

Deepwater Horizon Oil Spill Response Dataset

Centre for Offshore Oil Gas and Environmental Research (COOGER)
Department of Fisheries and Oceans (DFO) Canada

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1. Introduction

At the request of the United States (U.S.) government and BP plc, research scientists and technicians from the Centre for Offshore Oil Gas and Energy Research (COOGER), Fisheries and Oceans Canada (DFO) conducted at-sea monitoring operations with other scientific experts on board vessels R/V Brooks McCall and R/V Ocean Veritas to assist in a comprehensive environmental monitoring program in response to the Deepwater Horizon oil spill. In direct support of U.S. Environmental Protection Agency (EPA) directives to monitor the efficacy of subsurface oil dispersant injection and the fate and transport of dispersed oil as part a of the larger coordinated spill response operations, COOGER's primary operational objectives were to:

1. Find and characterize dispersed oil by LISST particle size analysis and fluorescence analysis.
2. Perform ongoing sampling and analysis to track the subsurface oil plume emanating from the blown-out Deepwater Horizon wellhead.
3. Verify plume modelling outputs provided by NOAA, SINTEF, etc.

Throughout this period from May 8 to August 24th the DFO COOGER team maintained a continuous monitoring program that recovered a total of 3197 unique water samples from 404 stations at depths down to 2000m for analysis of dispersant effectiveness by characterizing oil droplet size and UV-fluorescence (Fig. 1.1).

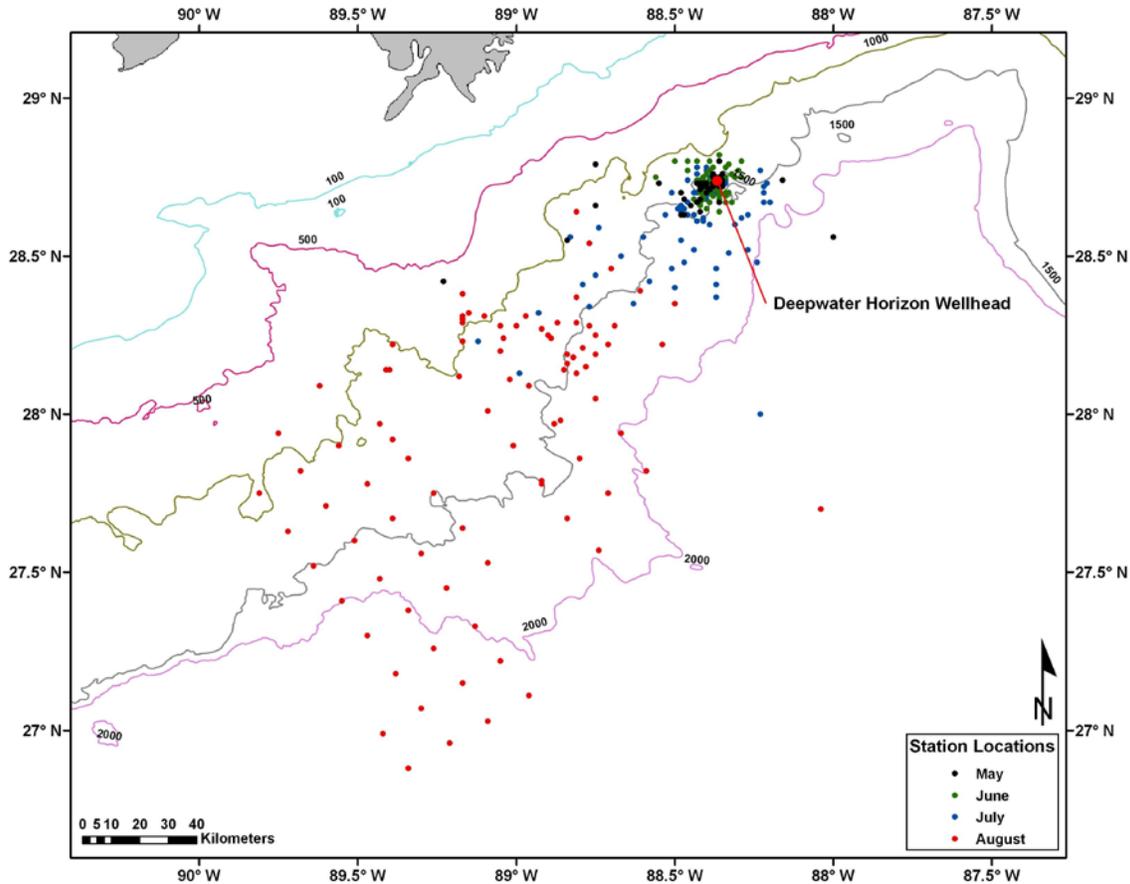


Figure 1.1: Deepwater Horizon spill response sampling stations labelled by month.

