

# CLASS PROGRAM

NAME \_\_\_\_\_ ADDRESS \_\_\_\_\_

SCHOOL \_\_\_\_\_ CLASS \_\_\_\_\_

		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	PERIOD 7	PERIOD 8
TIME	FROM TO ...								
MONDAY	SUBJECT								
TUESDAY	ROOM								
WEDNESDAY	INSTRUCTOR								
THURSDAY	SUBJECT								
FRIDAY	ROOM								
SATURDAY	INSTRUCTOR								

9¾ in. x 7½ in.

MADE IN U.S.A.

## DRY LAB

\*photocopy and scan  
at the end of every  
day  
→ optical engineer  
notes folder

5/9/13 - Make an inventory once full supplies  
arrive

### Accuri

- filter and tubes change at the end of  
each leg.

### TODO:

- run all calibrations + create a template
- re-test the thresholds w/ cultures

5/10/13 • changed fluidic bottle filters on:

- sheath fluid
- decontamination fluid
- cleaning fluid

• changed sheath filter (inside instrument)

• changed pump tubing on both waste (red)  
and sheath (blue) sides (inside instrument)

→ purge air after  
above changes  
(pg 28 inst. manual)

### Before cruise starts:

- ✓ replace tubing for pump  
and filter
  - re-calibrate Accuri (new  
beads)
  - cal. FlowCAM
  - get all cleaning solutions  
mixed + in place
- PRINT: - Revised protocols  
- Daily C-lists + routine

BAJ FSC

5/11

### For Flow CAM:

- S = bead & size calibration
- F = fluorescence calibration (says "for Acumi")
- E Appendix E in FlowCAM protocol - purple
- D, once
- every 2-3 weeks, or beginning + end of log
- measure tubing length

~~After~~ bleach

- 10% bleach solution - 10mL into 90<sup>mL</sup> H<sub>2</sub>O
- leave closed, w/ bleach sitting, flush in the morning
- have Marci look at data - is 50mL a good amount to run?
- V/F lid open

### Accuri

- clean SIP w/ squirt bottles
- do a set of calcs, inc. size before leaving
- also before leaving, make a new 6- and 8-peak stocks for first

Monday. 100

1000

CDOM sniffer:

Fluor. adds a point 2 μm filter to "reference" or "2nd" - change filter on wall once a week

\* make sure there is an empty bucket in lab at all times \*

Lee  
France  
cell

07 611 88986

From Lee:

- doc for FlowCAM -
  - revised Accuri protocol ✓
  - list of stuff that celine will get
  - log sheets
  - daily checklists
- send Lee screen shots and results when possible

\* culture location

→ email Bill Kelley re: insurance dates -

5/12/13

### Flow CAM

tubing length:

above flow cell = 30cm

below " " = 37 cm

- maybe 0.5cm on goes onto pipette tip

- Context files for:

- ① Manual Prime
- ② Sample
- ③ Cleaning

- Note - while water is running, cannot do "setup and focus"

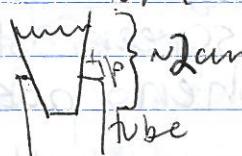
→ context files are done, need to

edit:

- volume to be run

size cal:

- prime w/~~the~~ DIW, until level is ~2cm  
above the bottom of the pipette tip:



- load a run sample context file
- change the sample volume to 1 mL
- change the basic size acquisition filter (Setup > Context > Filter (use ESB)) to min = 10, max = 2000
- pipette in 1mL of the 4x size mix
- run in auto image mode, save as; all size mix date
- load filters file
- make sure the histogram plot is correct.

filters should be: We only have 50!

~~15 μm +/- 0.7~~  
~~23 μm +/- 1.7~~  
~~50 ... +/- 2.0~~

h

5.95  
10.190

Bottles to label:

ALFA cleaning (amounts are enough for one cleaning)

① 1L DIW

② Detergent 50:1 (250 mL <sup>DIW</sup> and 5 mL deterg)

③ Bleach 50:1 (250mL <sup>DIW</sup> + 5mL bleach)

(DON'T USE STRONGER SOLUTIONS)

CDOM mapper

① DIW

② Detergent (5% solution) 10mL det., 190mL DIW

③ Methanol (50% solution)

④ HCl (10% solution)

ALFA  
CDOM M.  
FlowCAM  
Accuri  
Ultra  
IFCB

vol?

