IFCB Protocols for operation on Research Vessels

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Acknowledgements

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1 Initialization

Illustration of section 6 from the user manual will help to follow the steps described below.

1.1 Fill the two cartridges

⚠**Wear gloves as biocide might have gone in the filter.**
Use DI water or filtered sea water (FSW) depending on your samples
Start with one of the filters

1. Fill one of the big syringes through a blue tube with DI or FSW
2. Open the top and bottom valve of the filter (top does not need to be fully open)

⚠**Watch for the o-ring from the bottom screw (it can pop easily).**

3. Plug syringe in the bottom port of the filter catridge
4. Fill the cartridge, it usually takes about two full syringes
5. Close the top valve after filling with the first syringe
6. When ready with the second syringe open the top valve again and continue to fill until water begin to drip from the top port
7. Close the top of the cartridge, remove the syringe and close the valve of the bottom port

Repeat the operation with the other filter

1.2 Run debubbling

⚠**Put the intake and outtake tube of the instrument in a sample** (e.g. DI, FSW, bead solution)

1. Run on pump 1
   Tab Hardware > Pump 1 > On
   It might already be on
   Make sure Pump 2 is Off
2. Start debubbling (2 to 3 times)
   Tab Fluids > ☑Active is checked
   Tab Fluids > ☑Refill after debubble is checked
   Tab Fluids > Click on Debubble button
3. Repeat operation (1,2 and 3) for Pump 2
4. Turn Off Pump 2 and Turn On Pump 1

1.3 Fill Reagents (bleach & biocide)

⚠**Wear gloves**
Bleach Bag: Use cleaning solution (Contrad 5% + Terg A Zyme 1 % of total volume) to fill the bag
⚠ Bleach is deprecated seems to be at the origin of broken pipes on the IFCB, and would not be as good as other cleaning products: Micro90 or Cleaning solution.
Biocide: 400 ml, 5 % Sodium Azide solution (for a month long expedition, <100 mL is used)

1. Fill each bag of reagent by the intake port (transparent with blue zip ties) using the dedicated syringe, remove the extra air using the outtake port (gray and black).
2. Tie the two bags upside down with zip ties. (look at section 6.3.2 of Manual for more details)
3. It is recommended to strap the bags with the blue Velcro.

1.4 Fill beads solution

Prepare sampling solution: 130 ml 5 % Sodium Azide (⚠**Wear gloves**)
0.5 ml lab soap (Micro90)
80 µl concentrated bead suspension

1. Put absorbing paper on the electronic below the instrument's plastic syringe
2. Disconnect the tube at the bottom of the IFCB's plastic syringe that contains the beads solution
3. Plug the syringe to the instrument's syringe and fill it

1.5 Set instrument in housing

Setting the instrument in the housing is essential to protect it. Software consideration are the following:

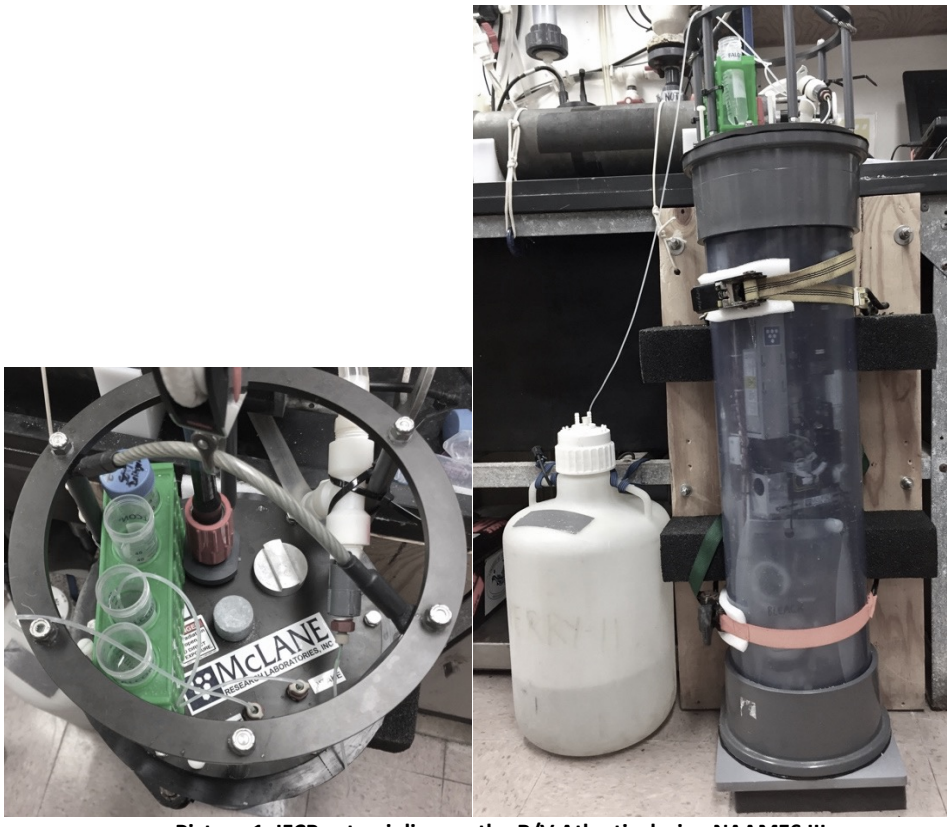
- Disable auto start for inline system (only done once on a computer stack)
- Make sure the IFCB will be able to connect to the network used for remote desktop. The network should be set to Office or Home.

Set instrument in housing:

1. Close exhaust and intake ports on top of the instrument with caps
2. Try to put the front (lasers + bags) to the same side as the laser sign on the can
3. Unscrew the 4 white screws on the top of the instrument
4. Turn off the computer of the instrument
5. Unplug all wires (screen, USB keyboard & mouse, power supply, ethernet)
6. Put the instrument in the housing and screw the 6 metal screws (tighten gently)

1.6 Set the inline system

The IFCB is set on a square piece of foam (~8 cm thick) and is strapped with a combination of soft and hard foam along a wall. To easily remove the IFCB from the housing, the top of the instrument was attached to the ceiling with a system of pulley and rope. There was no issue of vibration on the R/V Atlantis, the R/V Roger Revelle, the R/V Sally Ride, and the N/O Pourquoi Pas ?.
The IFCB is connected to the inline system via a 'T' shaped PVC tube. The intake tubing of the IFCB connected to the T-tube should be as short as possible to prevent cold water from degassing while passing in the tube. The T-tube should be set pointing downwards to let air escape. The intake tube of the IFCB going inside the T-tube shouldn't be too short to prevent drawing water from the stagnant area of the T-Tube. A 150 µm Nitex mesh is mounted on the intake to prevent clogging the instrument.
⚠ Check on the IFCB that the **time zone is on UTC** and that the **time is synchronized with the ship's server.**



