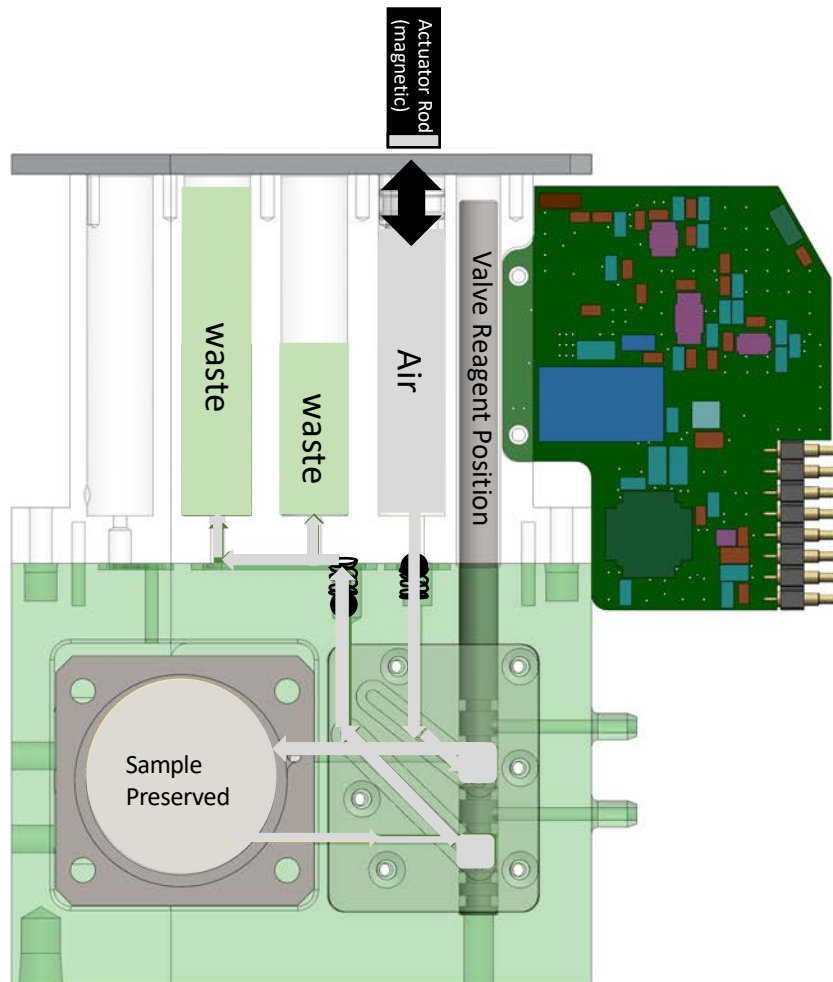
Archive Cartridge -- Features

- Connection to 3G core Sample Loop
- 5 Actuator Rods Actuate
 - 4 Syringes
 - 1 Valve (sample or reagent)
- 4 Syringes
 - Reagent/Air or waste