FlowCAM

sample bottles

① Filtered Seawater (0.45 μm filter)

② Surface Niskin 2 x 250 mL

③ DCM Niskin 2 x 250 mL

④ Bongo surface 50 mL

⑤ Bongo DCM 50 mL

⑥ Bleach 50:1 (0.5mL bleach + 25mL DIW)

⑦ DIW

Accuri

see log sheets

① Surface Niskin

② DCM

(flowthru may be taken @ other times)

③ Make 0.2 filtered seawater

Daily calcs: 8+6 peak beads, surecount + yellow beads  
Every other day: size cal w/ 4 diff sizes

: (add) of 200 μl  
 100 μl of Agarose onto 27 nmole DNA (ASIA)  
 DIW 11 ①  
 1:02 trapwash ⑤ 28°C  
 (100 μl DIW + 100 μl 2%) 1:02 trapwash ⑥ 0°C  
 (100 μl DIW + 100 μl 2%) 1:02 trapwash ⑦ 0°C  
 (100 μl DIW + 100 μl 2%) 1:02 trapwash ⑧ 0°C  
  
Program mode  
 W10 ①  
 1m OPI, 1m OI (mitules 2%) trapwash ⑤ ASIA  
 (mitules 2%) IonenM ⑥ MM100  
 (mitules 2%) DIW ⑦ MAXWFT  
 MM100  
 MAXWFT ⑧ DIW  
 2nd flow slow ⑨ DIW  
 (1st flow MM2%.0) 100% wash benefit ⑩  
 1:02 trapwash ⑪ 100% wash benefit ⑫  
 1:02 trapwash ⑬ 100% wash benefit ⑭  
 1:02 trapwash ⑮ 100% wash benefit ⑯  
 1:02 trapwash ⑰ 100% wash benefit ⑱  
 1:02 trapwash ⑲ 100% wash benefit ⑳  
 1:02 trapwash ⑳ 100% wash benefit ㉑  
 1:02 trapwash ㉒ 100% wash benefit ㉓  
 1:02 trapwash ㉔ 100% wash benefit ㉕  
 1:02 trapwash ㉖ 100% wash benefit ㉗  
 1:02 trapwash ㉘ 100% wash benefit ㉙  
 1:02 trapwash ㉚ 100% wash benefit ㉛  
 1:02 trapwash ㉛ 100% wash benefit ㉜  
 1:02 trapwash ㉜ 100% wash benefit ㉝  
 1:02 trapwash ㉝ 100% wash benefit ㉞  
 1:02 trapwash ㉞ 100% wash benefit ㉟  
 1:02 trapwash ㉟ 100% wash benefit ㉟

5/17/13 Made new bead solutions for Accuri:  
 - 6 + 8 peak  
 - Surecount + yellow standard  
 - Sizes (1, 2, 4, + 10 μm)  
 Running all calcs, editing protocol (small details)  
 TO RUN:  
 • New DIW - 2 min fast  
 SAVE • 8-peak  
 • DIW - 2 min  
 SAVE • 6-peak  
 • DIW - 3 min  
 SAVE • Count slow  
 SAVE • count fast  
 • DIW - 2 min  
 • Backflush/unclog X 2  
 • DIW - 2 min  
 • 1 μm size (fast and slow)  
 • DIW  
 SAVE • 2 μm size (fast AND slow)  
 • DIW  
 SAVE • 4 μm size (fast and slow)  
 • DIW  
 SAVE • 10 μm size (fast and slow)  
 • DIW  
 • Backflush / unclog X 2  
 • DIW

Cal files: 2 new cells

Count file:

Size file: 8 new cells (2 speeds ea. size)