Picture 1. IFCB setup inline on the R/V Atlantis during NAAMES III

2 During cruise

2.1 Recommended list of consumables

- Lab soap Micro 90 to clean the instrument (need to dilute at 2 %)
- Cleaning solution (Contrad 5% + Terg A Zyme 1 %)
- ~~Bleach to clean the instrument~~ (DEPRECATED)
- 150 µm Nitex mesh, small heat shrink tube
- beads and/or culture of phytoplankton (e.g. Dunaliella)
- 50 mL Falcon tubes

2.2 Connecting to the instrument

Two options are available to connect to the instrument:

- The instrument is outside the housing:
    1. Directly plug a screen, keyboard, and mouse to the USB port of the IFCB
    2. Start the IFCB by plugging the power supply
- The instrument is inside the housing
    1. Connect the Ethernet cable to a router
        - Note: if the IFCB was never plugged to that network before, the Remote Desktop might not be accessible. To enable remote desktop on the IFCB (follow procedure to connect to instrument when it's outside the housing), switch the network settings of the IFCB to work or home network (not public). In addition, if it's the first time you do a remote connection with the IFCB make sure that it authorize remote desktop connection from any computer (in system properties of the IFCB); there is a bug in windows and you might have to de-authorize and re-authorize the remote desktop a couple of times.
    2. With another computer, running Windows 7, remote desktop IFCB107

Remote Desktop can be found by searching for "remote desktop" in the start menu search

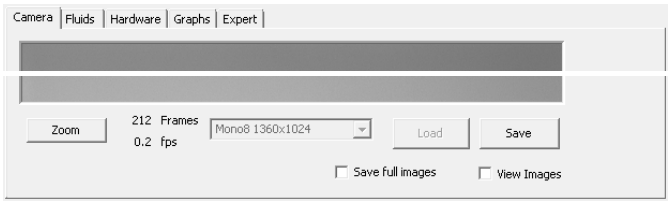
⚠ **Make sure that the intake is in the sample before starting IFCB acquire**

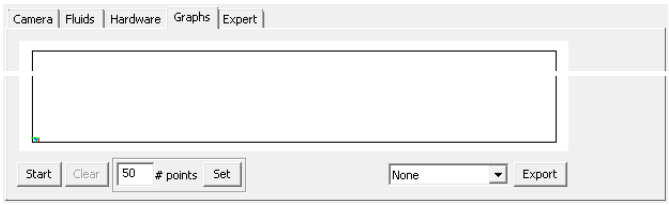
⚠ Check Temperature and Humidity frequently (prefer using the dashboard as the hardware tab might generate DAC errors).

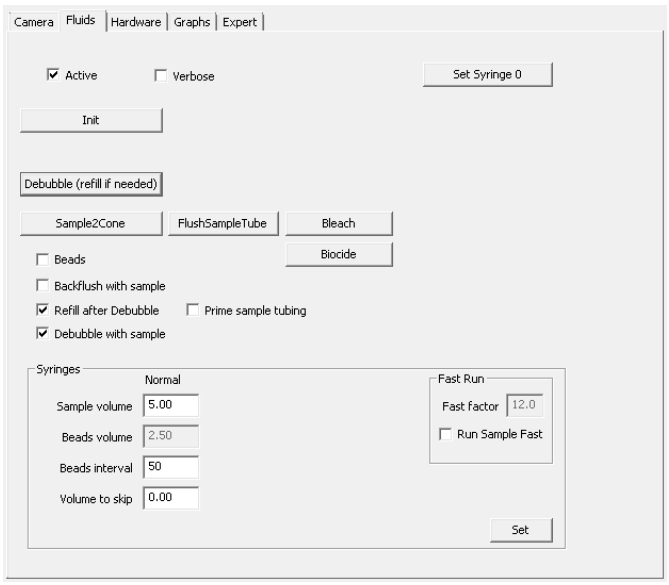
⚠ Do not plug IFCB on unprotected network, as virus protections and windows are not up to date.

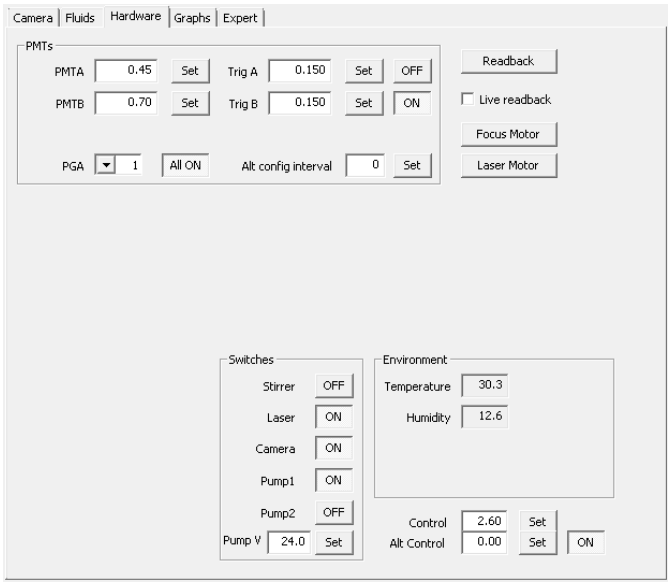
Note: When starting IFCB Acquire quickly setting the syringe to 0 prevents the IFCB from automatically starting the acquisition.

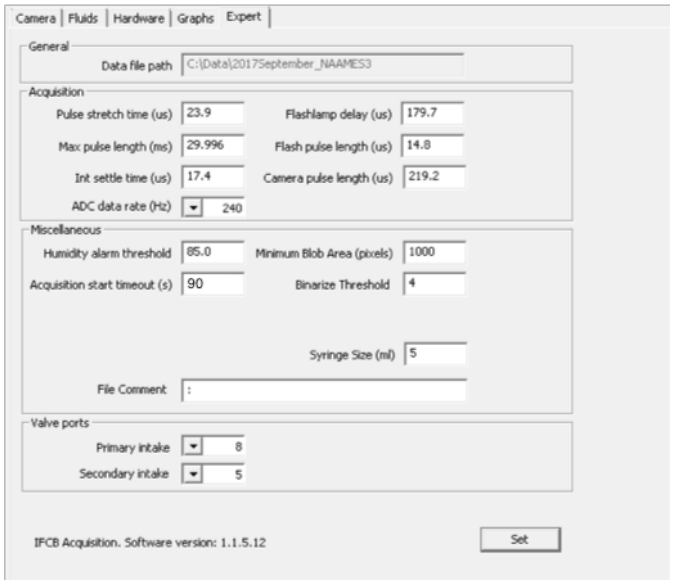
2.3 Default settings to run IFCB Inline

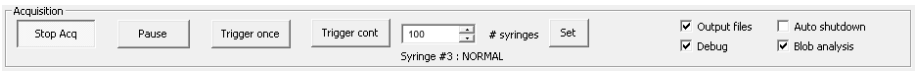












Screen shots from IFCB acquire setup to run inline samples. All parameters should be set similarly.

