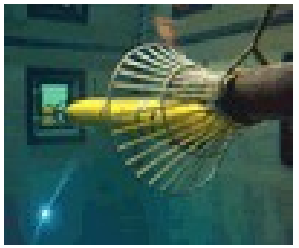
Monterey Bay Aquarium Research Institute

- Non-Profit Research Center Founded in 1987 by Packard Foundation
- Furthering marine research through the peer efforts of scientists and engineers
- 220 Employees (1/3 Science, 1/3 Engineering, 1/3 Administration)
- approx. $35 M/yr annual operating budget
- Located in Moss Landing, California
- Operates 3 full-time research ships plus numerous ROVs and AUVs
  - Two day boats operating primarily in the Monterey Bay
  - The swath vessel “Western Flyer” for longer missions further afield

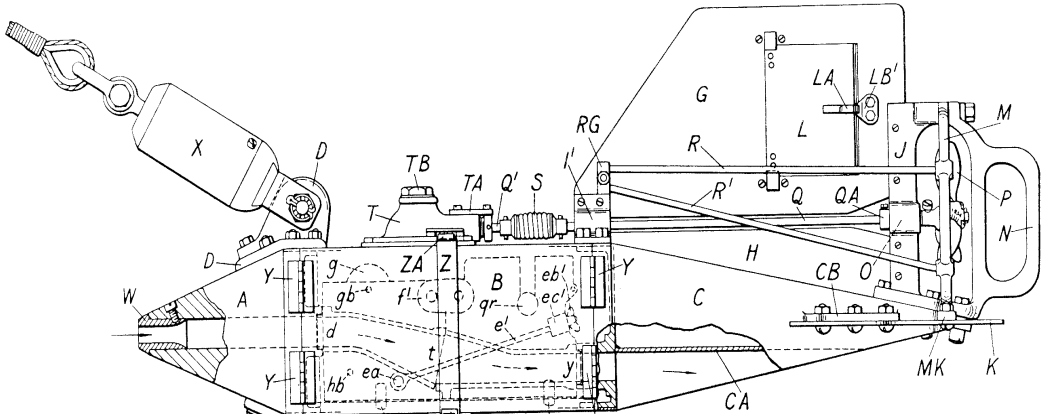


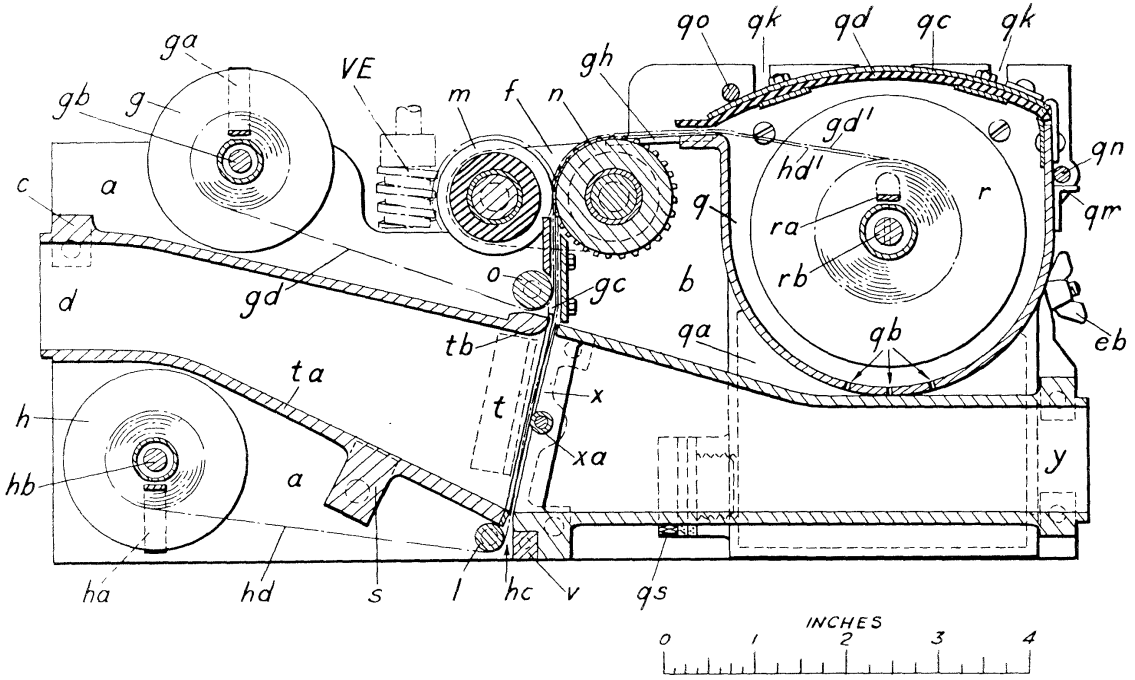
The Environmental Sample Processor

- An autonomous, robotic molecular biology laboratory
  - Typically operating underwater in a sealed pressure housing
  - Filters and concentrates particulate organisms from a raw water intake
    - i.e. → It pins bugs against a filter membrane
  - Then, either
    - Preserves and archives whole cells for later shore-based lab analysis
  - Or
    - Lyses (breaks open) cells to access their contents
      - Performing a variety of chemical assays in-situ that:
        - Genetically Identifying species, species groups or even strains
        - Detecting toxins
          - Such as Demoic Acid (causes shellfish poisoning)
        - Amplifying trace amounts of target DNA or even mRNA

  - Works with organisms ranging in size from bacteria to mussel larvae
  - Communicates assay results back to shore within minutes
  - May be redirected at any time from shore

A surprisingly old idea





Continuous Plankton Recorder (CPR)

First deployed on the R.R.S. *Discovery* 1925-27.