## TEST RUN OF ULTRAPATH

1/17/13 GOMaine water

- ran 1x cleaning cycle
- reference water ( $\sim 20\text{ psu}$ )
- \* file name: "test\_lorient\_r.txt" (r for ref)
- reference run:
  - int. time up to 385.0 to get a  $I_{MAX}$  of 75%
  - 200 cm pathlength
  - $I_{MAX} = 76\%$
- ref scan does not look too good.. need to use hplc water instead of DW?

estelleDIMIER

1/18/13 Bottles still to be labeled:

- For FlowCAM:
- FSW (between samples + flushing @ beginning)
  - Sample bottles  $\times 6$
  - Bleach solution

Accuri: sample bottles

5/19/13

## Departure Day

- mixed new cleaning, decon, + sheath fluid for Accuri
- add 10 cm to FlowCAM input tube length  
 $\rightarrow 0.078\text{ ID}$
- had to replace flow cell. was dirty on the outside.
- \* NOTE - tighten ~~screws~~ the screw facing directly at the user on the flowcell holder so that the flowcell will not move.

36 cm. below flow cell

30 cm above

- \* brand new flow cell is quite dirty on the outside of the glass...

- running bleach to clean inside
- lens paper used to clean outside of cell
- cleaning SIP on Accuri
- ran ~~6+8 peak~~ 6+8 peak & size caps  
 $\rightarrow$  (made new size bead stocks)

At sea:

- empty waste bottle
- empty Accuri waste

\* ASK Celine:  

- disposal of glass tubes?  
 $(at sea)$

- 5/24/13 Station #155  
LOCAL TIME 0730  
0730 Turned on FlowCam P+L, Accuri, Ultrapath  
0740 Running sheath on Accuri to clear any bubbles. (3x5 min)  
Made FSW w/flo-thru H<sub>2</sub>O  
Put fresh HPLC-grade H<sub>2</sub>O in Ultrapath bottle.  
Checked V-path ref salinity: 34 psu  
→ Local salinity = 35.3  
flow meter for IFC B + alfa showed 0.0 l/min;  
opened the valve under the bench more  
0800 →  
0830 Running 6+8 peak beads on Accuri  
- Put beads back in fridge, also put in V-path ref. H<sub>2</sub>O  
\* Error message on COOM mapper: Run-time error!  
- emailed Alan, will wait for response. Overflow  
Running count standard cal on Accuri  
0843  
0900 → Rinsed 0.2µm disk filter w/ 120 mL of HPLC water (two 60mL syringes full).  
Wrapped disk filter in foil until use.  
0910 Running a cleaning cycle on V-path:  
Citraxx → MeOH → HCl → HPLC H<sub>2</sub>O (wait for bubble; one min each)  
0930 Got ref water from fridge; filling wave guide (Ultrapath)  
0945 Got surface samples for V-path from Rosette cast (3x 250 glass amber bottles) \*filtered those  
② 0.2 µm disk into 3 other bottles

5/24/13

Stn 155 cont.

1010

Not getting a great ref. scan. Cleaning again.

1129

Cleaning cycle 4 times. ref. is ~0.0

✓ except in the UV - much higher.

- running the niskin samples.

ask JF → should the pump be running while the

sample scan is going?

- COOM spectra #1 looks okay, maybe can just subtract the ref in post-processing?

Running:

Cleaning

Ref

\* all samples run

Sample 1

w/ 200 cm pathlength

Cleaning

Ref

Sample 2

Cleaning

Ref

Sample 3

end: { Ref

Cleaning

- left w/ water in tubing

1215

finished @ 1215

cont.

1237

Filtered ~~coom~~ samples thru 300 µm mesh w/ FlowCAM plastic funnel.

- 4' bottles: 2x250 mL surface niskin  
2x250 mL DCM niskin

- new pipette tip on Flowcam funnel.

1241

Make filtered SW (from flo-thru) w/ 45 µm disk filter + syringe, to flush FlowCAM w/.

1421

Running FCAM surf trigger mode

1608

Finished running Accuri surf. + DCM (w/ beads in each for conc.)  
- not too much in water.