Archive – Water Filtration Concentrate Particulates



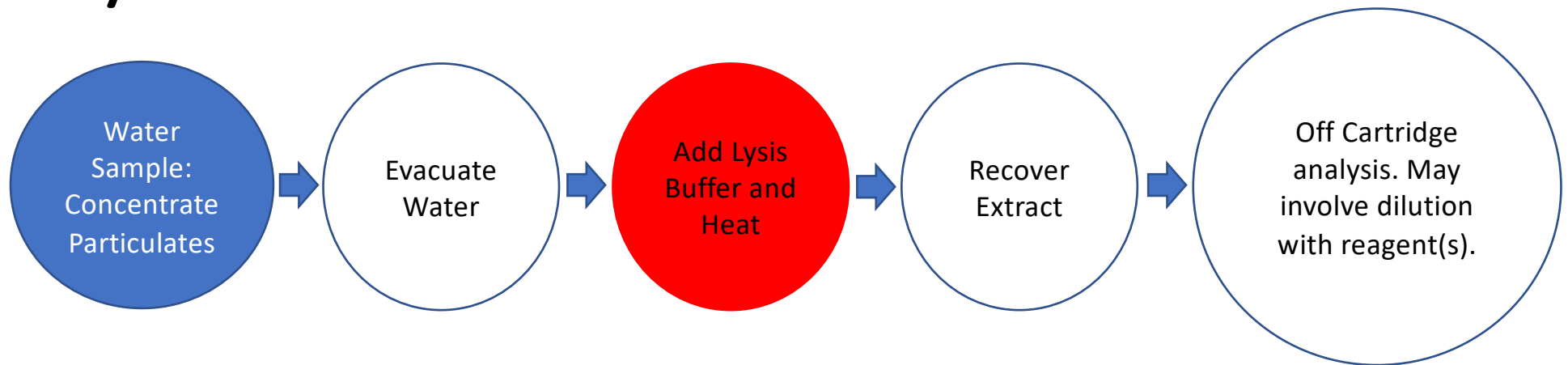
- Sampling (surface to 300m)
 - Test psi range 0-500 psi
 - Multiple 25 mm filter porosities/types
 - Sample path under vacuum in 3G/LRAUV.
 - Sample enters top of filter first before full fluid path through cartridge is opened.
- When sampling is complete move valve to reagent position.



Archive – Preserve Sample

- Preservative pushed in and displaces the water to waste. Preservative reaches >95% concentration.
- Incubate
- Refill Syringe 1 with air and displace preservative