Design driven by need to document "patchiness" of zooplankton

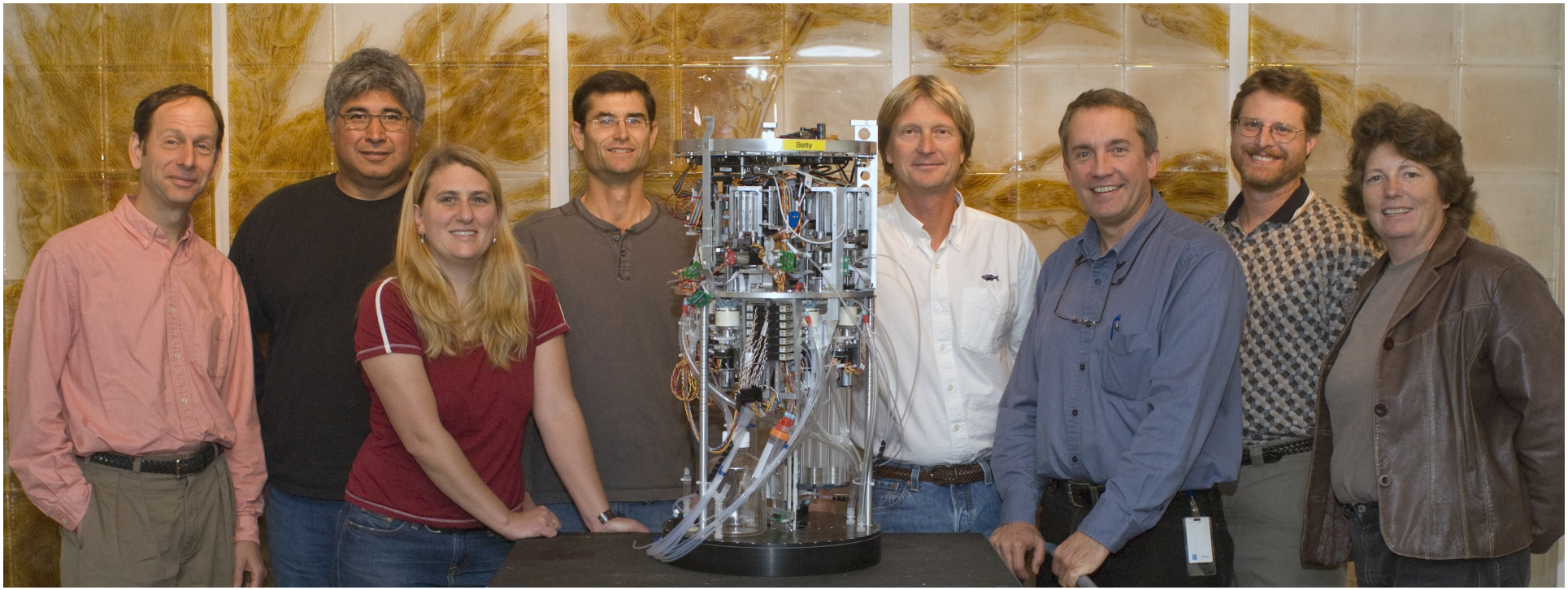
Many modifications over the years

- took ~10 yrs to become "operational"

ESP Applications

- Scientific Research
  - Better understanding the ocean, river, and lake ecologies
  - Detecting genes and volatile gene products that cannot be preserved for later
- Monitoring Harmful Algal Blooms (HABs)
  - Traditional lab based assays often sound the alarm too late to be useful
  - Beaches get closed well after initial onset of toxins or even after they are gone
  - Presence of “red tide” doesn't necessary correlate with toxins
- Aiding Aquaculture
  - Random screenings after harvest can be avoided if water is continuously monitored
  - Fisheries can be quarantined in response to ESP's early warning of contamination
- Monitoring quality of drinking water
  - In reservoirs for trace toxins
- Sewage treatment monitoring
  - For increased fecal bacterial counts, et. al.
  - EPA will soon mandate such higher rate, targeted monitoring
- Much more effective when results are delivered within hours rather than days or weeks

ESP Gen 2 Team



Brent Roman Chris Preston Chris Scholin Doug Pargett
Roman Marin Scott Jensen Jim Birch Cheri Everlove

Not Pictured: Jason Feldman

ESP Pucks

- Many different internal functions
- All hold some sort of filter membrane
- All the same exact external dimensions
  - This is key to robotic handling
- Current pucks snap together rather than screwing together

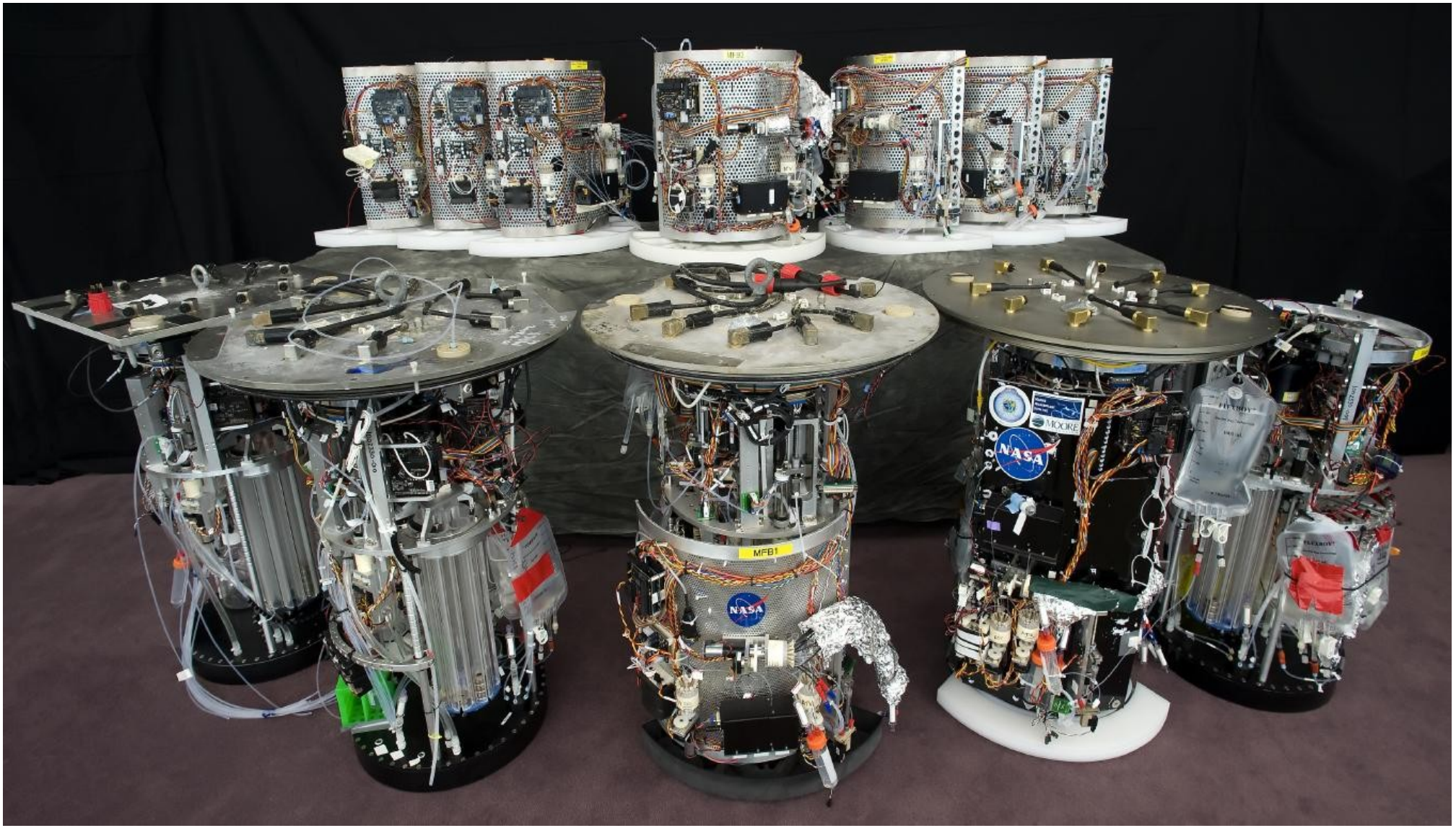
ESP Assays and "Analytical Modules"