The database we provide contains 9 tables which include all data used to conduct our analyses.

1. [Chem_Data] – Hydrocarbon chemistry data. All units converted to $\mu\text{g/l}$.
2. [CTD] – Complete CTD trace data.
3. [CTD_Bottle] – CTD trace data matched to Niskin bottles based on date, time, and depth.
4. [LISST_InSitu] – LISST data for *in situ* deployments May 10, 11, 15, and 17, 2010. Column descriptions including units found in Table 1.1.
5. [LISST_R/V_Brooks_McCall] – Discrete LISST data collected aboard R/V Brooks McCall. Column descriptions including units found in Table 1.1.
[LISST_R/V_Ocean_Veritas] – Discrete LISST data collected aboard R/V Ocean Veritas. Column descriptions including units found in Table 1.1.
6. [Station_SpatialData] – Date, depth, location, and distance from wellhead (calculated using Arc GIS 9.3) data for each R/V Brooks McCall and R/V Ocean Veritas sampling station.
7. [UV_Fluorescence] – UV Fluorescence data collected aboard the R/V Brooks McCall and R/V Ocean Veritas. Raw fluorescence units.
8. [Wellhead_Daily] - Daily oil flow and recovery, and dispersant application data.

Table 1.1: Column names and descriptions for LISST data tables.

| | |
|------------------|--|
| FileName | Filename of raw data file – used as unique <i>in situ</i> tow identifier. |
| ScanNo | Scan number within tow. |
| 1:32 | Volume concentration (in $\mu\text{l/l}$) for size class 1 through 32. |
| LaserTransSensor | Laser transmission Sensor [mW] |
| BattVolt | Battery voltage in calibrated units [V] |
| AuxIn1 | External Auxiliary input 1 in calibrated units [mW] |
| LaserRefSensor | Laser Reference sensor in calibrated units [mW] |
| Pressure | Pressure in calibrated units [m] (not available for discrete analysis) |
| TempDegC | Temperature in calibrated units [C] (not available for discrete analysis) |
| DayHour | (Day*100 + Hour) at which data taken |
| MinSec | (Minutes*100 + Seconds) at which data taken |

2. Monitoring Methods

2.1 Discrete LISST-100X Small Particle Analysis

During the Deepwater Horizon spill response, COOGER devised operational protocols and conducted a field survey of the dispersed oil droplet size distribution using two Laser *in Situ* Scattering and Transmissometry instruments (LISST-100X, Sequoia Scientific Inc., Seattle, WA). The LISST-100X particle size analyzer (Type C) is an optical device that measures the size and volume of particles in a given sample based on the physical properties of light as it is scattered off of a particle. In order to achieve this, a laser beam is directed across the sample chamber and any of this light scattered by particles present within the sample is focused by a specialized lens onto a series of detector rings numbered 1 through 32 (Fig. 2.1). Light intensity readings for the 32 discrete rings is processed with the manufacturer-provided inversion algorithm to automatically calculate volume concentrations (in $\mu\text{L/L}$) for 32 particle size bins, along with output of 10 other parameters including laser transmission sensor power, laser reference sensor in calibrated units, pressure, temperature, computed optical transmission over path, and beam-attenuation. The 32 particle size bins measured by the LISST-100X are logarithmically spaced across the analytical range, the upper size in each bin being 1.18 times that of the lower (Table 1).