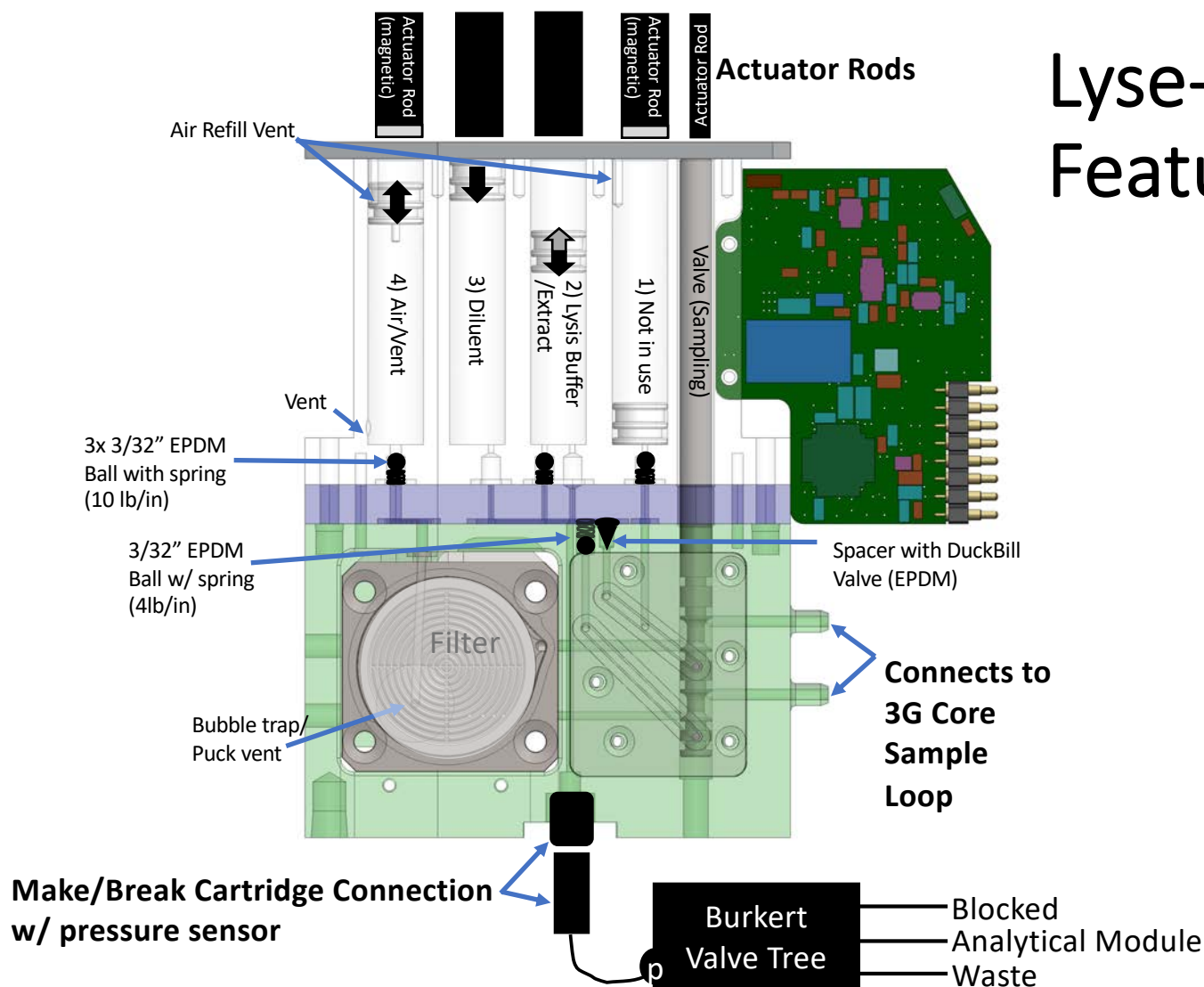
Lyse-n-Go Process



- Requirements

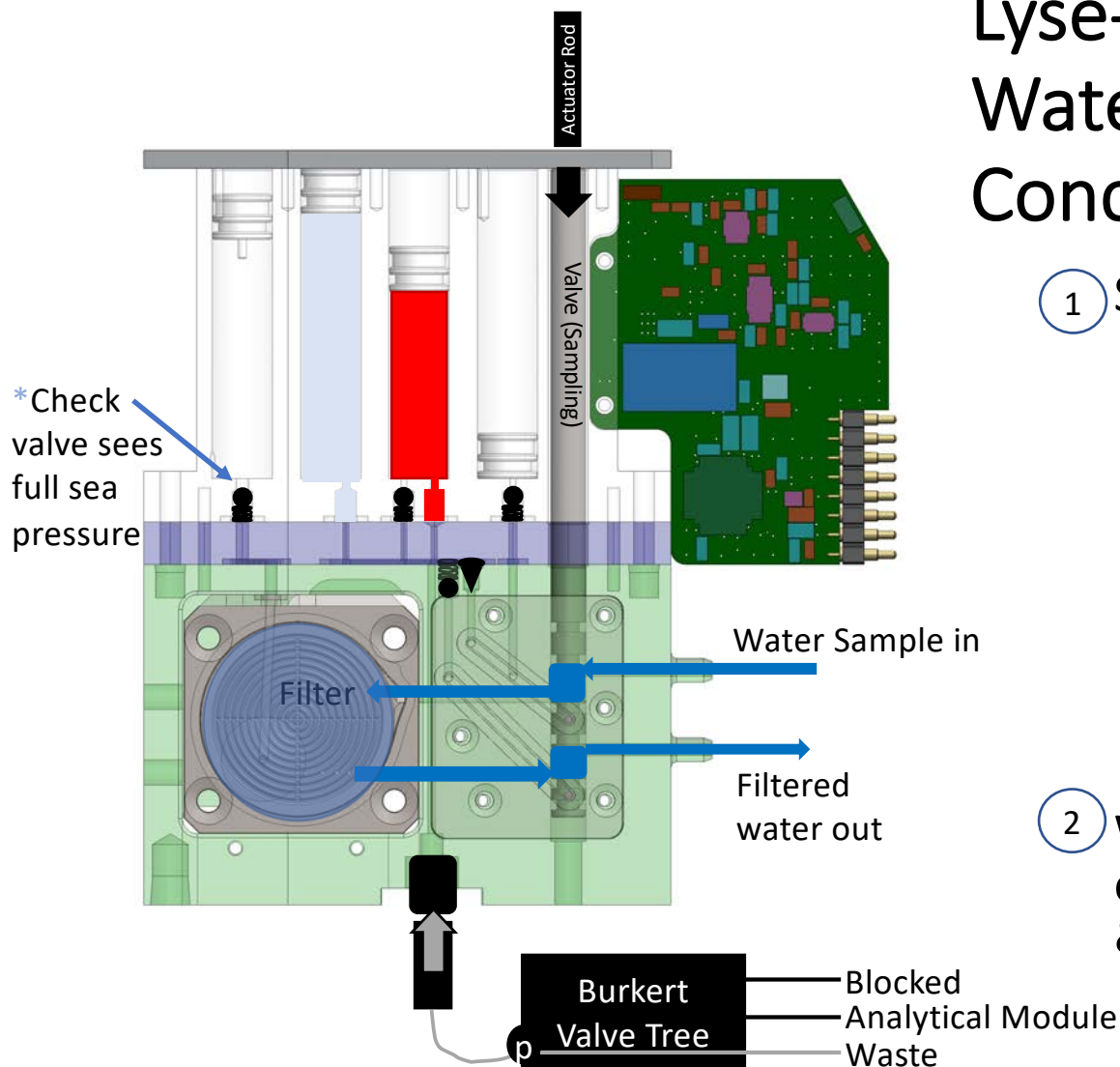
- Pressure range 0-500 psi
- Temperature 3C – 30C
- Multiple 25 mm filter porosities/types
 - 30 μm – 0.1 μm
- Chemicals: seawater, freshwater, 10% bleach, 10% HCl, silicone oil
- Deliver lysis buffer and recover cell lysate.
- Perform dilution
- Deliver a bubble free extract off cartridge for analysis.