- Core ESP performs the most common assays without external modules
  - Whole Cell Archival (i.e. preserving cells with alcohol)
  - Sandwich Hybridization
  - Screening for common toxins (demoic acid, etc.)
- More exotic assays are performed in ancillary "Analytical Modules"
  - Interface to core ESP via standardized electrical and fluidic connections
  - Core ESP designed to support up to 3 analytic modules

- The Microfluidic Block (MFB) is the first and, so far, only analytic module
  - Prepares tiny (< 1uL) volumes of chemical cocktails
  - Primarily to support PCR for amplification of genetic material
    - But may be used as a front end of other, similar low-volume assays
  - Mounts on curved mesh panel around the core ESP

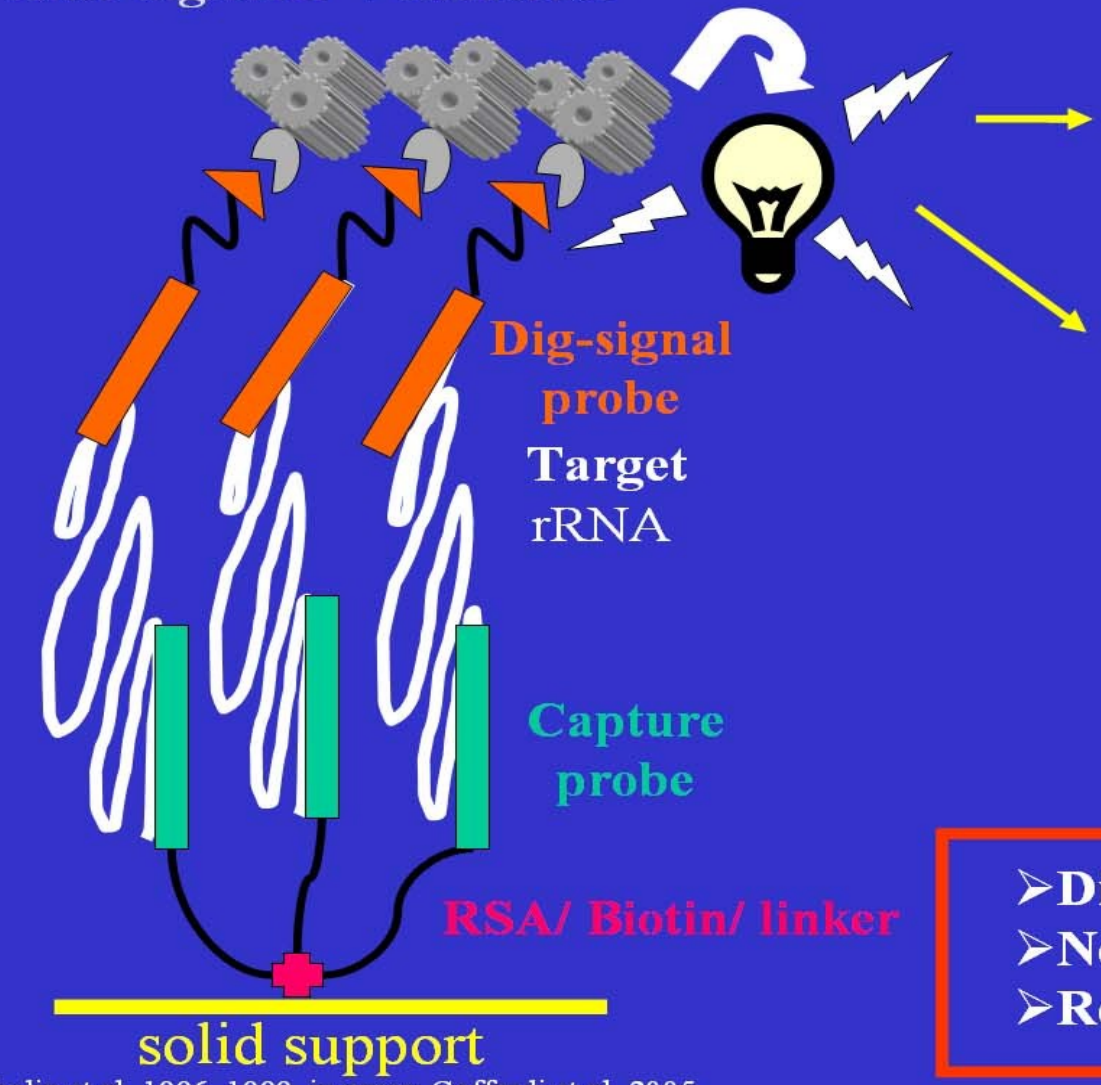
- A couple academic collaborators outside MBARI are developing others

ESP Gen 2 Production Run



MBARI

Sandwich Hybridization



Scholin et al. 1996, 1999, in press; Goffredi et al. 2005

Imaged array

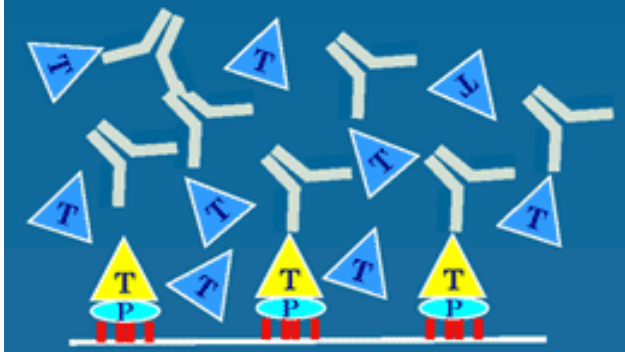
Photo courtesy of A. Haywood

Verification by matching 96-well bench run

array spot intensity ∝ absorbance (450 nm)

- **Direct capture of target sequence**
- **No purification required**
- **Reagents stable at room temp**

Domoic Acid (DA) competitive ELISA

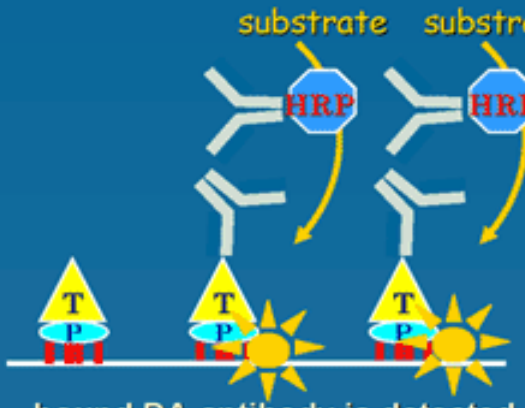


toxin in the sample (blue triangles) competes with toxin-protein conjugate (yellow triangles) immobilized on membrane array for DA antibody in solution