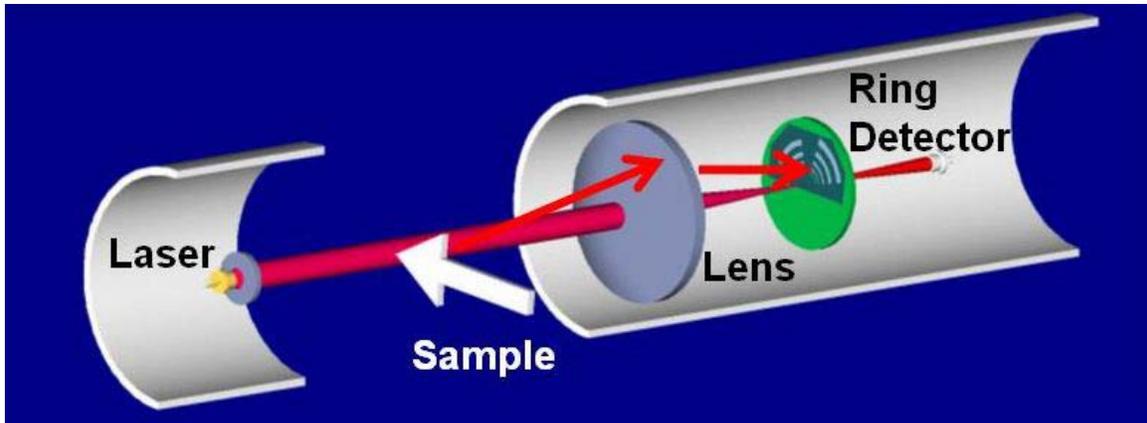


Figure 2.1: Basic illustration of the LISST-100X optics system showing the laser. Source: Sequoia Scientific

Initially during the spill response, both LISST units were aboard the R/V Brooks McCall. Due to the extreme water depth surrounding the Deepwater Horizon wellhead, the LISST units could not be deployed directly to the bottom (The LISST-100X units used for the Deepwater Horizon spill response could only be deployed to 300m). Instead, the first LISST unit was equipped with a small test chamber (120 ml) that allowed it to conduct bench-top particle size analysis. This unit remained aboard the R/V Brooks McCall for the entire response operation.

The second LISST unit was initially deployed directly into the water column at the surface mixing layer from the R/V Brooks McCall in order to carry out *in situ* monitoring of possibly dispersed surface oil between May 10, 2010 and May 17, 2010. As of May 27, 2010 this instrument was fitted for bench-top analysis and transferred to the R/V Ocean Veritas when that vessel began field operations so continuous monitoring could be maintained in the Gulf of Mexico.

Table 2.1: The minimum, mean and maximum limit of each size bin in microns for the LISST-100X particle counter.

| Size Bin # | Min | Mean | Max |
|------------|--------|--------|--------|
| 1 | 2.50 | 2.73 | 2.95 |
| 2 | 2.95 | 3.22 | 3.48 |
| 3 | 3.48 | 3.79 | 4.11 |
| 4 | 4.11 | 4.48 | 4.85 |
| 5 | 4.85 | 5.28 | 5.72 |
| 6 | 5.72 | 6.23 | 6.75 |
| 7 | 6.75 | 7.36 | 7.96 |
| 8 | 7.96 | 8.68 | 9.40 |
| 9 | 9.40 | 10.24 | 11.09 |
| 10 | 11.09 | 12.09 | 13.08 |
| 11 | 13.08 | 14.26 | 15.44 |
| 12 | 15.44 | 16.83 | 18.22 |
| 13 | 18.22 | 19.86 | 21.50 |
| 14 | 21.50 | 23.43 | 25.37 |
| 15 | 25.37 | 27.65 | 29.93 |
| 16 | 29.93 | 32.63 | 35.32 |
| 17 | 35.32 | 38.50 | 41.68 |
| 18 | 41.68 | 45.43 | 49.18 |
| 19 | 49.18 | 53.61 | 58.04 |
| 20 | 58.04 | 63.26 | 68.48 |
| 21 | 68.48 | 74.65 | 80.81 |
| 22 | 80.81 | 88.08 | 95.36 |
| 23 | 95.36 | 103.94 | 112.52 |
| 24 | 112.52 | 122.65 | 132.77 |
| 25 | 132.77 | 144.72 | 156.67 |
| 26 | 156.67 | 170.77 | 184.87 |
| 27 | 184.87 | 201.51 | 218.15 |
| 28 | 218.15 | 237.78 | 257.42 |
| 29 | 257.42 | 280.58 | 303.75 |
| 30 | 303.75 | 331.09 | 358.43 |
| 31 | 358.43 | 390.68 | 422.94 |
| 32 | 422.94 | 461.01 | 499.07 |