Lyse-n-Go Cartridge -- Features

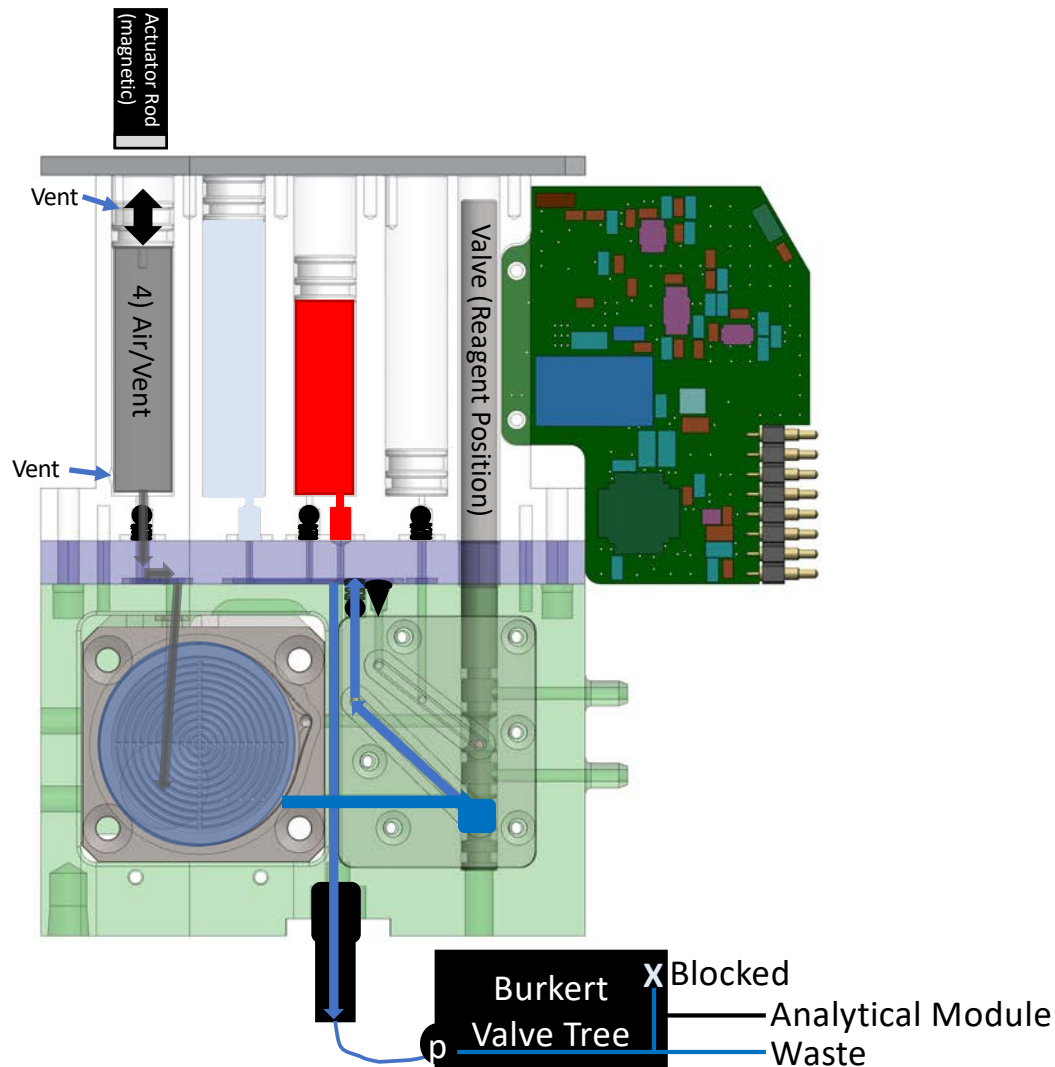


- Connection to Sample Loop
- 5 Actuator Rods
- 4 Syringes
- 1 Valve (sample or reagent)
- Make/Break cartridge connection to downstream components (waste, analytical module)