View Images (Camera tab) and *Start* (Graph tab) should be run occasionally to make sure that the IFCB is operating properly. However, they should stay off the rest of the time as they are slowing down the system and affecting the quantification abilities of the IFCB.

The gain of *PMT B* (Hardware tab) is set between 0.65 and 0.70 during cruises (for IFCB107) this parameter can be modified at the beginning of the cruise if the number of trigger is too low or the particles that triggers are too small to be recorded as ROI. However, PMT settings should not be modified often as it alter the quantification abilities of the IFCB. Note that those values are specific to each instrument.

Expert tab: No parameters should be changed in that tab, except the *Data file path* that should be set to the directory of the current cruise.

The *temperature* and *humidity* of the IFCB should be monitored frequently and stay below 35 °C and 20 % respectively. Prefer using the dashboard as the hardware tab can cause DAC errors.

With these settings, the instrument will clean itself automatically approximately once a day (every 50 syringes) running beads, biocide and bleach. However, the auto-clean is not enough. Every time the IFCB is stopped for an extended period of time (> 2 hours), inject biocide with both pumps on and if time permits, let the pumps run 15-20 min before closing IFCB acquire.

2.4 Run a sample

There is carry over from one sample to another depending on the concentration of the previous sample. It is good practice to flush (i.e. rinse) the system one to three times without recording the data. When doing so it's possible to save time running the sample fast and with lower volumes (1 mL instead of 5 mL). Note that the entire syringe barrel won't be rinsed if the volume is less than 5 mL.

1. Turn On desired trigger (PMT A: scattering and/or PMT B: chlorophyll *a* fluorescence)
2. Set the number of syringe to run
   >200 to run several days on the inline (~50 runs / day)
   0 or 1 to run a discrete sample
3. Check that settings are set according to previous section
4. Start acquisition

When ending last sample from underway: change # syr set to 0 and it will finish that sample and be done. You get a complete file while not having to sit around and watch and wait for it to finish.

2.5 Cleaning the instrument

Cleaning should be performed each time the IFCB will be stopped for an extended period of time (>2 hours) or daily when running continuously (it's done automatically with the bead run). To clean the instrument run each of the following step twice, once on each pump.

⚠ Make sure intake is submerged in solution because the IFCB will pump fluid for flushing.

1. Run the cleaning solution (Contrad 5% + Terg A Zyme 1 %)
   In tab Fluids > button Bleach
   It will empty air from tubing
   Takes ~5 min
2. Run biocide
   In tab Fluids > button Biocide
   Takes ~1 min

2.6 Run bead solution

The bead solution is used to adjust the focus of the camera, its alignment, and troubleshooting the instrument. Cultures of *dunaliella* are also very good for the same purpose and recommended for the focus adjustments as leaving cells tend to be in a different focal plane from beads.

1. In fluids tabs check boxes as follow:
   ☑ Beads
   ☐ Backflush with sample
   ☐ or ☑ Refill after debubble ☐ Prime sample tubing
   ☐ Debubble with sample
2. Set sample volume <5 mL (2 mL is usually enough)
3. Set number of syringes to 0
4. Start acquisition

2.7 Other

When plugging back the inline system after running discrete samples, it's a good idea to prime the intake to remove any air that would have accumulated in the line. It can be done by running a sample fast as in the section 2.4.
A run is ~20 min, if you check debubble it will take ~22 min.

2.8 Stop the instrument

1. Wait for the acquisition to finish. Stopping the acquisition in the middle of a run is fine but will prevent to use the sample for quantification analysis. A trick consists in setting the number of syringes to 0 and it will finish that sample and be done.
2. If the acquisition is stopped manually, empty what is left in the syringe with Fluids Tab > Init
3. If the IFCB is stopped for an extended period of time (>2 hours) **inject biocide** (Fluids Tab), if you have time, leave the pumps running for 15-20 minutes after running biocide.

⚠ Do not close the software without stopping acquisition, otherwise the current file will be lost.

3. Set the number of syringes to 0 to prevent IFCB Acquire to start sampling automatically next time it is started.
4. Close IFCB acquire
5. Turn off the IFCB:
   - From a Remote Desktop Session:
     a. Open command prompt (write cmd in the search area of the start menu)
     b. Type: shutdown /s
     c. The Remote connection window will close automatically (it takes about 30 seconds)
     d. Wait 2 minutes for the IFCB to shutdown properly and disconnect the power.
   - If directly plugged on the instrument (screen, keyboard & mouse):
     a. The IFCB can be turned off as a normal computer.

3 End of cruise

- Fill the two filters with DI water. Alternate running biocide and cleaning solution during a few days (48 hours).
- Before turning off the instrument make sure to set the number of syringes to 0.
- Leave DI water in the filters to prevent flow-cell from drying and in case of leak: DI is nicer than FSW.
- Put caps on the intake and exhaust of the instrument on the top plate to prevent the flow-cell from drying. In case caps are lost: another option is short piece of Si tubing with knot at end.
- Tape the desiccant bag on the bottom plate of the IFCB to prevent it from moving around during shipping and getting punctured.
- Remove reagent bags, in case a line breaks during shipping that minimize the amount of fluids going around.

4 Setting up the dashboard

The dashboard runs on a separate computer that needs to be on the same network of the IFCB. The dashboard is running in a virtual machine (VirtualBox with Ubuntu). To start the virtual machine (VM), start the software Git Bash. Enter the following commands:

```
cd .\ifcb-dashboard
vagrant up
```

To stop the virtual machine before shutting down the computer:

```
vagrant halt
```

The dashboard is accessible on the same computer as the virtual machine at this address:

http://localhost:8888

If you would like to connect to the dashboard from a different computer, you have to adjust the configuration file dashboard_conf.py in the ~\Documents\GitHub\ifcb-dashboard directory.
Replace the line that reads:

```
DASHBOARD_BASE_URL='http://localhost:8888/'
```

With:

```
DASHBOARD_BASE_URL='http://mycomputer.something.edu:8888/'
```

Where `mycomputer.something.edu` is the ip address of your computer or the url to access it.
Note: don't change WORKFLOW_URL.
Now restart the dashboard (make sure that the virtual machine is on):

```
vagrant ssh
sudo service apache2 restart
```

To access the administration section of the dashboard go to admin at the bottom of the main page. The default login is admin@whoi.edu and the default password is 12345678.
It is possible to force loading the last data in the administration section going to Time Series > Accede.