At each sampling station, discrete water samples were collected at various depths using a series of remote-triggered Niskin bottles attached to Conductivity Temperature and Depth (CTD) rosette which was deployed from the *Brooks McCall* or *Ocean Veritas* using a deck-mounted winch. Individual sampling depths were selected by looking at CTD downcast measurements of CDOM, and dissolved oxygen and assigning bottle firing points to depths where peaks were observed (analyzed on LISST & UV-Fluorometer), and then spreading the unassigned bottles out over the remainder of the water column with one bottle being triggered at 2 m depth at every station (LISST analysis only). In addition to these Niskin bottle samples, a surface (~0.5m) sample was also collected by casting a bucket attached to a rope over the side of the vessel (LISST & UV-Fluorescence analysis). Once the rosette returned to the surface and was safely on deck, each of the Niskin bottles was sub-sampled using disposable containers (when required, these disposable containers were again sub-sampled into a glass vial for UV-Fluorescence analysis) and brought to the LISST-100X for particle size and concentration analysis. For

each sample, a mean concentration value was calculated from 20 independent measurements (labelled “scans” in data table) by the LISST-100X instrument (data collected and recorded once per second). Standard operation procedures developed for the Deepwater Horizon LISST analysis can be found in the appendix of this report.

During field operations two major challenges were encountered.

1. Variation of the seawater temperature of the samples collected from different depths in the water column and the ambient air temperature. The water temperatures vary widely from close to freezing (4°C) at maximum depths to very warm water at the surface (30°C). Stratification of the water inside the small chamber was causing laser beam reflection represented by a false signal of the presence of larger particles (Mikkelsen et al., 2008; Styles, 2006).
2. Possible misalignment of the LISST-100X (#1215 - Brooks McCall, and #1174 - Ocean Veritas) optics which occurred at some point during operational deployment may have caused unscattered light from the laser to be incorrectly directed towards the inner detector rings resulting in an anomalously high light intensity reading on those rings. During the inversion calculation made by the LISST to derive particle concentrations at the various size ranges, this false inner ring reading was propagated into the lower size bins. Depending on the extent of instrument misalignment, a greater number of the large particle size bins was compromised.

The problem of stratification within the sample chamber was corrected in late June 2010 by introducing a full-path mixing chamber which stirred the sample during analysis and prevented stratification within the analytical chamber. Previously collected data affected by this issue was manually corrected in order to remove readings that showed signs of interference. A ‘NumOfScansInAvg’ field has been added to the final dataset that indicates the number of individual scans used to calculate the final concentration values.

In an attempt to address the misalignment problems encountered during operations, both LISST-100X instruments were returned to the manufacturer, Sequoia Scientific, for thorough examination. During their analysis, it was found that one instrument (#1174) was out of alignment beyond acceptable limits while the other (#1215) was only slightly misaligned. Technical experts at Sequoia advised that data collected using instrument #1174 could not be considered reliable and that larger particle size bin data from instrument #1215 should be rejected.

LISST data tables contained within the DFO_COOGER_DWH_DATASET.mdb database have been flagged to reflect the findings above. Entire rows (all particle size bins) which have been flagged for exclusion from analysis due to the identification of a problem have been identified as such using the [Exclude] and [Exclude_Note] columns. A unique exclude code number was used for each problem which lead to individual scans being excluded from further analysis (Table 2.2). In addition, particle size bins 26-32 for data originating on the R/V Brooks McCall and all data for the R/V Ocean Veritas has

been converted to text format and appended with an asterisk following the value (eg. #.##*) in order to indicate its unsuitability for data analysis.

Table 2.1: Exclude code values and associated notes used in the DFO COOGER Deepwater Horizon Database

| Exclude | Exclude_Note |
|---------|---|
| 1 | Over Range |
| 2 | Temperature Effect |
| 3 | SumSP>Mean+3xStDev |
| 4 | Station OV001 removed due to CTD hitting bottom |
| 5 | OV123-127-identical particle concentrations |
| 6 | Scan No. >20 |

2.2 *In-situ* LISST-100X Small Particle Analysis

Near the beginning of the monitoring program, one of the particle size analyzers (LISST-100X) was deployed at 3.5 m depth along side the Brooks McCall for *in situ* analysis of the droplet size distribution of the dispersed oil. Figure 2.2 displays the deployment of the LISST-100X from the R/V Brooks McCall.