Lyse-n-Go Cartridge – Water Filtration Concentrate Particulates

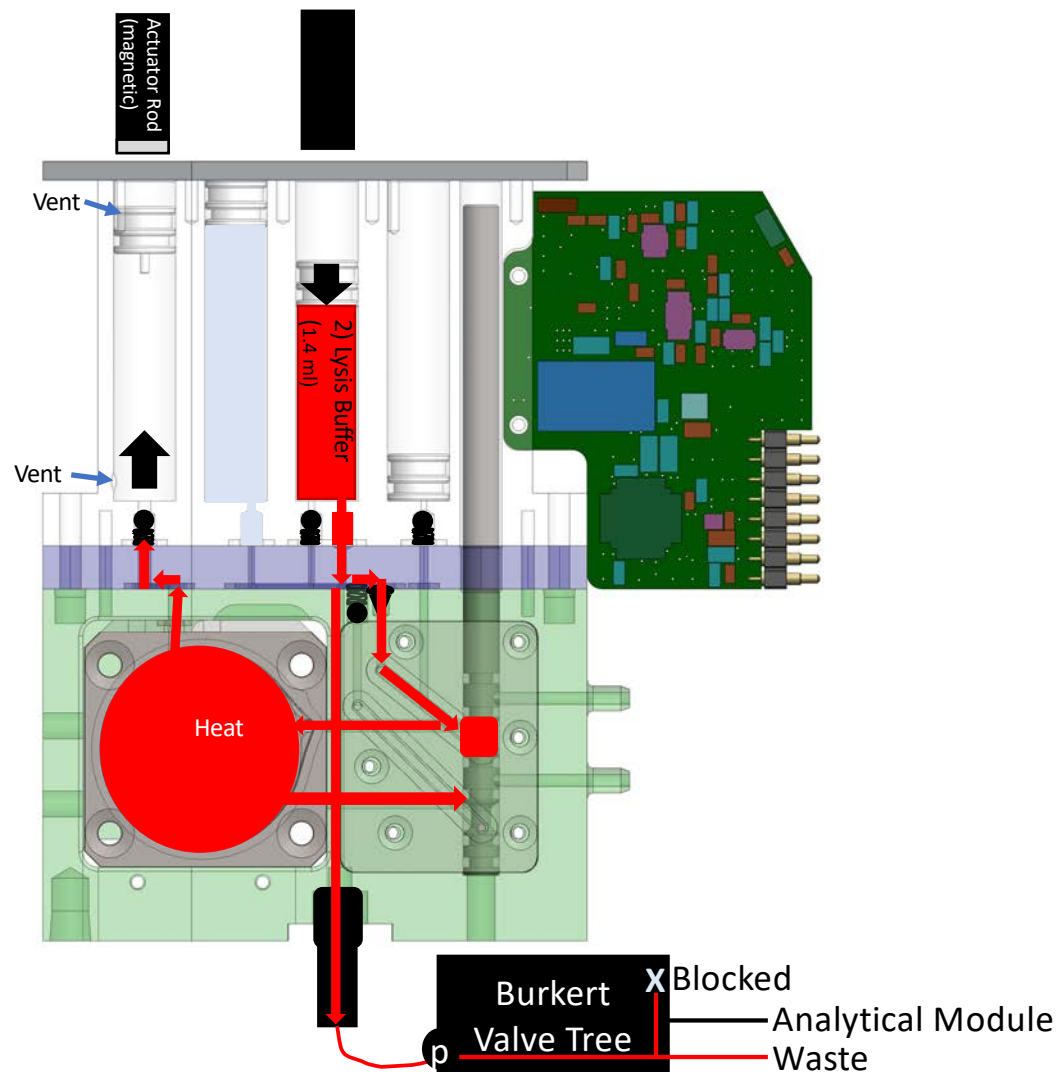


- 1 Sampling (surface to 300m)
 - Test psi range 0-500 psi
 - Multiple 25 mm filter porosities/types
 - Sample path under vacuum in 3G/LRAUV.
 - Sample enters top of filter first before full fluid path through cartridge is opened.
- 2 When sampling is complete engage handoff (open to waste) & move valve to reagent position.