Set-up the dashboard shared directory (to update dashboard every ~40 min):

1. Create a folder data in ~\Documents\GitHub\ifcb-dashboard\
2. Create a sub-folder with your data set for example
   ~\Documents\GitHub\ifcb-dashboard\data\IFCB107_NAAMES03
3. Use a tool such as SyncToy to synchronize this folder with the IFCB shared data
   a. Set windows task scheduler to repeat the task frequently (~10 min)
4. Go in the time series section of the administration part of the dashboard
   http://localhost:8888/admin#/time_series
5. Add a new time series:
   a. Label: IFCB<serial_number>_<cruise_id> (no space)
   b. Description: <cruise_name> <month> <year>
   c. Enabled: True
   d. Live: True
   e. Path: /vagrant/data/<name_of_sub-folder>
      Ex: /vagrant/data/IFCB107_NAAMES03
   f. Data Type: raw
6. Save & Click on Accede Button, the following message should appear:
   Data found, accession initiated for <Time_Series_Label>
7. To accede data automatically set a crontab in the VM or with the Windows TaskScheduler:
   curl http:/localhost:8888/<Label>/api/accede

More information on the dashboard are available on:

- Wiki (https://github.com/joefutrelle/ifcb-dashboard/wiki)
- FAQ (http://mclanelabs.com/imaging-flowcytobot/ifcb-user-group/)
- Joe Futrelle (jfutrelle@whoi.edu) developer of the dashboard

⚠ A strong internet connection is required to install the dashboard as VirtualBox and Ubuntu will be downloaded, don’t wait to be at sea.

5 Troubleshooting

5.1 Horizontal alignment

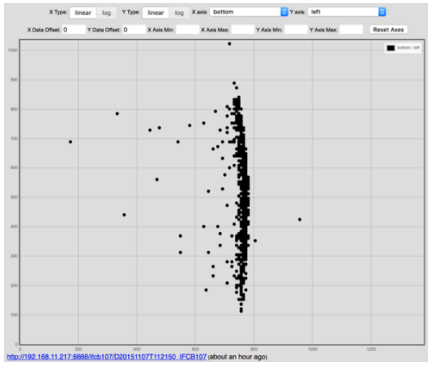


Figure 1 - Late horizontal alignment

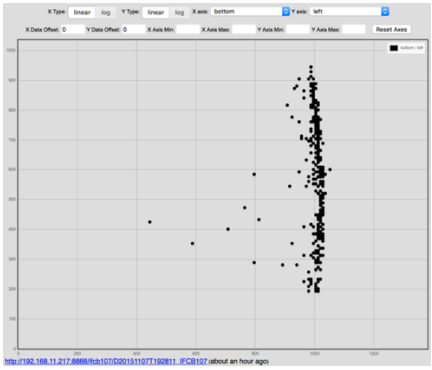


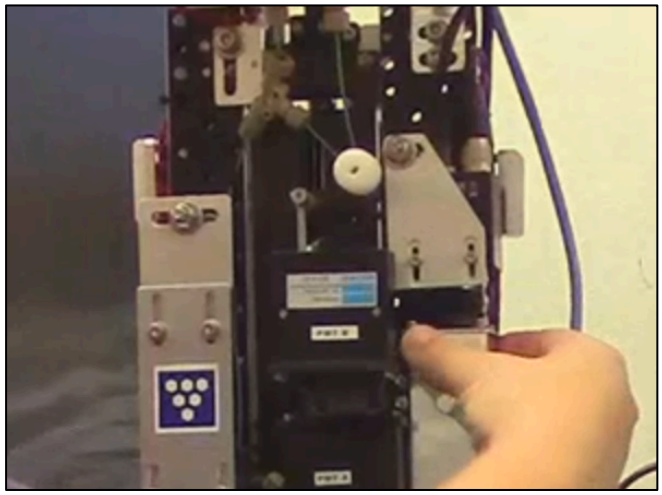
Figure 2 - Good horizontal alignment

The two figures above show a good and a late trigger timing. This can be adjusted in the Expert tab, by setting Flash lamp delay to a shorter or longer time. The acceptable range of value is approximately 60 to 200 microseconds.

Note: Expert tab might not appear on your software. If this is the case: close the software IFCB Acquisition. Go to C:\Program Files\McLane\IFCB\ open ifcb.cfg and line 64 replace 0 by 1. Restart the software IFCBacquire.

```
64)    1              : expertTabVisible
```

If you can't set the horizontal alignment properly with the software, you have to open the housing of the IFCB (after stopping acquisition). Then unscrew the four screws on the right column holding the plate below the camera (see figure below or minute 10 in video MVI_0190.MP4) and loosen the nut holding the vertical camera plate. Adjust the camera position, using the real-time visualization of particles in the Camera tab check the box "view images" while running a sample. It is recommended use a sample containing a high concentration of small cells such as *Dunaliella*. Big cells and low concentration makes it harder. The bead solution works relatively well too. Be careful to keep the horizontal alignment (check it in the graph tab). More details on this procedure can be found in the manual at the section System Alignment Step 4 – Adjust Camera Position page 114 or A-6.



Picture 2 - Camera alignment

5.2 Vertical alignment

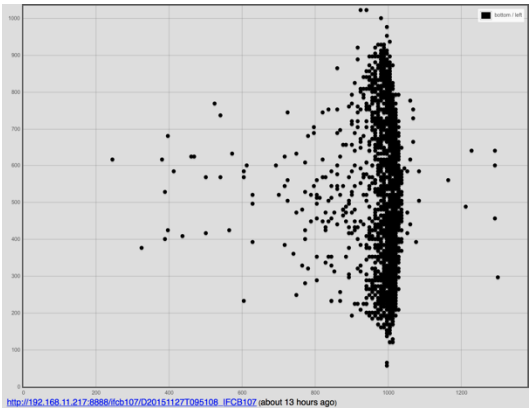


Figure 3 - Good vertical alignment

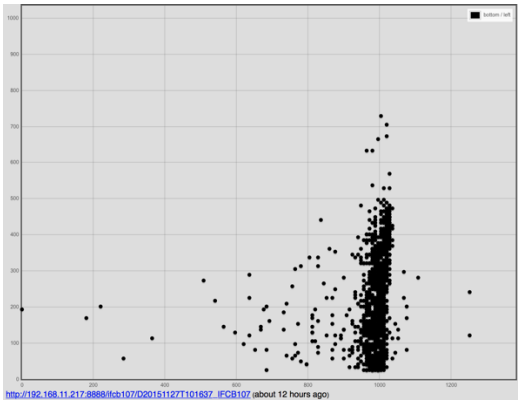


Figure 4 - Bad vertical alignment

The vertical alignment (see figure above) suddenly shifted. Many long chains of *Chaetoceros* were observed when that happened long ROI (see picture below).