Figure 2.3 illustrates the transect lines on May 10 and May 11, 2010 following subsurface injection of chemical dispersants on those days (time of dispersant injection unavailable). Figure 2.4 displays the LISST transect lines on May 15 to 17, 2010.



Figure 2.2: *In situ* deployment of LISST-100X particle size analyzer at R/V Brooks McCall.

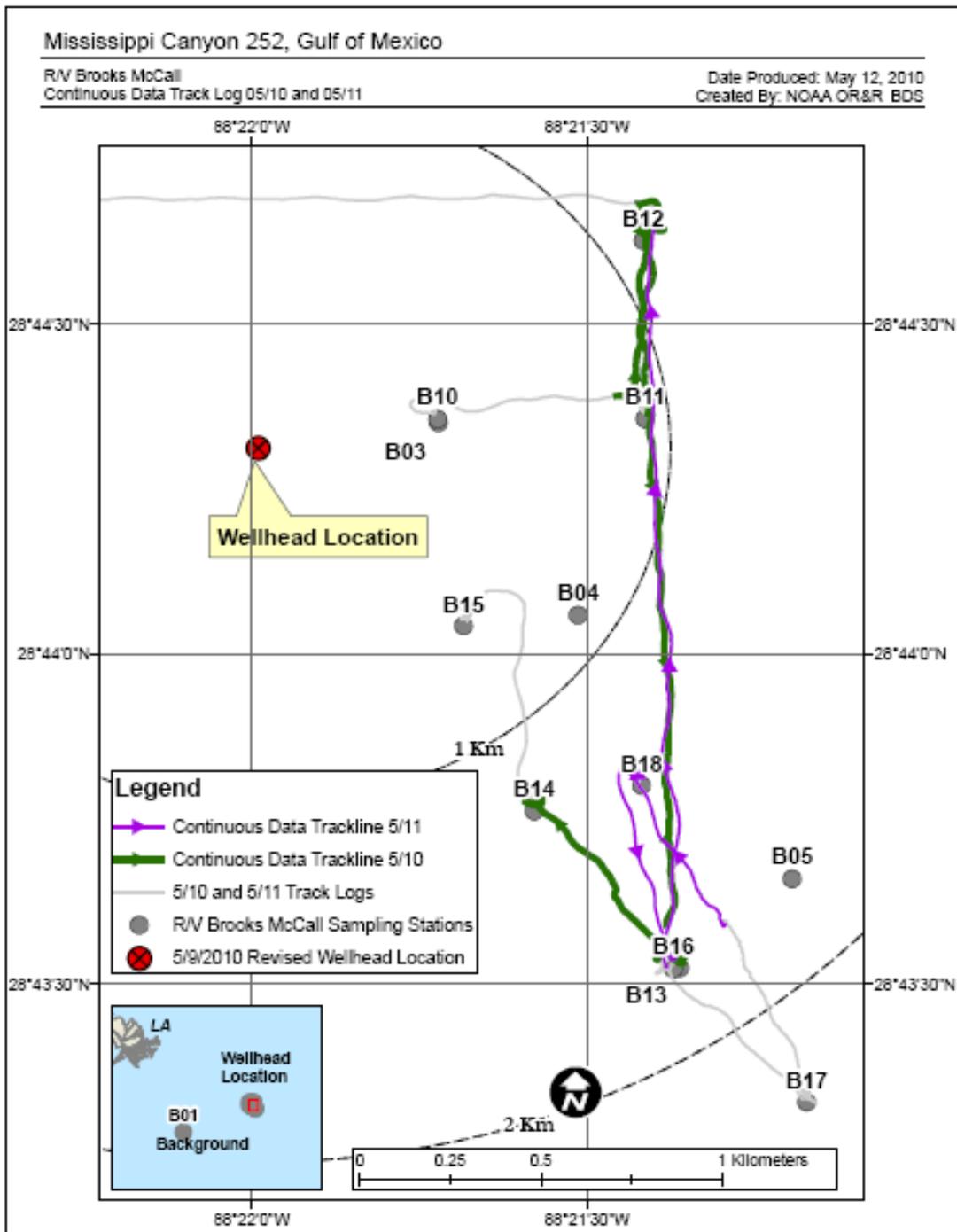


Figure 2.3: Transect lines of the *in situ* deployment of LISST-100X on May 10 and May 11, 2010 around the wellhead location.