FCAM is running, ~30 mins per sample  
(for 50 mL of sample)

1625

Accuri shut off (finished its automatic cleaning)

FlowCAM running. Still to do on it:

- DCM auto mode

- conc. 0.2 water (net) A mode

- conc. 20 water (net) " F mode

\* movement of the boat causes slight shifts in the flowcell + fluid inside...

the background calibration is very sensitive, so this means the software starts seeing dirt on flowcell as cells to image. Problematic.

cont.

17:00 email from Alan - he also gets the same error messages; working on a software fix. Also recommends not using Port A since it's not pumping + thus could be drawing air into the system.

17:30 Running FCAM conc. 0.2 μm water (surf). Most stuff ~~decreased~~ is small, rarely the chains we saw w/ the surf. niskin. maybe cells are damaged in the conc. water process?

18:00 Finished 0.2 conc. water. Did not filter thru 300 μm net. Started 20 → water (net). Did filter thru 300 μm net. - much more big stuff / chains in 20 → water.

18:35 Finished 20 → net water. Many phyto. cells (+ chains)

18:54 Trying to get IFCB into good focus. (using the focus motor under "hardware" tab.)

19:31 Running meso (~800 m depth) samples in flowCAM\*. trigger mode = basically zero cells (did not filter first meso bottle... did filter second "A" bottle... do not expect a diff. for flu reason.)

cont.

20:27 running meso on auto mode

- test on CDOM mapper for ALAN: run sample in place of ~~flu~~ ref H<sub>2</sub>O.

LOCAL TIME Station day 2

0851 CDOM mapper crashing frequently. Screen shots sent to Alan.

0900 Started a new ALFA file: "alfa-20130525-070000" → made on 5/17  
Running peak + count cols on Accuri.  
- Also size cols (solutions made on 5/19)

Fluor. cal on FlowCAM...

0921 CDOM mapper crashed again. (all new databases created since cruise start on 5/19 are due to software crashes)

0927 looks like some bubbles in IFCB images...  
- stopped aqu, running "debubble and refill".  
0935 - re-started

Accuri 6+8 peak runs look ok, not perfect.  
Try making new bead solutions from

running Accuri ~~size~~ col - looks good  
count

1014 Running accuri size cal. looks not quite as good as prev. runs - make new size stocks for next size cal.

1046 size 4um beads ~~do~~ are dramatically reduced in count.  
→ discarding all bead solutions.

1126 Cleaning Ultrapath

- took "dark scan" (w/shutter off)
- and "Reference scan"

5/26/13 Ran Ultrapath w/ flow-thru 1100 local time

5/27 Ran Ultrapath w/ flow-thru 1800 local time

5/28 1200 - made new reference water, replaced old ref water in CDDM mapper, also added new HPLC water. Started a new CDDM mapper database.

- crashed again, before crashing, spectra did not look good.

14:51 took a flow-thru sample  
- made new 6+8 peak Stocks, size stocks, daily count standard,  
- Running Accuri Cals.

local time

1800

Ran ultrapath, w/ cont. flow water  
temp =  $10.39^{\circ}\text{C}$   
Sal = 35.3  
Sal ref = 35 psu  
200 cm pathlength  
77% Durax  
385 int. time.  
lat =  $59.379^{\circ}\text{N}$   
lon =  $-9.3258$

Accuri:

- ran all cal's: peaks, counts, sizes
- doesn't look like too much in the water sample from Cont. flow
  - 2 min FL3 slow:  $26.3 \mu\text{m}$
  - " " fast:  $130.8 \mu\text{m}$
  - " FSC slow:  $26.3 \mu\text{m}$

Flow cam: running 200mL @ 1.5m  
time start: 14:20 (from flow-thru) m

duration: 2 hrs 12 min

# of particles: ~~094708~~ 28,770

vol <sup>processed</sup> sampled: 199.6

ESD: mean = 27.25

min = 10.00

max = 99.94

fluid vol imaged = 29.644 mL  
1.5 mL/min

To do on wed 5/29:

- 50 mL @ diff. speeds in FlowCAM test.
- Run Accuri - first make sheath fluid
- Run ~~ultrapath~~ ultrapath (new ref water + HPLC water for cleaning)
- FlowCAM fluor. cal?
- Accuri cryovials?
- make ALFA cleaning solutions
- test of new vs. old ref water

5/29/13 Took flow-thru water:

0802 UTC

60.6374°N

-8.2697°W

t = 9.8233 °C

s = 35.3 psu

~~0830 started~~

UTC time from now on

0830 - started a 50 mL @ 1.5 mL/min w/ flow-thru water on mm-1 FlowCAM (speed test)

0907 started next 50mL run on FlowCAM, @ 0.75 mL/min.

- made new liter of Accuri sheath fluid
- made bleach + detergent solutions for cleaning ALFA.

5/29

sent ~~ALFA~~ ALFA screen shots to Mark, and accuri screen shots to Lee

1020 - Started zooprocess for yesterday's 200 mL run and today's 2x 50 mL runs.

1217 - mixed new ref water w/ fresh HPLC water; 34 psu local sol = 35.3 psu

1253 - running ~~count~~ peak ~~count~~ cals on Accuri  
- turning on + cleaning U-path.

1324 - running count cals on Accuri  
- FlowCAM, upath, Accuri ready for rosette H<sub>2</sub>O

1407 started running FlowCAM surface Auto-mode, 200 mL, 1.5 mL min-

1426 - running ultrapath  
IMAX = 75%

1443 - running Accuri samples

1503 - finished running ultrapath + Accuri

5/30 Faroe Islands

0700 Cleared ALFA (flow-thru is stopped)



5/31/13 Underway again from Faroe Is.

1700 Re-started flow-thru + all instruments, except CDom mapper. Still having problems, in touch w/ Alan Hails.

Focus motor:

2012 >> 350

6/1/13

1000 Email from Alan w/ several things to try on CDom mapper...no luck; still having error message/ software crashing. Also, now the software seems to stop and we get "not responding" in task manager at the first reference cycle.

1010 Starting a new ALFA file w/ new settings: PDP shots = 75  
Blue Int. = 0.7  
Green Int. = 3

"alfa\_20130601-101000"

No sample today - waiting for int'l wi

Next to do:

Accuri

- 5 min Sample runs
- extended cleaning cycle

FlowCAM

- try 100mL (max raw images = 99999)
- process + sort data ✓
- Fluorescence cal

→ inventory of glass vials

→ send Lee IFCB images

1500 Power outages on all instruments.

Re-started ALFA

Re-started IFCB

0800 STATION 157

- mixed new ultrapath water
- local sal = 35.0
- ref sal = 35 psu

\* DO ACCURI EX. cleaning @ end of the day.

- started Accuri

- changed all re-useable tips

0820 - started a new ALFA file

6/2/13

- replaced white pad under Accuri SLP

0900 Running FlowCAM fluorescence cal

0920 Running Accuri 3 cal

- very slight doubling in peak cal  
- count cal look good

1000 Flowcam fluor. cal. stopped at 30 mins...  
the context file overrides the  
stop conditions (apparently)

1005 Started running flowcam Auto-image.

100 mL  
1.5 mL/min

99999 max raw images

1045 starting Accuri Samples

11:20 Finished Accuri  
started FCAM fluorescence run  
- lots of tintinids

1142 Running extended clean of  
flow cell on the Accuri  
~~- did not hear pump running,  
and then the~~

6/2/13

1314 Accuri restarted w/ D/W  
(message for extra start-up time = expected)

- Ultrapath start running

- Processing FlowCAM images w/ ZOOG**process**

1326 Accuri Shutdown

\* Added the CDOM barcode to the comment  
like on the TIDAS DAQ software.