Email from Ivory Engstorm:

This may be a partial blockage within the injection needle or a bubble stuck in the manifold. In this case, the core stream can be biased toward one side. It sounds like you have already run the standard cleaning procedures and this may have only partially helped.

I have had blockages that I was able to clear mostly using Clorox. First, I turn off the sheath pump and run two "sample2cone" of 5mL each of 10% Clorox. Then I run a sample of the same 10% Clorox at 2X speed. Usually one or two samples of Clorox can break up the blockage. Finally, I will turn the sheath pump back on and run a couple of "debubble" runs. This should allow any remaining material to be pulled back into the syringe and ejected to exhaust.

Alternatively, there may be a stubborn bubble in the manifold that is redirecting the core stream. To cure this behavior, first turn off the sheath pump, then run two "sample2cone" of 20% Micro90 at 5mL each and allow to sit in the manifold for a couple minutes. Finally, turn the sheath pump back on and perform two "debubbles" and this should remove any air in the manifold.

I hope this helps; I'll be traveling tomorrow and will have only occasional access to email, but will check my messages when I can.

5.3 Bubbles and Horizontal alignment

The IFCB ran for a few minutes or several hours in the air? Debubbling is not working? The position of the ROI are “random”? There is certainly air in the sheath fluid.

Email from Taylor Crockford to solve the issue:
Bummer about running so much air. I'm going to include quite a few things to try in this email.

It is likely that a lot of air has built up into the filter cartridges. This is not necessarily the end of the world, but could potentially be the culprit of these bubbles. Has it been rough weather during this post-air sampling? It could be that the level of air in the filters is tolerable during calm conditions, but once the ship starts rocking, occasional bubbles could be dislodged from the filter system and make their way to the flow cell. There are ways to deal with this by taking the instrument out of the can, but but we usually try to avoid that while at sea if possible. Because in general, often times when we start taking things apart, it ends up causing different problems than what we started with.

Are the bubbles are always at the end of the sample run? How big are these bubbles you're talking about? If they're giant bubbles then yes, definitely a problem. If the bubbles are small, the images look good, and roi position looks good, then maybe don't worry about them. Just keep a closer eye on the files.

First thing I would do is check if roi position looks good for each run. It is possible that images look great but the core is actually out of position and you're missing triggers. (I've had this happen before.) In this case, bad roi position is definitely an indicator that bubbles are causing flow problems. An easy way to check position is using the dashboard. Usually your 'view' is set to 'mosaic' because you are looking at images, but you can click on 'plot' and that will show roi position. Roi position (bottom vs left) is the default plot that comes up. The axes defaults are the full field of view with xlim=0-1400 and ylim=0~1030. The core should be towards the right side of the x-axis (maybe around 1,000 give or take), and pretty centered in the y-axis. If the core is out of position, then you need to deal with the air because it is causing problems. Check the position of several files. If position looks okay and fairly constant, and the bubbles are a few and somewhat small, then maybe you don't have to worry about it. Just keep a close eye on things. I will send a separate email with an example image to make sure this email goes through.

You can also use the plotting function to investigate if the bubbles are being run at the end of each sample. If bubbles are always at the end, then this would suggest that the syringe is injecting air at the end of each run. There are a number of different options on want to plot. Roi position is just the default. Select x-axis = TriggerOpenTime and y-axis = width or height. This gives you a timeline of the size of each roi over the course of the entire sample. Then you can use the mouse to drag a selection box over any part of the plot. Drag the box around the end of the run. This will give you the set of rois along the right side of the screen. Also, if the bubbles are giant and at the end of the run, then the plotted points will infer this just from size. Don't select a ton of points all at once because this can bog down the browser.

One thing to note is that running many debubbles in a row can actually be problematic because you end up creating a sort of negative pressure situation in the system. There is a one-way valve in the exhaust line that prevents back sucking water into the system. So each time a debubble is run, you're sucking up water without adding water back into the system. If you do this several times it can start causing degassing within the instrument. **A way to get around this problem is to alternate running a debubble and running a sample fast.** It is definitely important to have 'debubble with sample' checked which is great that you have already done that. And I agree multiple debubbles is the first thing I would try, so maybe try several rounds of debubble/inject sample fast. To run fast, in the fluids tab there is

a run fast option on the bottom right. I generally leaving the 'fast factor' at 15 and just check the box and press set. Definitely don't save these runs because flow will be bad and rois will be out of focus and all over the place. I wouldn't go any faster than 15x fast.

If you are seeing bad roi position (and thus bad flow), another possibility could be you have a stubborn **air bubble stuck somewhere around the flow cell. In this case, I find it helps to run a little detergent.** I like to use a dilute (~2%) solution of micro which helps change the surface tension inside the cone/flow cell area. To do this,
1) turn off sheath and inject 1-2ml of 2%micro, can run normal or fast
2) with sheath still off, run debubble debubble
3) Start running sample fast and turn on sheath part way through. If you have view rois on, sometimes you can see a large bubble get sucked upwards through the quartz.
4) debubble sheath on
5) now run a fair bit of seawater to flush the micro from the system. I generally use ~5-6 samples of 5ml filtered sea water 15xfast

I know this was quite a bit of information. Please let me know if you have any questions. Try some of these things and let me know how it works out. I have a more advanced list of options if this isn't working but like I said we try to avoid taking the instrument out of the can if we can get around it.

In summary:

1. check roi position
    a. can you tell if bubbles are frequently at end of sample?
    b. are the bubbles giant?
2. try several sets of debubble, run 3-5ml fast
3. run a bit of micro
4. try debubbling sets again
5. decide if bubbles are not that big a deal and just keep a close eye on things if you're satisfied with the runs
6. buck up and get ready for more advanced troubleshooting

Additional trick from Taylor: once I had a stubborn bubble and tried injecting 2% micro and then debubble with pumps off. The first attempt failed, but the 2nd attempt I physically shook the ifcb with my hand (gently). It was out of the can so I could see that the little bit of movement helped dislodge the bubble during the debubble.

Email from Ivory Engstorm:
Sorry for the late reply; I'm out on the west coast at a conference. Taylor pretty much summed up what I would have suggested. If ROI position or focus are variable, then it is likely that small bubbles are disturbing flow or alignment is slightly off.

When I have had to deal with bubbles I will generally turn off the sheath pump and perform a sample2cone with 5% micro90 and let that sit for a couple minutes. After a few minutes, turn on pump1 to flush the system and run a few seawater samples with debubble enabled.

Just to confirm, only pump1 is active correct? Having both pumps on will cause varying performance.