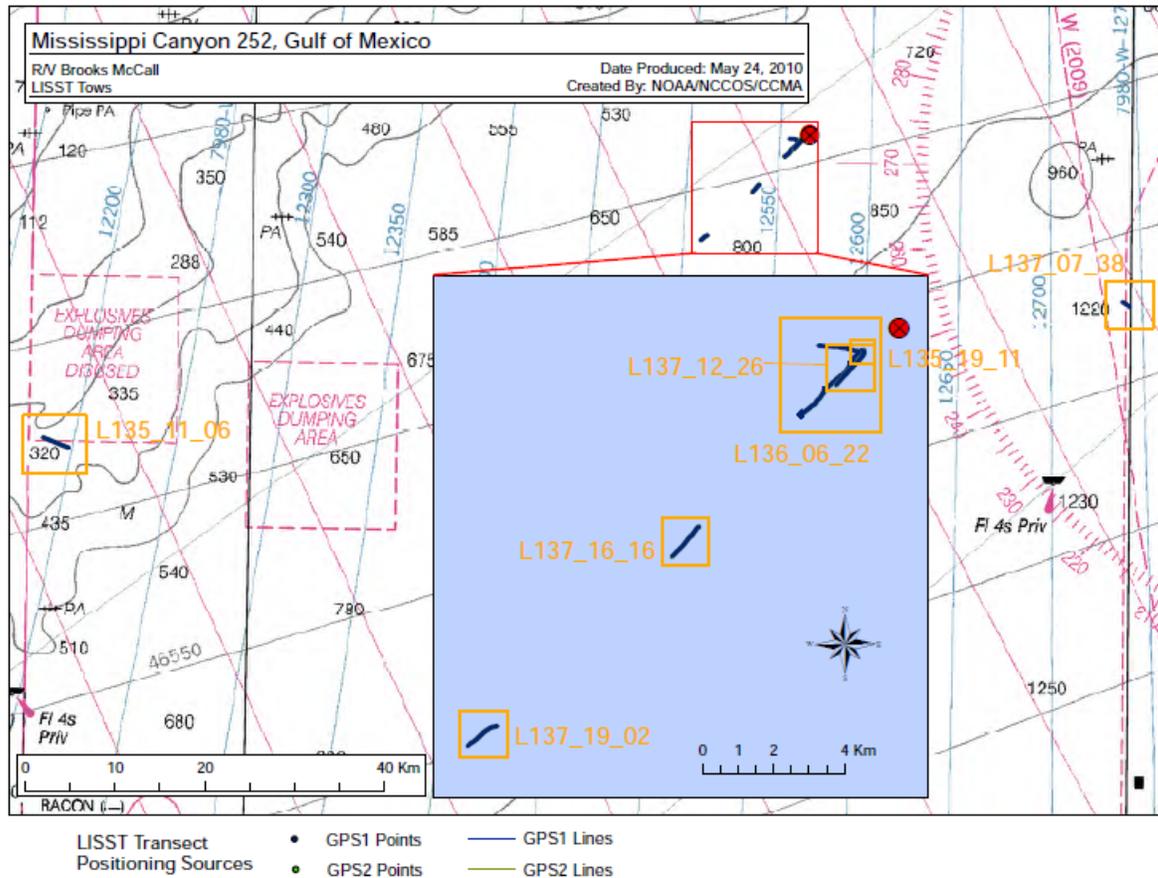


Figure 2.4: Transect lines of the *in situ* deployment of LISST-100X on May 15 to May 17, 2010 around the wellhead location.

2.3 UV Fluorescence Analysis

UV fluorescence analysis was also performed on a 1292 samples (primarily plume samples identified by LISST, CDOM Fluorescence, or DO) of discrete Niskin samples collected aboard the R/V Brooks McCall and the R/V Ocean Veritas. The majority of the UV-Fluorescence analysis was conducted immediately after sampling using the Quantech Life Sciences Fluorometer. Samples collected at R/V Ocean Veritas stations 1-55 were acidified, stored in glass vials, transported, and then analyzed aboard the R/V Brooks McCall due to a shortage of 450nm filters aboard the R/V Ocean Oeritas). The Quantech was configured with interchangeable filters to transmit an excitation UV beam at 280 nm into the sample, and to measure emission fluorescence from the sample at wavelengths 340 and 450 nm.