1410 Finished ultrapath

\* changed 0.2 μm filter on wall for CDOM  
Mapper mapper

CDOM mapper cleaning:  
H<sub>2</sub>O, det, air, meth, HCl

1820 CDOM Mapper:

Ran for ~20 min to get bubbles out  
of new 0.2 μm filter. took off orange  
caps; tubes just in bottles w/ parafilm  
on top. Primed all ports; started

\* Run-time error 13 at end of cleaning  
cycle (before ref)

- changed all cleaning to "0" in parameters  
(and cycles between cleaning = 100).

- manually collecting dark + ref, then set  
of sample spectra.

6/13;  
-make ref water  
-Accuri peak+count cal (no size)

6/3/13 STATION 158

0630 Made new ref water for v-path

CDOM mapper crashed in the night;  
restarted.

0800 Accuri count cal: made new 6+8  
peak vials; 6 peak had doubling,  
re-made a new one, still has  
slight doubling - tried adjusting  
SIP.

V-path ref H<sub>2</sub>O in the fridge.

0830 ~~Restarted~~ New ALPA file started  
Checked IFCB Images (processed)

1100 Cleaned v-path.  $I_{MAX} = 67\%$   
- changed int. time to 390 msec.  $I_{MAX} = 70\%$  now.  
- changed to 400 ms, 71%  $I_{MAX}$

\* Use Monitor under "Scan" (or button)  
40.5 sec, 75%  $I_{MAX}$

1242 Started FluxCAM auto surf.

100 mL 90000 max images  
1.5 mL min<sup>-1</sup> 20 μm min size

6/3/13

14:15 Accuri + FCAM are running  
- making Accuri & fluid solutions

1506 Accuri shutdown

1815 ~40 mins left on last FCAM run  
(when done, start processing w/ ZOO process)  
→ need more log sheets

Focus on IFCB - email from Lee + Rob.

Inventory of under the bench:

Green bag #1 = 10 packs of glass vials = 250 vials  
" " " #2 = 8 " " " = 200 "

Ultrapath - don't forget to check the "monitor"  
button, also Reference scan should be w/ ref  
water.

1840 Another FCAM sample: 20-180 DCM net

6/4/13

07:55 V-path ref water made (35.5 psu)  
\* Flow thru water taken (0755 UTC)  
~~67.1954°N 0.23786°E~~  
temp = 8.75°C sal = 35.15 psu  
(HPLC not ready, re-taking more water later)

iceberg  
thomas\_leeuw@umit.mann.edu

6/4/13

1115

Finished V-path w/ flow-thru.

\* Every day from now on (except  
fill station or CTD cast):

Take a flow-thru sample @ the 2021  
Same time as HPLC, DOC, and  
nutrients (so, those three plus  
Accuri, FCAM, + V-path are  
all run w/ same H<sub>2</sub>O).

1348 Zooprocess validation from 6/2/13

Raw images:

# of Anthids: 8, 8, 8, 2, 5, 6, 6, 7, 10,  
9, 10, 5, 9 tot. 93

File = flowcam\_20130602-stn157-S-A (raw)

Work images:

# of Anthids: + + + + + + + + + + + + + +

+ + + + + + + + + + + + + + tot

file = 20130602-stn157-s-a

Meso FlowCAM:

67 10.05 6905

0 13.064

B<sub>2</sub>O<sub>2</sub> = 400 vol conc D<sub>2</sub>O  
45.4 vol pump 50 vol

Comment = 40000

B<sub>2</sub>O<sub>2</sub> = 50 vol. conc.

xx vol. pump

~~100,000~~ comment

ee

6/5/13

Station 159 (short)

=surface only

1330 Made ref water

- Started CDOM mapper  
→ crashed, error b/ overflow  
- restarted

1350 calibrating Accuri (peaks)

1600 count cal + sample (Accuri)

1615 FCAM Auto started

CDOM mapper is still going; spectra look  
generally correct but noisy (?)

1650 Did de-bubble + refill on IFCB; restarted a

1720 After debubble + refill, positions in  
the ROI vs. peak plot look good.

FCAM capture x vs capture y looks  
good. Also, changed the min.  
diameter to 20 μm... 10 gets  
too much junk (unidentifiable)

1857 CDOM mapper still running, data looks  
okay? proper general shape  
but noisy?

Mon 6/6/13 \*check flascam & comp. free space!!