The only other cause I can think of for varying ROI position would be misaligned horizontal laser position. You may try adjusting remotely while running beads to optimize the position.

Another email from Taylor Crockford:

It sounds like the cone is/was completely full of air and the system is having a tricky time filling it back up. Often times if there's a ton of air, you will manage to get some fluid into the cone and then the sheath will sweep it away and the cone will be empty again. I would suggest:

1. inject sample with sheath off
2. turn sheath on for about 30sec
3. turn sheath off
4. inject sample
5. repeat maybe 3 or 4 times

If you inject this much water into the system with the sheath off, it would be best to use filtered seawater if possible. If sheath is off then you're basically injecting a bunch of junk into the system that will sit around in the cone and other places and was not filtered. Not the end of the world if you don't use FSW because it will eventually get filtered out, but would be good practice.

If flow still isn't great, then set up the software to do a series of run sample fast with debubble before sample. You can just set it up to run for an hour or so and come back (like 40 samples or something). Or if you want to sit there and check after several, whichever you feel.

There could also be a bubble stuck just below the flow cell. I find those bubbles particularly tricky to get out. That's when the micro trick can be really helpful.

5.4 Camera Alignment

I could not get the camera properly align screwing the four flange screws on the panel, in fact, to get a good alignment I just screwed one of the flange screws of and the top nut. The three others being covered by the plate. The flow-cell chamber is properly aligned and looked exactly like in the picture page A-4 of the manual Rev_.18.A.08. Is there anything else I could adjust to be able to screw the four flange screws ?

Answer from Vinnie Ferreira

There are circumstances where a shim is added to the silver mirror which reflects the image 45 degrees up to the camera. I am not sure if your instrument contains such shim. It can be identified by a small piece of plastic material on the top side of the mirror varying in color depending on thickness. I would start by just checking if there is a shim but not removing it as this would also change the angle of the laser light reaching the PMTs. You can try removing and reinserting the silver mirror as sometimes this may result in a better fit and affect the image framing.

5.5 Horizontal and Vertical alignment

If an improper alignment of the ROI is noticed, it can be caused by a low speed of the sheath fluid caused by the sheath pump running slowly. A simple way to check for this is by using the other pump, if ROI are properly aligned with the other pump it means that the original pump has an issue and needs to have its voltage reset (email below) or be replaced.

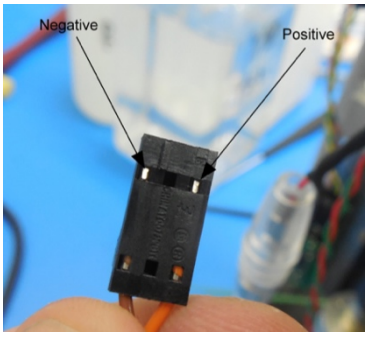
Email from Ivory Engstorm:
Here are instructions I have for resetting the sheath pump voltage.

1. Download TeraTerm at (https://en.osdn.jp/projects/ttssh2/downloads/59957/teraterm-4.80.exe/) and then copy the file to a USB drive and drag onto the desktop of the IFCB (TeraTerm should already be available on desktop of IFCB107)
2. Extract/run the installer, installing on desktop is fine.
3. Launch the program (TeraTerm), and click cancel when the new connection window launches.
4. Click on Setup >> Terminal
5. Change both New-Line Receive and Transmit to be "CR+LF" and check "Local echo." Click OK to apply settings.
6. Now click to Setup >> Serial Port
7. Verify COM1 is selected, change Baud rate to 115,200 and leave other values at defaults. Click OK to apply changes.
8. This will launch a session where we will talk directly to the housekeeping board (note that IFCBacquire cannot be running while we do this).
9. To query the digital potentiometer that sets the pump voltage type " $E " and press Enter (no quotes).
10. This will return the digital value at this component ("128" I expect)
11. First flash the chip to half-scale output by typing " $E128 " and press enter (no quotes or spaces).
12. To flash the chip with correct value type " $E255 " and press enter (no quotes).
13. Now when you repeat the query command " $E " the response should be 255 (which is full scale, 24V).
14. If the command returns the correct value, then go ahead and quit the program and launch IFCBacquire, you should be all fixed now.

Hopefully, the voltage just dropped when you experienced the DAC error.

Did you try changing the value to 128 and then back to 255? We've found that sometimes the chip in question will report 255 (full scale) but that it is only reading back the EEPROM value. This is why I added a line to change to half-scale (128) then back to full scale (255). You can always verify the sheath pump voltage by measuring the DC voltage between the pins of the harness (unplug from pump and measure across pins, picture attached).

Generally speaking, if the core is wide this doesn't suggest a flow-cell alignment issue but rather decreased velocity of the sheath fluid (by way of low drive voltage), density/temperature/salinity contrasts, a blockage or bubble in the system. Also, if the "run sample fast" check is active under fluids you will produce a very wide core.



5.6 Acquisition restart unexpectedly

Symptoms

A few seconds/minutes after starting the acquisition, the IFCB stops the acquisition and starts a new acquisition. The log of the IFCB shows the following error:

```
ERROR IN FluidicsQuery: unexpected return string in opto
```

This event happens randomly, some acquisitions work well, others crash.

Explanation from Ivory Engstorm

It seems that each sample run lasts only 90 seconds, which is the default timeout for the sample to restart when no triggers are received. This is an error recovery routine for times when the camera doesn't sign on correctly; we can restart the event and see if it is functioning correctly. However, the software is reporting 'blobs' and triggers so it shouldn't b restarting the sample run.

The error lines are actually expected and don't suggest a hardware issue. This is part of the code where we need to send a command a few times to get a response from the fluidic bus and typically one or more bounces will be seen as an error. I'm sorry that this happens because it is misleading but normal behavior.

I would double-check that both 'blob analysis' and 'output to file' are checked on the lower right of the main window; or try unchecking and re-checking the boxes to make sure the config file is getting the correct value.

Looking more closely in your file log it does look like a number of the "restarts" were associated with "0 records." So this is expected behavior; the system is not receiving triggers and thus thinks there is a camera error and restarts.

I would double check the alignment to see that you can trigger off of beads. Often, I find that after shipping, the laser-horizontal needs a slight adjustment to get triggering again.

To disable the restart behavior for testing; simply change the value of "acquisition timeout" in the expert tab to something larger than 90 seconds (anything over 1200 would mean the timeout never fires during sample analysis for a 5 mL syringe).

5.7 DAC errors

The logs (on the right of the window) displays: “Error writing DAC: sent …”, this error happens sometimes after visiting the Hardware tab. If the error does not solve by it-self when running the next sample, restarting IFCB acquire should solve the issue. However, if you notice alignment issues after encountering DAC errors, go to section 5.4.