1

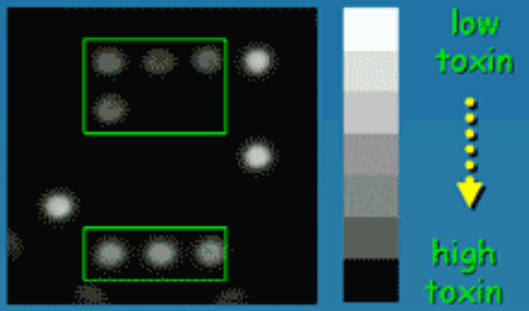
free DA antibody and that bound to toxin in the sample is washed away, leaving only the antibody bound to the toxin-protein conjugate on the membrane array

2



bound DA antibody is detected using a second antibody attached to an enzyme (HRP), which produces a chemiluminescent (i.e., light) signal when provided with a substrate

3



the light signal is imaged using a CCD camera; array spots in green boxes show presence of DA in sample; spots outside of boxes are controls; data are transmitted to ship and/or shore and stored onboard ESP; assay time ~1 hour

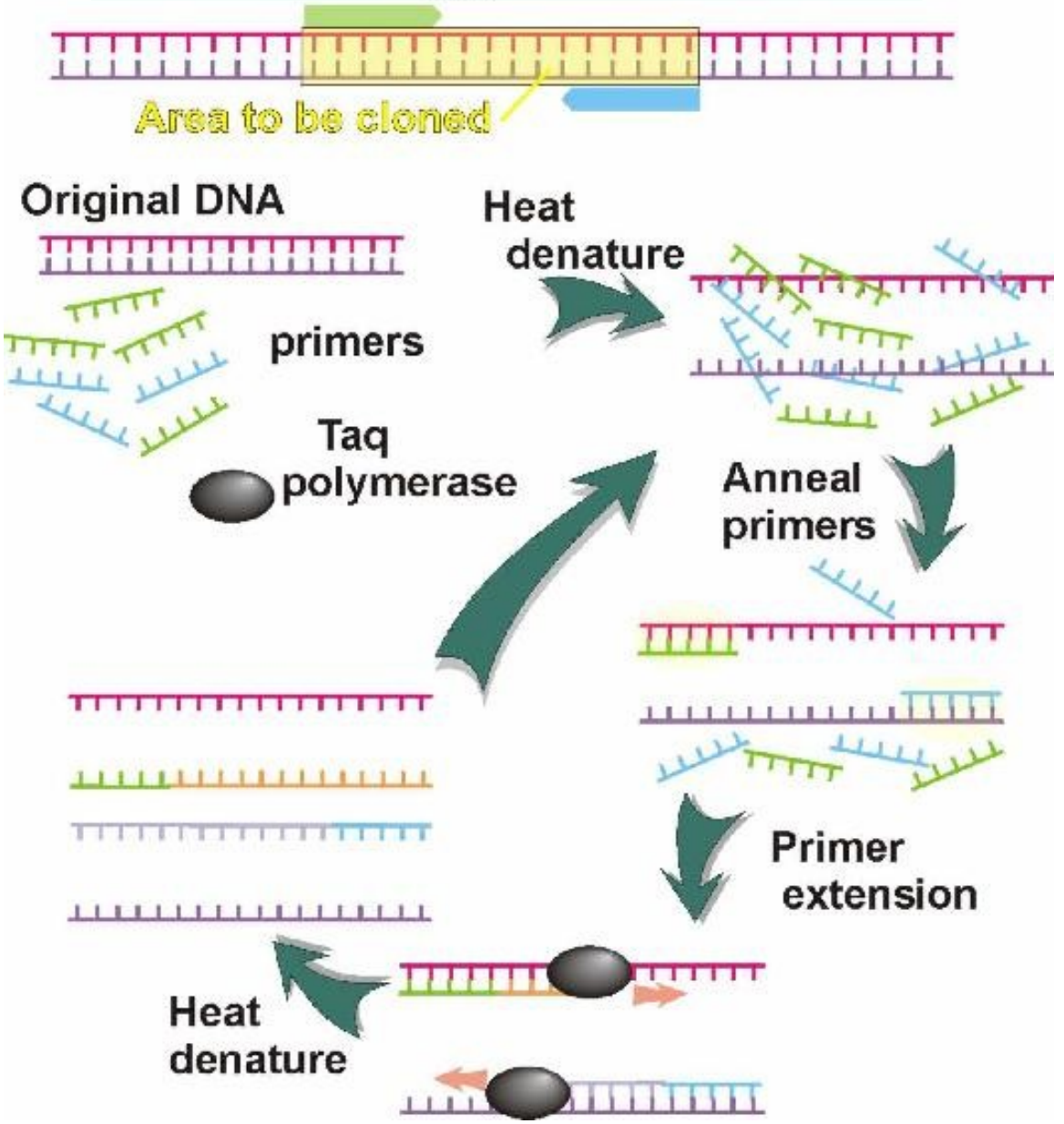
PCR:
Polymerase Chain Reaction

- Artificially copies targeted DNA fragments.
  - Much as DNA is copied during cell division
  - Start and end sequences (primers) must be known
  - Middle of DNA may be unspecified
- Number of copies grows exponentially
  - Roughly 2x per PCR cycle
- Requires precise, rapid heating and cooling
- Some nucleotides fluorescently labeled for signaling
  - QPCR measures # of cycles needed to reach detection threshold

- Very sensitive
- Extremely susceptible to contamination
  - Requires careful front end sample preparation in lab, clinical settings and law enforcement