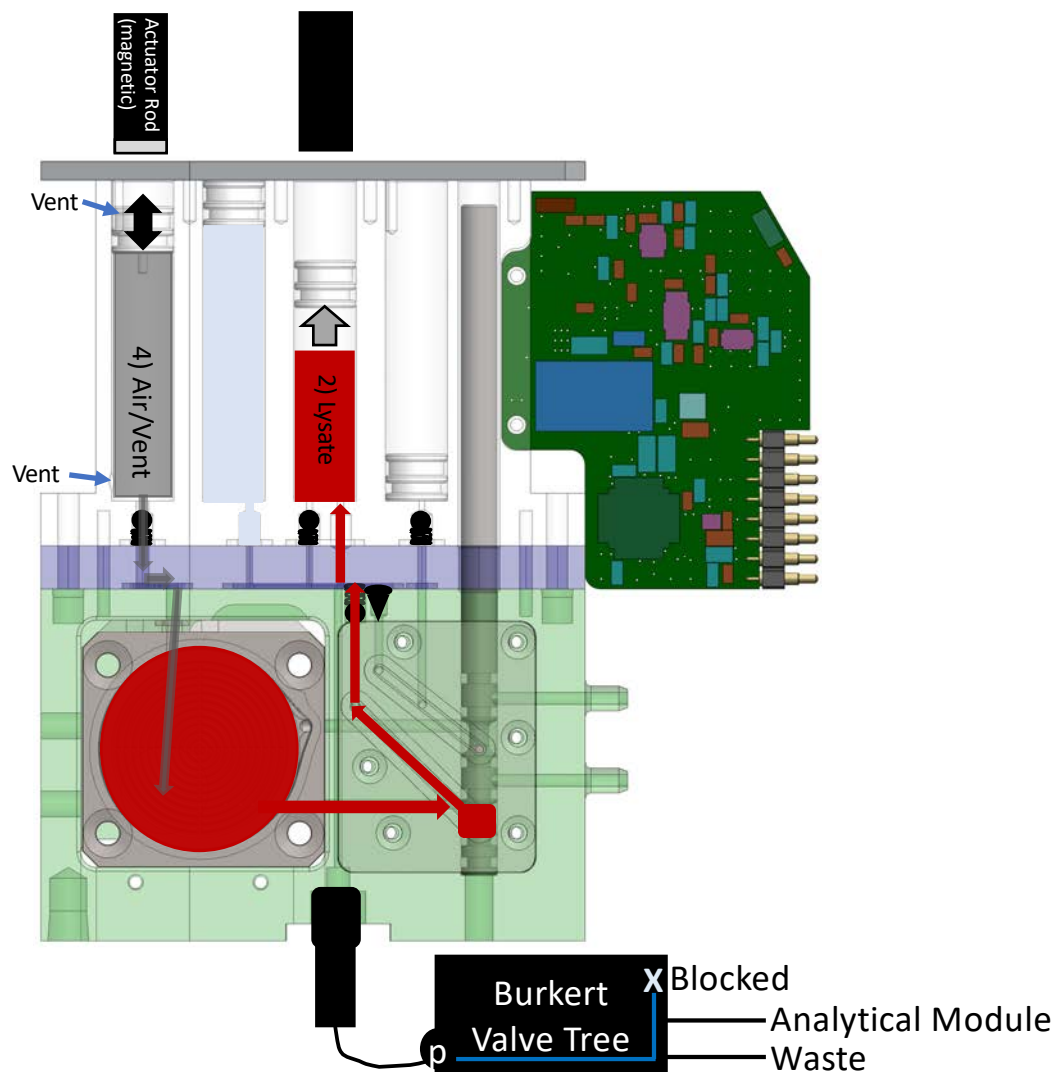
Lyse-n-Go Cartridge – Evacuate Water

- ③ Refillable air/vent syringe (4) used to push water to waste
 - All other plungers in syringes backed up with actuator rods
- ④ To remove remainder of water, must break bubble point of the filter.
 - Burkert tree at blocked
 - Repeated cycles with air/vent syringe (4) until reach specified psi
- ⑤ Drain Water to waste
- ⑥ Vent top of puck
 - Plunger below bottom vent & boss of plunger displaces check valve