5.8 No triggering and camera view does not change

The IFCB does not trigger anymore when you activate continuous triggering you can't see anything flowing, even if the camera operates normally. This is certainly due to a bad focus of both the Laser and the Camera, what might have happened is that for no reason the focus motor of the camera and the laser turned all the way in one direction making both the camera and laser completely out of focus. Start by setting the focus of the camera, Appendix Performing System Alignment, Step 1-2 (from IFCB user Manual). Note that it can help to run a sample with a highly scattering solution (e.g. few drops of Malox or milk in 45 mL of DI) and activate the continuous triggering to confirm that focus is adjusted properly. Then proceed to the laser horizontal and focus adjustment Appendix 2, Performing System Alignment, Step 6 (from IFCB User Manual). Note that it took -150000 steps of focus motor to see the laser beam again.

Email from Matt Bach

You want the laser beam low as that correlates to being higher are you are seeing a flipped view. This allows the particles to pass through the laser than the camera will take a phot base on what you flash lamp delay is. You can move the laser vertically by rotating the red thumb screw on the back side of the IFCB, just about the computer stack. You can adjust this and see if you increase your gains on you ROIy plot, but also pay attention to where your images are located on the camera tab. You want them in the 1/3 section to the right, to ensure if you get any chain like organisms they will be entirely in the field of view.

A good reading for bead is about 0.45 – 0.5 for PMT A and 0.5-0.55 for PMT B, the gain can be adjusted accordingly and the threshold is usually set to 0.15v for both.

5.9 Replace the Camera

After shipping some points appeared on the camera (make sure it's on the camera and not the optics or flow-cell), I have a spare camera with me and tried to replace the camera. The issue I have is that the "new" camera capture images continuously instead of taking picture on a trigger. I noticed the orange LED keep blinking really fast as soon as the acquisition is started with IFCB Acquire.

Explanation from Vinnie Ferreira

Here is the procedure for changing the settings of the new camera.

1. Launch IFCBacquire and ensure you are not running a sample (#syringes = 0)
2. From the start menu, launch GigViewer (Allied Vision Technologies > GigViewer)
3. In the Cameras window, select the only entry under Host and click the wrench icon (show camera's attributes)
4. Find the ConfigFile node
5. Click on ConfigFileIndex, choose 1 from the dropdown at the bottom of the window and click the check button
6. Find the Acquisition > Trigger > FrameStart node
7. Click on FrameStartTriggerMode, choose Syncln1 from the dropdown at the bottom of the window and click the check button
8. Find the ConfigFile node
9. Click on ConfigFileSave and click the ConfigFileSave button at the bottom of the window
10. Click on ConfigFilePowerUp, choose 1 the dropdown at the bottom of the window and click the check button
11. Close GigViewer and IFCBacquire and then relaunch IFCBacquire. The camera should now be set to trigger properly.

5.10 Adjust Camera Brightness

The images are darker than before, is there any brightness and contrast settings on the camera ?

Answer from Vinnie Ferreira

There is an option on GigViewer to increase the camera gain. A default gain of 6 should produce adequate brightness. Here is how to change it:

1. Launch IFCBacquire and ensure you are not running a sample (#syringes = 0)
2. From the start menu, launch GigViewer (Allied Vision Technologies > GigViewer)
3. In the Cameras window, select the only entry under Host and click the wrench icon (show camera's attributes)
4. Find the ConfigFile node
5. Click on ConfigFileIndex, choose 1 from the dropdown at the bottom of the window and click the check button
6. Find the Controls > Gain > Gain Value node
7. Type in 6 on the Gain Value textbox at the bottom of the window and click the check button
8. Find the ConfigFile node
9. Click on ConfigFileSave and click the ConfigFileSave button at the bottom of the window
10. Click on ConfigFilePowerUp, choose 1 the dropdown at the bottom of the window and click the check button
11. Close GigViewer and IFCBacquire and then relaunch IFCBacquire. The camera should now be set with a higher gain.

Recommendation from Taylor Crockford

Try to keep the camera gain as low as possible and adjust strobe voltage before adjusting camera gain.

5.11 Camera not found

Case I: When we restart the IFCB it says that "there is not exactly one camera found, no camera found". We found a work around by unplugging and re-plugging the ethernet cable on the camera while IFCB Acquire is open. Do you have the fix to this one ?

Answer from Vinnie Ferreira
Newer versions of IFCBacquire implement a 12 second wait period before the first acquisition to allow the camera enough time to negotiate its connection with the ethernet port. If you can update IFCBacquire to version 1.1.5.15 or newer, you can take advantage of this feature. Your described workaround is performing this same task but I understand it is very inconvenient to have to unplug/plug the ethernet cable. Sometimes, waiting about 20 seconds after launching IFCBacquire and before starting an acquisition will allow the camera data connection to stabilize without the need to cycle the ethernet connection.

Case II: The camera was never setup with the current electronic stack.

Explanation from Ivory Engstorm
The camera and network adapter both need to be on the same subnet to communicate with eachother. Typically we configure the network port as following: 169.254.1.1 / 255.255.255.0 / blank gateway, and we configure the camera to be: 169.254.1.2 / 255.255.255.0 / 0.0.0.0

Here is an excerpt from our setup document. You may find that you need to set the network adapter connected to the camera as "dynamically assigned IP" in order to communicate and configure the camera if necessary.

1. In order to power up the camera, the IFCB acquisition program must be run.
    a. Start “IFCBacquire” from the desktop shortcut.
    b. The Ethernet status lights should indicate that the camera has been powered up.
        i. The connection will likely fail until the camera is configured (i.e. ‘startAcquire will not run).

2. Start “GigEIPconfig”
    a. The camera should show up in the dialog window now.
    b. Select the camera and click the “change” button
    c. Select “Use the following IP address:”
        i. IP address: 169.254.1.2
        ii. Subnet mask: 255.255.255.0
        iii. Default gateway: 0.0.0.0
    d. Click “OK” to commit changes.
        i. The camera list should refresh and show the new address as “fixed.”
    e. Close “GigEIPconfig”