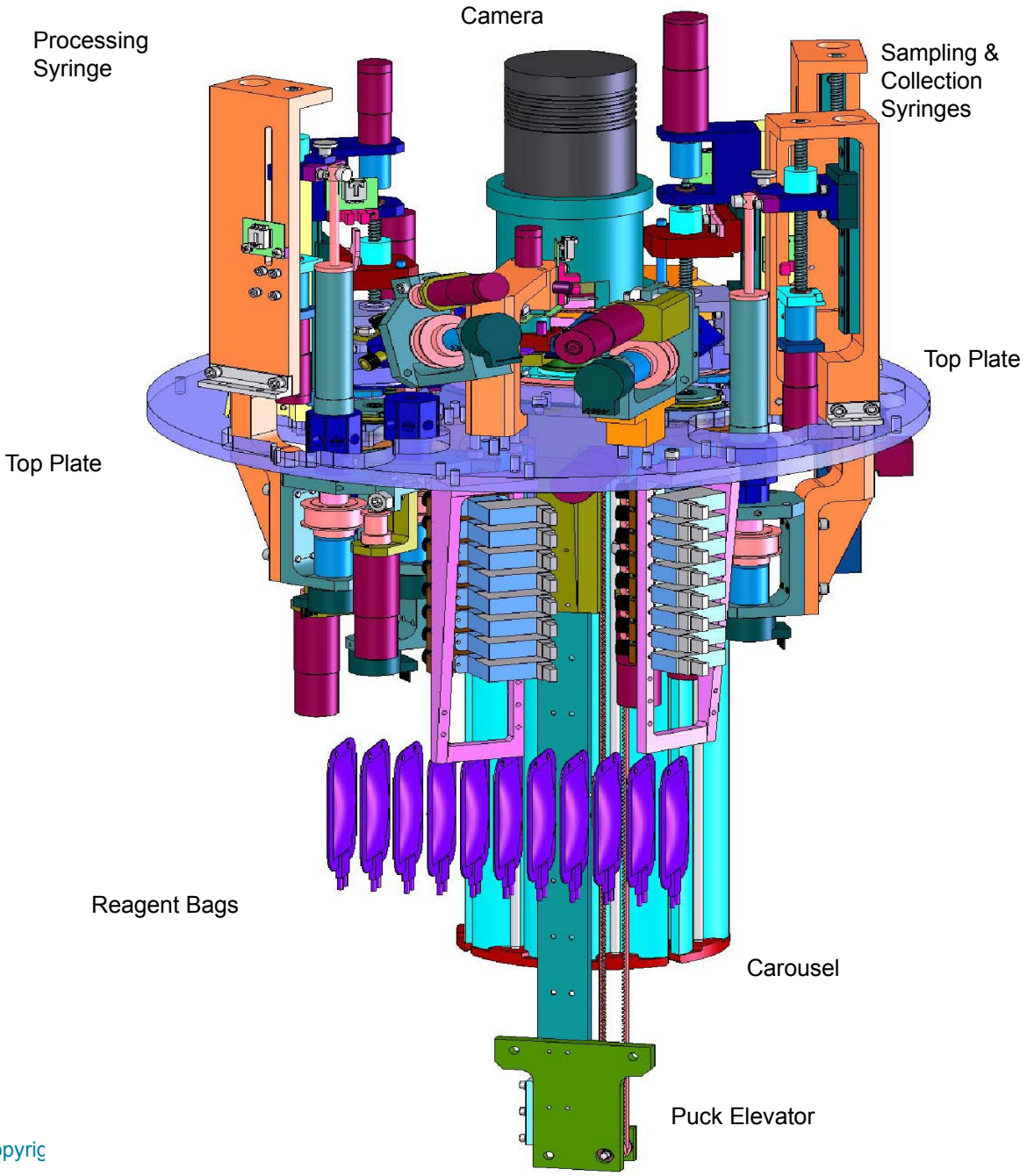
- ESP is first successful PCR in an underwater marine instrument