Tmnw: clean ALFA (when flow thru is stopped)

- send screen shot to LKB
- validated predicted vignettes
- email re: CDOM mapper

6/6/13 Station 160 (short station)

0700 made new ref water (and replaced  
psu = 35      CDOM mapper  
local = 35.17      ref + HPLC - H<sub>2</sub>O)

0725 Prepped bottles w/ stickers, got new  
empty logsheets  
Turned on Accuri

0730 Running Accuri peak cals  
U-path ref water dishes

0745 checked IFCB images

0755 IFCB focus is ev, x settings MAF

350 >> 350 >> 350 >>

350 >> 350 >> 350 >>

350 >> 350 >> 350 >>

350 >> 1400 pos

350 >> 1750 pos

(all objects now out of focus, going  
back the other way)

Back to zero pos



IFCB focus:

6/6/13 -350 pos  
-700 pos  
-1050 pos  
-1400 pos  
-1750 pos

0800 Rosette arrived - go to get H<sub>2</sub>O + filter CDOM

0825 -2100 pos  
all objects out of focus  
-1400  
-1050 some in focus now  
-700  
-350  
-300  
-250  
-200 getting better  
-150  
-100

0830 Turn off U-path, start cleaning cycle. First put  
Accuri + FCAM samples in fridge.  
Added new MeOH + citranox to cleaning bottles  
(pour outside)

0841 IFCB - made small motor step=20  
-80 pos  
-60 pos Some still out of focus  
-40  
-20 most in focus  
0 pos

6/6/13

0833 IFCB focus (u-path running also)

+20 pos

70 pos

Small step 10

50 pos most things in focus (best yet)

60 pos

50 pos

Small step size = 5

45 pos

40

35

0930 Finished u-path. Dumping waste.

0940 Started FCAM-A (made filt. seawater  
for primary + filtered sample thru  
300  $\mu\text{m}$  mesh).

Making Accu count standard  
- filtering accu sample + FSW

0950 Started a new ALFA file  
Running count + size cals on Accu

0955 IFCB focus end on pos = 55  
- check processed image files  
from after this time later.

1100 Running FSW + Samples on Accu.  
Running FCAM Trigger mode  
Running CDOM mapper w/ new .exe!  
- spectra look pretty good, emailed Alan one.

6/6/13

1300 After lunch - did all lab dishes + started  
Zooprocess on FCAM computer.  
\*Always check disk space on FCAM  
comp. before running samples\*

→ new Z: drive on FCAM computer - for this  
is the back-up drive. In general,  
work on the C: drive, which is  
the local FCAM comp.

158 0603 - 0746 67.082

W 0°.150

A -20 SRF 37.04 mL

① 6/7/13 Same as yesterday. - short station 161  
Made new sheath fluid for Accu  
Did a longer manual cleaning on  
CDOM mapper.

0944 IFCB debubble + refill

6/8/13 Short station #162

0800 CDOM mapper - water <sup>(fluids)</sup> appears to be going in but not coming out... open later to look inside.

- Made new Accur size stocks
- peak cal's have doubling, tried backflush + unclog, did not work, removed SIP to clean top w/ squirt bottle. Running DIW for 15 min (did 2x B-flush/unclog first)
- Accur peak cal looks a little better after SIP removal + cleaning



6/9/13 Long Station #163

0630 made ref water - 35 psu  
local = 34.8  
temp = 21°C

0700 New ALFA file  
checked IPCB images - lots of blobs, mostly small single cells, many not in focus.

CDOM mapper: opened yesterday, is leaking inside somewhere. Unplugged per Alan's instruction + waiting on him for more info.

6/9/13

0922 "Backflush with sample" on IPCB (Stop acq. first)

0940 Checked IPCB camera - screen was black, \* stopped acq., unchecked backflush, start again.  
\* nevermind - checked "view images" and it was fine.  
LOTS of blobs post-backflush!