UV-Fluorescence analysis was conducted by pipetting 3.5 ml of sample into a 1 cm path-length methacrylate disposable cuvette, allowing the sample to approach room temperature, then inserting the cuvette into the fluorometer for analysis at emission wavelengths of 340 and 450nm. Six independent readings were obtained for each sample at each wavelength from which an average for each emission wavelength was calculated

for use in further analysis. Standard operation procedures developed for the Deepwater Horizon UV-Fluorescence analysis can be found in the appendix of this report.

Due to the infeasibility of acquiring a field blank with which to calibrate the fluorometers, only the raw fluorescence values were collected. As such, both instruments show differing baselines, and the absolute range of intensities coming from one unit differs from the other. Care must be taken when analyzing data from both instruments simultaneously.

It should also be noted that fluorometry samples collected early in the response effort aboard the Ocean Veritas (Stations OV001 through to OV055) were transported to the Brooks McCall for analysis as there was a shortage of 450 nm emission filters at that time.

Revised standard Operating procedures for LISST-100X and UV Fluorescence analysis can be found below.

2.4 Hydrocarbon Chemistry, CTD Profiles, and Daily Oil Flow and Recovery and Dispersant Application Data

During our work, a number of external datasets were used in order to facilitate more in-depth data analysis.

- Hydrocarbon chemistry data were collected directly from BP then adapted to match the sample ID scheme used during COOGER field operations.
- Individual CTD data files for the R/V Brooks McCall were retrieved from the NOAA National Oceanographic Data Center (<http://www.nodc.noaa.gov/General/DeepwaterHorizon/oceanprofile.html>) and merged by COOGER into one data table.
- Daily oil flow data were obtained through the government “Oil Budget Calculator” document from October, 2010; and recovery, and dispersant application data taken from BP “External Affairs Requested Data_Supplemental Request 7-30”.

3. Standard Operating Procedures (SOPs)

3.1 Revised LISST-100X Standard Operating Procedures

LISST-100X Particle Size Analyzer Standard Operation Procedure (SOP)

The Sequoia Laser *In Situ* Scattering and Transmissometry (LISST-100X) instrument uses a laser diffraction technique to determine *particle size distribution* (PSD). The LISST consists of an optical system producing a

collimated laser beam, a detector array, electronics for signal pre-amplification and processing, a data storage and scheduling computer, and a battery system.

List of Materials

- User's Manual with software disk
- LISST-100X instrument
- Full path mixing chamber
- Magnetic stir-bar
- Stir-bar retriever
- Communication cable
- External power cable
- Bench top instrument stands
- Allen wrench set
- Pre-moistened alcohol wipes
- De-ionized water (should be at room temperature)
- Duct tape or rope/string to anchor LISST and laptop to workbench

Bench-top SOP

Setup

1. Remove the instrument stands and set them up on a flat bench top working surface.
2. Remove the LISST-100X from the case and set it on the stands. The LISST-100X unit may need to be affixed to instrument stands using tape or string/rope if analysis is being done at sea.
3. Remove the full path mixing chamber from the case, attach the flexible tubing and tubing stop clamp, and hook-up and plug in stirrer controller.
4. Slip the mixing chamber between the optical windows of the instrument such that these windows can be submerged for calibration and analysis; be careful not to damage the rubber o-rings which make the seal between mixing chamber and optical windows. (See Appendix F of the User's manual for more details on chamber installation).
5. Check and adjust the mixing chamber and the spacer to make sure that the chamber is sealed well to both optical windows and will not leak when filled with water.
6. Add magnetic stir-bar to mixing chamber.

7. Remove the communication cable from the case. Connect the 5-pin underwater connector of the communication cable to the LISST-100X, and the 9-pin DB-9 connector to a laptop personal computer.
8. Install the LISST-100X software including the calibration files specific for the instrument. For ease of operation, create a shortcut to the LISST program on your desktop.