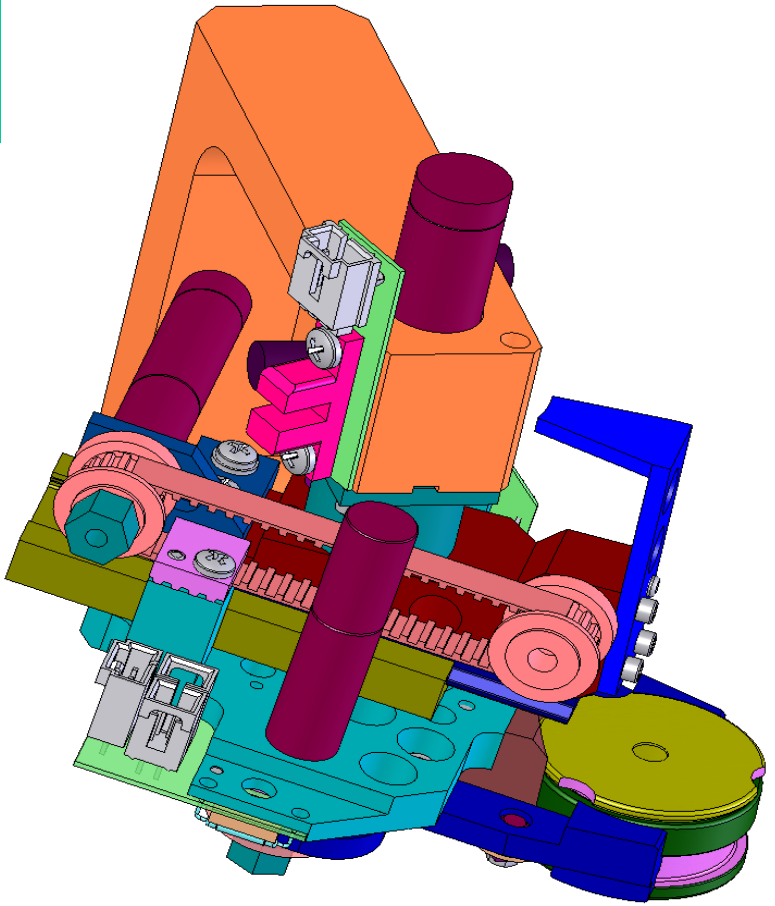
Polymerase Chain Reaction

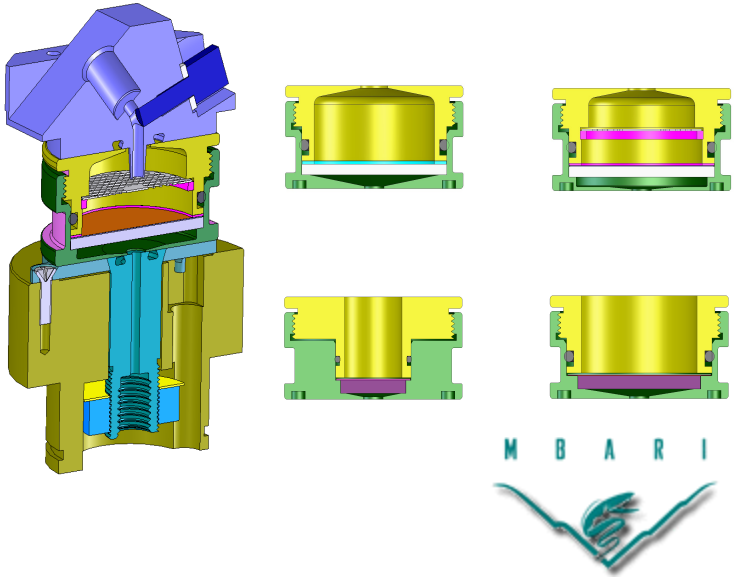


http://www.ocf.berkeley.edu/~edy/PCR.jpg

ESP Core
Solid Model



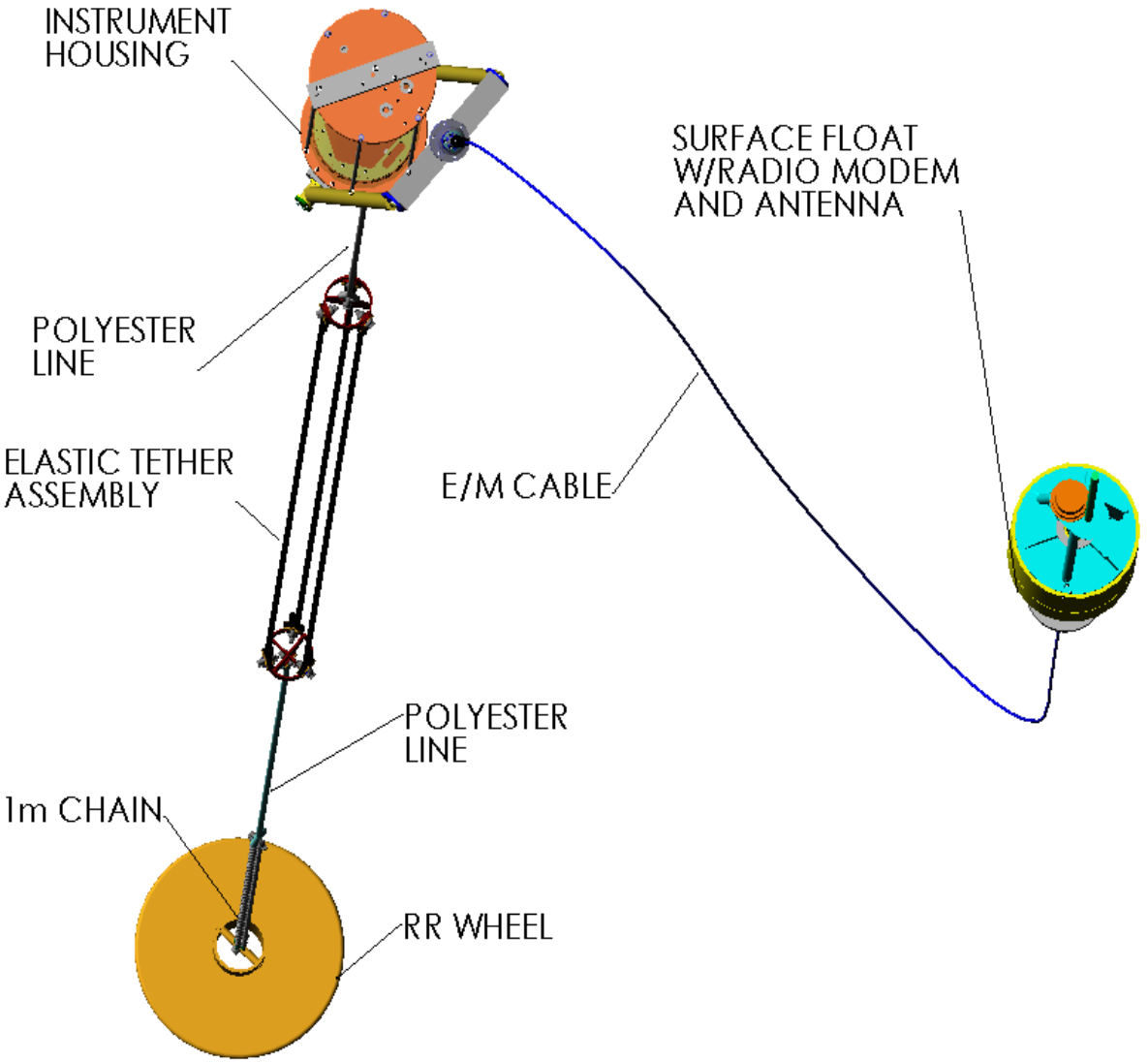




M B A R I

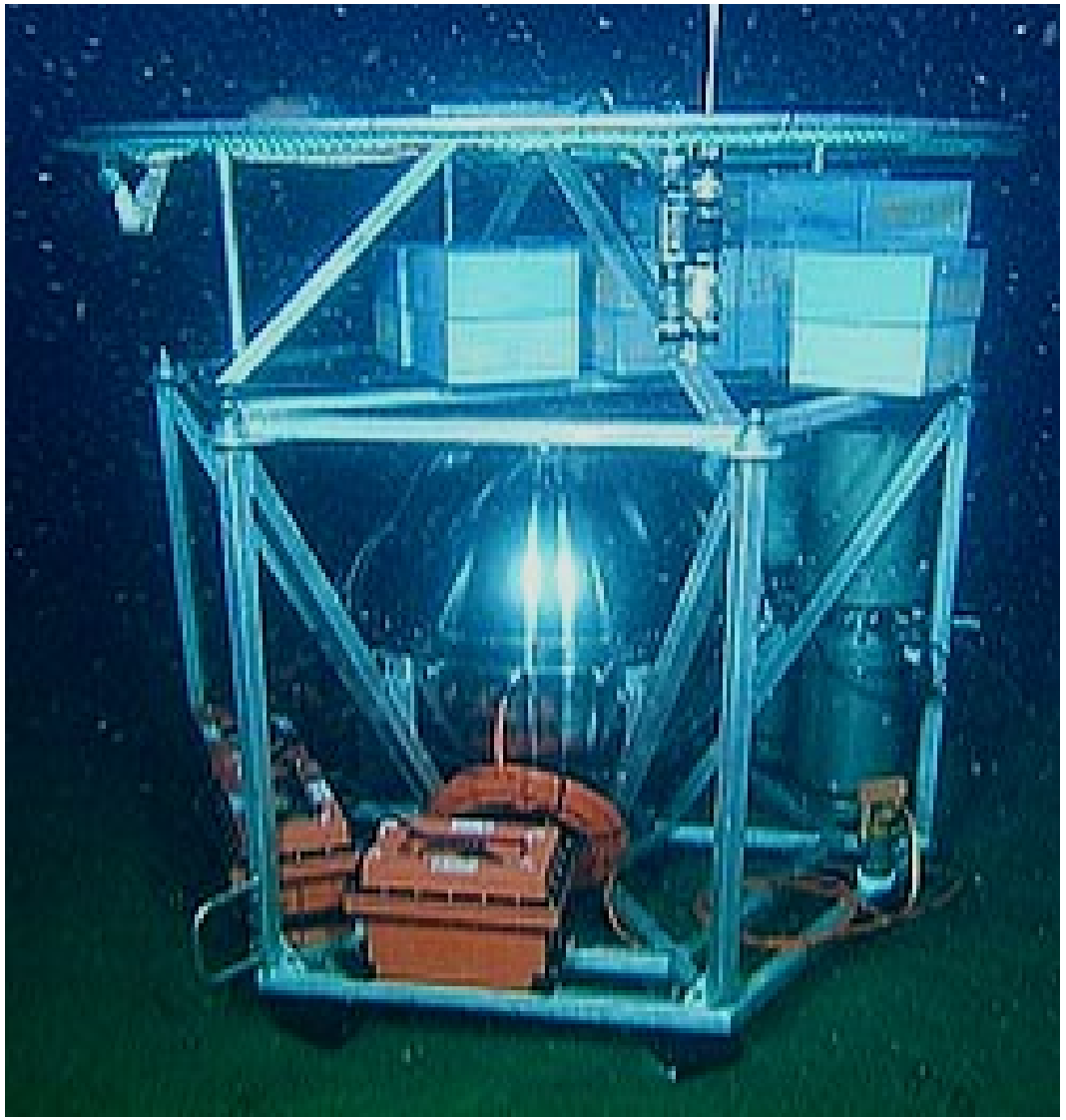
ESP Core
Puck Handling Movie

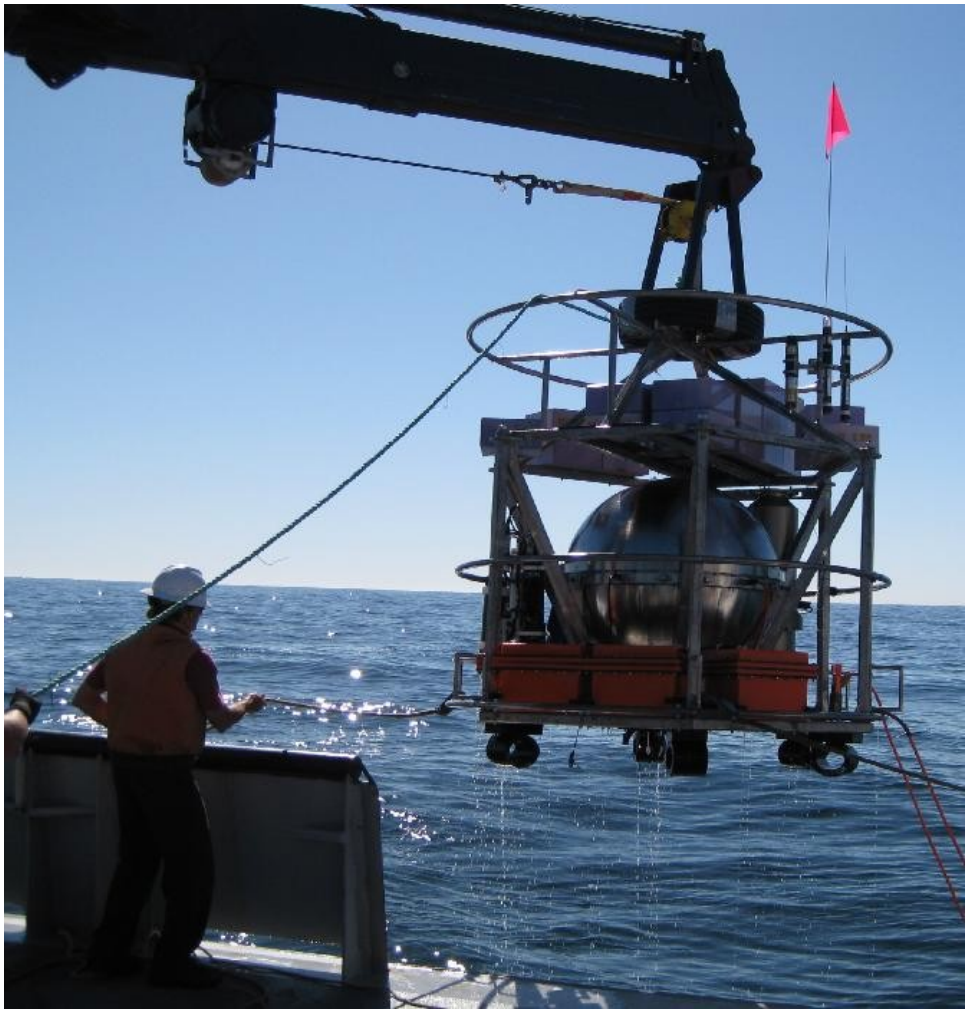
Shallow 10-30m Mooring



Shallow Deployment Movie

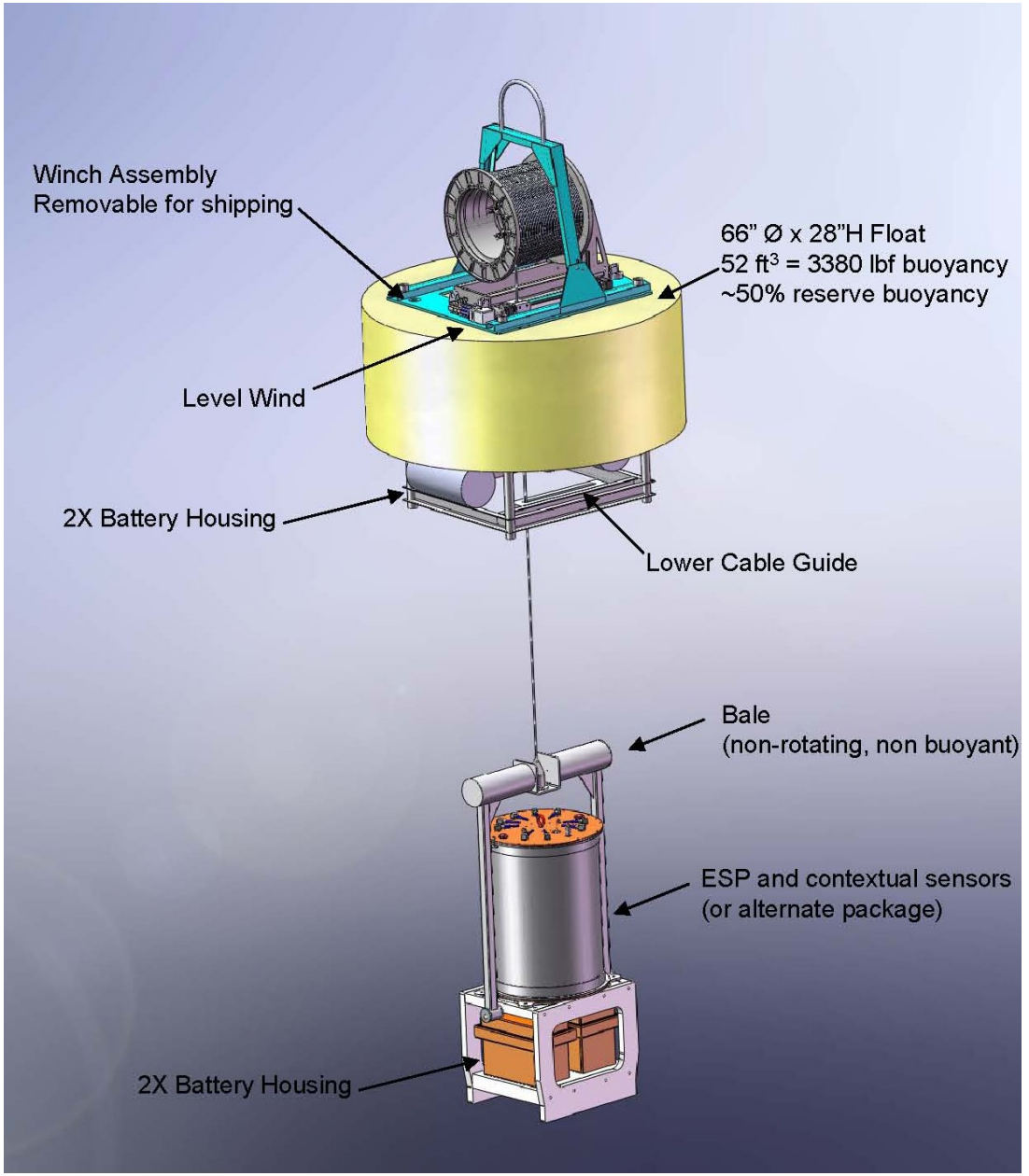
Deep 10-4000m ESP



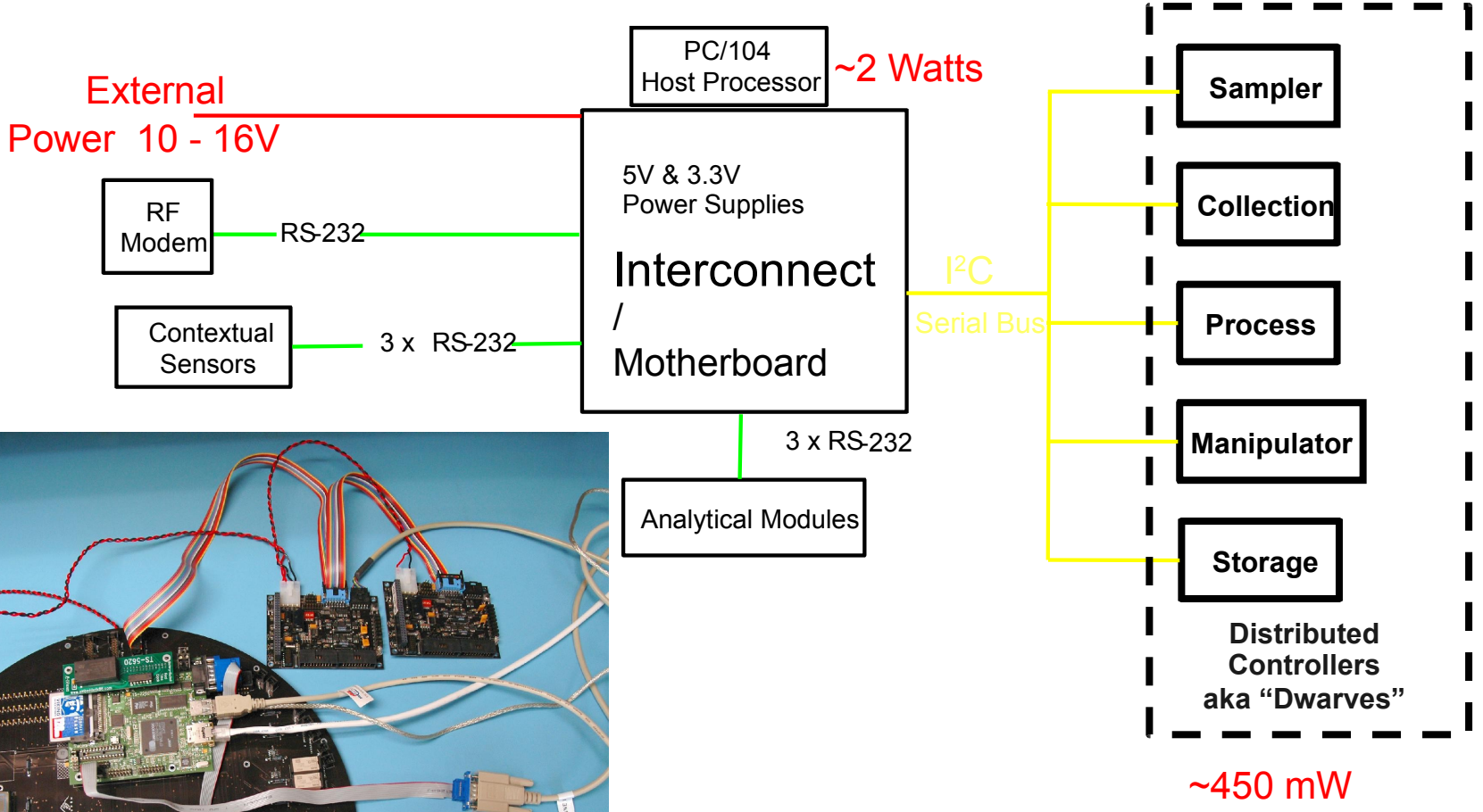


Deep Water ESP deployed at 800m in Monterey Bay

ESP Drifter
Solid Model



2G ESP
Electrical Block Diagram



Serial Buses

- RS232 is still mainstay for “traditional” oceanographic instruments
  - One port per device
  - RS-422 can do multi-drop, but...
    - No networking standards
    - High quiescent power due to required pull-ups

- I^2C Bus is Low-power, multi-master