Many particles in the water today - for the first time I had to use two filters to get thru the CDOM filtering

#163

Running FCHM. File naming error:  
flowcam-20130609-stn163-S-A SHOULD BE:  
flowcam-20130609-stn163-B20-A  
AND: flowcam-20130609-stn163-S-A=true  
is the real file of that name.

1730

# Make new 6+8 peak beads.  
Flipped top o-ring on SIP.  
Re-running cal's.  
Looks bad - droplets.  
Tightened SIP collar. Looks good!  
Re-running FSW + Sample.

6/10/13 2<sup>nd</sup> day of long station-only meso water from rosette.

Using cont. flow water for Accuri, Upath, FCAM (also meso in FCAM)

- Made new Accuri sheath fluid

Lab room is hot today. (heaters are on)

Accuri cals still look good.

Upath ending IMAX was 83%?

Why so much higher? Ask Joanne.

6/10/13 Daily cast will be @ ~2pm

0923 Running extended cleaning solution on Accuri

1600 Noticed IFCB was not restarted after last night's AC-S cleaning - start aqg.  
Ultropath reference spectra look good.

AC-S filtered flow is back up to ~7l/min (was down to ~1 yesterday)  
after new filter.

6/12/13 Short station. #165

0730 Made new ref. water.

0800 Started up Accuri, running peak cals. Added more decon + cleaning fluid to bottles (did not mix more; already had some in mixing bottles).

- mixed more diluted bleach for FCAM cleaning.

0900 U-path ref is exactly at zero all across...?  
→ axis values were off - needed click on the small "LY" button to make it pink instead of the "LX" button being pink

- had to click the button again. Data + ref. look nice.

1030 ~~disk~~ IFCB debubble + refill; backflush w/sample

6/13/13 LAST station! #: #166 (Daily/short)

w/backflush left on on IFCB, data look good... maybe this will help?

- O N. M.
- 6/13/13 - List of things to tell next day lab person → dry lab report  
 - FlowCAM # of vignettes

6/15/13 w/ Joannie in Tromso

- 1) ALFA cleaning &
- 2) COOM Mapper (get it working?)
- 3) FlowCAM (change flowcell + calibrate) + syringe cleaning
- 4) Accuri - change filters + pump tubing
- 5) Ultrapath data
- 6) chemical inventory + dilutions

ALFA: • 2 cleaning cycles. The 2<sup>nd</sup> was done to try to get a better spectra as we still could see a little peak ~ 625nm

• the 2<sup>nd</sup> cleaning cycle improved it a bit but still see that little peak

• Mark from Ali's email & Alexander think it could be due to air in the instrument. They suggested filling the instrument with MilliQ water via gravity (no pump) & MilliQ bottle as high as possible. Joannie & I will try it today (17 June)

2) COOM Mapper: its works → we tried with sampled offshore water (flow COOM) and were not successful. We added the tec. drops and got a beautiful spectra ... then diluted with filtered sea water.

3) <sup>Accuri</sup> <sup>filtered</sup>  
 - The sample (seawater + tec. drop) was analyzed with ultrapath (50cm pathlength): same shape for spectra but relative values were a little higher with COOM Mapper C410nm: COOM Mapper 1.17 1/m ultrapath 0.7 1/m

• We noticed with COOM Mapper unconstant (no replicable) spectrum within its 10 samples!

(-) For unknown reason, do not continuously work? software

4) FlowCAM: <sup>We</sup> Change the flowcell & clean the syringe & filled the bleach 10L bottle & calibrate for size & count beads.

- Created a new context file: run-sample.context Tromso.ctx

5) Accuri: changed filters into bottles + inside Accuri  
 - we changed tubing into 2 pumps inside Accuri  
 - We will (17 June) look at results and Ali will demo.

5) Ultrapath data looked good.

6) Chemicals: 2.5L Citanox to be diluted

5L Methanol or more to be diluted  
(Dilute) Fill up the 5L 250mL bottles 0.1N (or 1%)

See Mats, Geraldine, Sophie and/or Mancino at NPI  
or Gerald or Claudia at CIVAC plan for fumehood