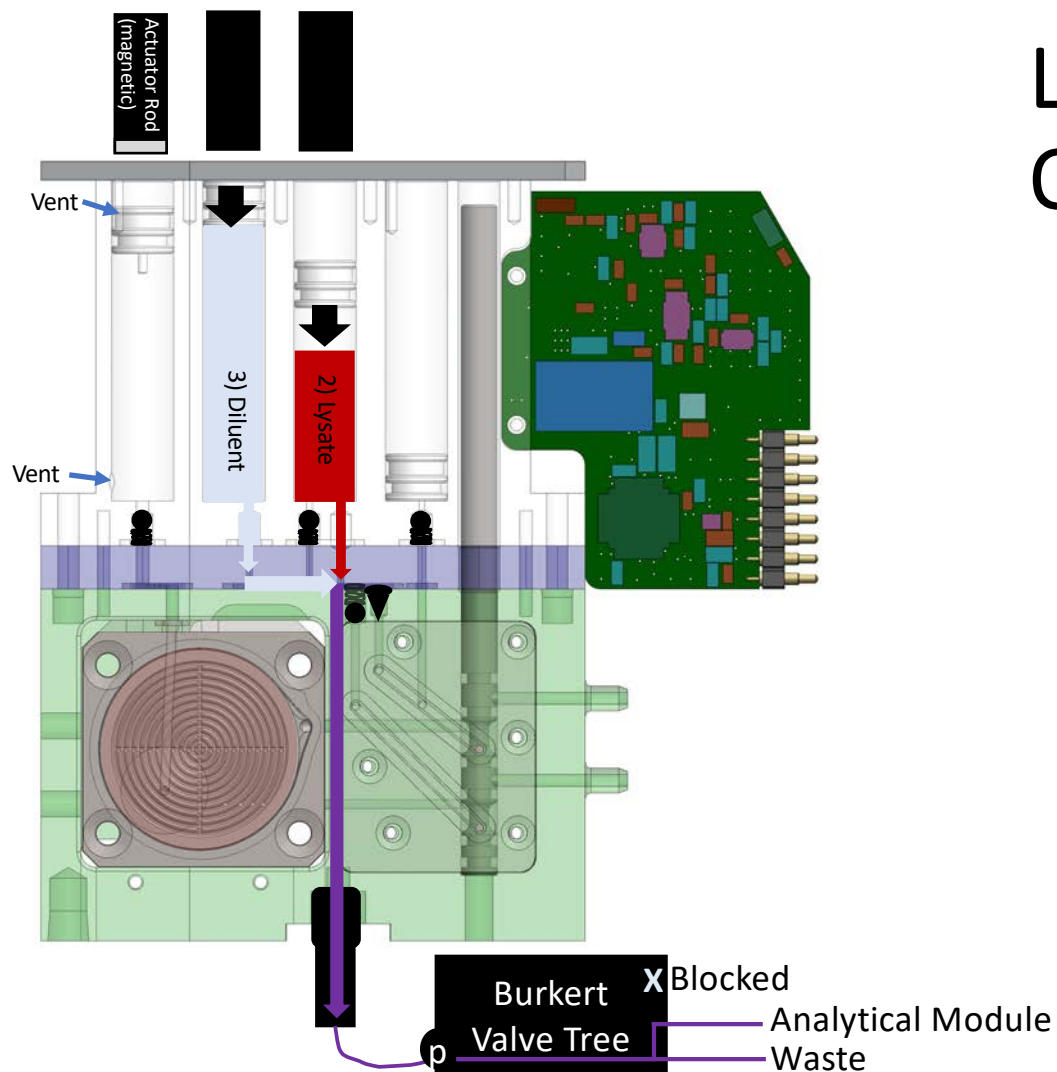
Lyse-n-Go Cartridge – Add Lysis Buffer & Heat

- ⑦ Fill off cartridge line with lysis buffer (300 μ l)
 - Puck top vented, open to waste
- ⑧ Fill majority of filter chamber with lysis buffer (syringe 2)
 - Puck top Vented (syringe 4)
 - Burkert tree at blocked
- ⑨ Pressurize filter chamber & Heat
 - Burkert tree at blocked
 - Puck top sealed. Plunger moved above vent with pressure balanced move with Syringe 4 & Syringe 2.
 - Drive syringe 2 to empty.
 - No bubble over filter
 - Heat to 85°C, 14 minutes



Lyse-n-Go Cartridge – Recover Extract

- 10 Recover 900 μ l of Extract
 - Pressure filter chamber with Air/Vent Syringe
 - Draw back on actuator rod (2) when pressure exceeds threshold. Plunger moves due to pressure.
- 11 Relieve pressure in system
 - Open to Waste
 - Vent Puck Top



Lyse-n-Go Cartridge – Off Cartridge Analysis

- 12 Syringes 2 & 3 push at different rates to “mix” reagents at appropriate dilution
- Initial mixture pushed to waste before pushing to analytical module