  - A real network
  - Broadcast medium
  - Susceptible to electrical noise
  - We add CRC checking
  - Direct support in some msp430 models

- USB for Wi-Fi and Low Light Camera

Software Design Overview

- Full ARM Linux – older 2.4 kernel
  - 32MB DRAM, 16MB RAM
  - Busybox squeezes common Unix commands into just 4MB flash
  - CPU and memory resources comparable to 2003 vintage cell phone
- ESP application written entirely in Ruby scripting language
- Linux and Ruby are not deterministic enough
  - Ruby's garbage object collection causes occasional ~300ms delays
  - This Linux kernel has poor interrupt latency
  - > 5ms measured during intensive writing to Compact Flash drive
  - Serial ports with large (64 byte) FIFOs required to prevent data loss
    - 115kB for 20ms = approx. 50 bytes
- The MSP430 based “dwarves” close all real-time control loops
  - Distributed control to the rescue !!

MSP430 based “Dwarves” do all the real-time control

- Each one dedicated to a modular subsystem
- All communicate over the I^2C serial bus
- Identical hardware and Firmware to ease maintenance
  - Dual servo motor (PID) control
  - One 5Amp Thermal (PID) controler
- Daughter boards support 4 Rotary and 8 Solenoid valves
- Whole board uses approximately 70mW quiescent power.
- Implemented with a TI MSP430169 (on-chip I^2C controller)
  - Draws only microamps
    - 60KByte Flash, only 2KByte RAM
    - Coded in 'C'
    - No real-time kernel, lots of intricate interrupt service routines
    - Get a newer (2xx) part of you intend to work with I^2C
      - Silicon bugs in x169's I2C serial controller cost months

Demo

- Sample “Air”
- Photo a glowing puck
- Shuttle carousel elevator
  - Capture SE status and graph
  - Play with PID parameters