3. Modify the Ethernet port settings for use with the camera:
    a. Navigate to ~/Control Panel/Network and Internet/Network Connections
    b. Rename the primary port “LAN1 - Camera”
    c. Rename the secondary port “LAN2 - Network”
    d. Right click “LAN1” and click “Properties.”
        i. Select “Internet Protocol Version 4” and click “Properties”
        ii. Select “Use the following IP address:”
            1. Set to:
                a. IP Address: 169.254.1.1

b. Subnet Mask: 255.255.255.0
c. Default gateway: blank

iii. Close the network connections / IPv4 windows

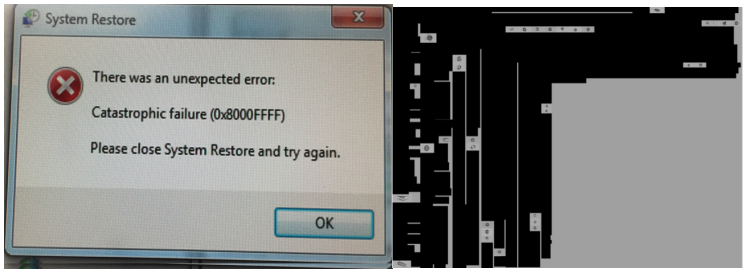
e. Navigate to ~/Control Panel/Hardware and Sound/Device Manager
f. Navigate to “Network Adapters” and select the “82567LMGigabit Adapter”
    i. Select “Properties” and “Advanced” tab.
        1. Set “Interrupt Moderation Rate” to “Extreme”
        2. Set “Jumbo Packet” to 9014 Bytes
        3. Set “Receive Buffers” to the maximum allowable (Typically 2048)
        4. Set “Transmit Buffers” to “256 bytes”
        5. Click on the “Power Management” tab.
        6. Uncheck “Allow the computer to turn off this device.”
        7. Click “OK” to commit changes

g. Navigate to “Network Adapters” and select the “82567L Gigabit Adapter”
    i. Select “Properties” and “Power Management” tab.
        1. Uncheck “Allow the computer to turn off this device.”
        2. Click “OK” to commit changes
    ii. Close Device Manager

4. If the program is still running, stop and restart “IFCBacquire.”

5. Configure camera
    h. Start GigE Viewer
    i. Select camera under “Host” and click wrench icon to access configuration
    j. Go to the ConfigFileIndex and set to “1”
    k. Click on ConfigLoadFile ‘1’, enter
    l. Set ConfigFilePowerUp file to ‘1’
    m. Set “Acquisition\Trigger\FrameStart\FrameStartTriggerMode” to “SyncIn1”
    n. Set “Controls\Gain\GainValue to ‘1’
    o. Click on ConfigFileSave to save changes.
    p. Close configuration window and exit GigE Viewer.

5.12 Unexpected error, Catastrophic failure: Replace Computer Stack



March 30, 2018
It's the third time on the expedition that we have to restart the IFCB because the bins saved on the SSD are bad.
Either a few bytes are shifted in the roi files or they are all set to 0 (I've attached a screenshot of what it looks like on the dashboard, there is the same issue with IFCB Viewer). After a few bad bins IFCB Acquire will be unresponsive and eventually Windows too.

Once that happen the remote controls becomes extremely slow and it's not possible to establish a new connection it tries to secure the connection forever. I also notice once that the files in a directory where missing and if you tried to create a new file it will not show up. However, after rebooting the IFCB they would magically appear again. I wonder if there is either bad sectors on our SSD or an issue with the registry of Windows on our IFCB.

April 1, 2018 (Fools' day but the IFCB wasn't joking)
The IFCB stopped last night and we could not access it through remote desktop and had to stop it, turning off the power. We could only connect to it opening the can and plugging directly to it. At first the network adapters were showing in network adapter settings of windows, but right clicking to get the status would give us a blank pop-up, no ip address… (I never saw that with windows before). However, plugging and unplugging the ethernet cable would turn blue or gray the network adapter. I forced set the adapter settings to a valid fix ip address and we could see the ifcb on the network but not connect to it with remote desktop or access the shared folders (it would give us errors). I then tried the troubleshoot network from windows which suggested that the driver were corrupted or missing. After that I tried to disable and re-enable the adapter settings and for some reason both network adapters disappeared and would not re-appear after a reboot. I also tried to to open internet explorer and a number of error showed up. However, IFCB Acquire seemed to work okay.

At this point I'm pretty sure there is an issue with our instance of windows, either some bad sectors or a corrupted registry. I'm planning to change the electronic stack later today.

Recommendations from Ivory Engstorm
Actually, we made an entire new spare electronics stack. That stack includes the CPU, power supply, hard disk, Analog and Housekeeping boards. I loaded it fully with software and identical settings as the IFCB was originally shipped. It should be a complete "drop-in replacement" for the current stack, but will require removing a few of the wiring harnesses to the IFCB hardware.

Keep in mind that all hardware must be fully connected while the IFCB is completely powered off. "Hot swapping" hardware while powered up could damage the stack. Seeing as most of the hardware is working, I don't believe there is a significant risk of damaging the spare stack.

One thing to consider is that you can copy the "IFCB.cfg" file to a USB-drive from the current stack and transfer that to the new stack once it is installed. This would retain all of the syringe zero settings,

PMT settings etc. However, any networking changes or additional software that was installed will be missing from the new stack. As IFCB Acquire software was likely not updated on the spare electronic stack it's also a good idea to copy it.

Alternatively, replacing only the damaged board should be the fix, but there is some risk of course to rebuilding the stack. I'll leave that decision to you guys and how comfortable you are in doing a partial swap or full stack swap.

I would definitely reset the zero for the syringe in order to get reliable fluid control. Simply find the bottom of the syringe travel by hand with the instrument turned off and then set zero as soon as IFCBacquire is started.

I don't think you will have any problem running the newer version of software from the desktop, all the program needs is the configuration file to operate normally.

Note from Taylor Crockford:
The PMT settings are specific to each Analog board. After changing the entire stack of electronics, copying the configuration from the "old" stack to the "new" stack, won't be enough, PMT settings will have to be re-tuned.

Appendix A: Daily checklist

- Check that the acquisition is **running** and *output files* checkbox is checked
- Daily **Cleaning** done: check section 2.5
- Check **Date and Time** of IFCB
- Check **Humidity**: on the dashboard, it should be fairly constant and below 15%. If humidity rises quickly, there is a leak in the IFCB. Troubleshoot immediately. Small changes might occur with changes in temperature.
- Check **Temperature**: on the dashboard. It should stay fairly constant between 28 and 33 °C. If temperature is higher than 40 °C, they might be some hardware failure, cool down the IFCB.
- Look at the **images** on the dashboard and check the followings:
    - Alignment
    - Focus
    - Bubbles
    - Make sure *view images* (Camera tab) is unchecked on IFCB Acquire.
- Check **ROI Position** on the dashboard: look at the alignment of the region of interest (ROI). Troubleshoot if necessary.
- Check **intake**: uncontaminated recent seawater is flowing without bubbles.
- Check **exhaust**: the carboy is not full

Note: Both the temperature and humidity can also be checked from the Hardware tab of IFCB acquire however preferer using the dashboard as going on the hardware tab of IFCB Acquire can generate DAC errors (which typically occur during high trigger rate).