Software Configuration

1. Start the LISST software by clicking on the program shortcut.
2. Click *LISST>Connect* (using *LISST* menu or by clicking icon that looks like a traffic light) then select *LISST>Wake up LISST* (or by clicking icon which looks like a sunny field). A dialogue box will then appear counting down from 138 seconds, which is the maximum time required for the instrument to wake up.
3. Click on *File>Settings* then select the *Output* tab and check all of “build a binary particle size file (.psd)”, “build an ASCII particle size file (.asc)”, and “build and ASCII raw data file (.log)” then click *OK*. This step instructs the LISST software to automatically create a raw LOG file, and processed PSD and ASCII files for each sample run.

Rinsing

Rinsing the full path mixing chamber is a 4-step process.

1. Drain the previous contents of the mixing chamber (when not in immediate use, mixing chamber should always be left filled with de-ionized water) by opening the stop-clamp which is located on the drain tube.
2. Wipe mixing chamber, magnetic stir-bar, and both optical windows well with a pre-moistened wipe. (This step only needs to be performed after running surface samples or other highly oiled water samples)
3. Perform two “small rinses” with de-ionized water. A “small rinse” entails leaving the stop-clamp opened and pouring only enough de-ionized water into the mixing chamber to fully cover the bottom, then letting it drain completely. Do this twice.
4. Perform one “big rinse” with de-ionized water. Close the stop-clamp and fill the mixing chamber to the top with de-ionized water then open the stop-clamp and let the mixing chamber drain completely.

Checking Background

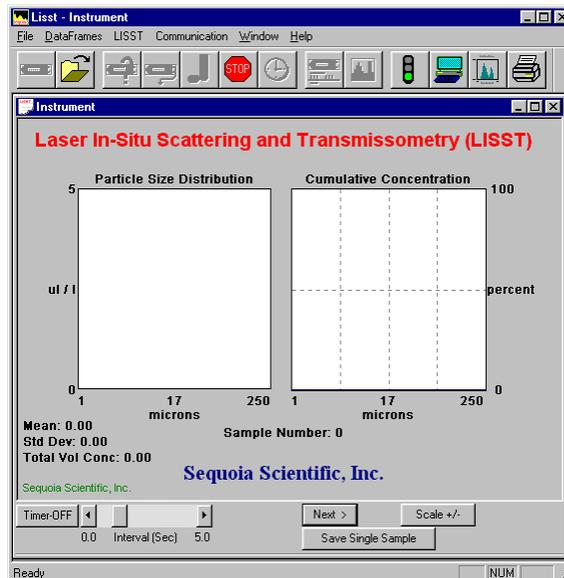
1. Create a new data directory for the day and/or subdirectory for the station as required - refer to example directory structure provided as reference.
2. Connect-to and wake-up the LISST if not already done (as described above) and turn on magnetic stirrer.
3. Rinse (as per instructions above) and fill the mixing chamber with clean de-ionized water.
4. Double check that there are no leaks between the mixing chamber and the optical windows on the LISST.
5. Turn on stirrer and set dial to approximately 120 revolutions per minute (RPM). Because the stirrer motor is controlled by an analog dial, achieving the correct RPM will need to be done through trial-and-error.
6. Acquire background scatter profiles by selecting *LISST>Collect Background Scatter Data* (or by pressing the  button on the toolbar) and, when prompted, select the factory background scatter file (looks like `factory_zsc_####.asc`, with “####” being the serial number of the instrument). Once selected, this factory background will be shown on the displayed axis. The background scatter measurement is critical to good instrument performance and will also check the overall health of the instrument. It will verify that all of the systems are functioning and that the optics are in proper alignment.
7. Press the *BEGIN Collection* button. The software will now acquire 20 samples from the LISST and superimpose the mean collected background scatter data over the factory baseline values (specific to each instrument). If the values are acceptable they should be saved in the daily root directory (refer to example directory structure if needed) by clicking the *Accept and Save* button and entering a filename (format: `YYYY-MM-DD_bkg.asc`) when prompted.
8. If there is a problem, an error message will be displayed and in most cases corrective action will need to be taken. eg. cleaning the mixing chamber and optical windows with a pre-moistened wipe, rinsing with de-ionized water, ensuring that the current laser power is close to the factory laser power, or, in some cases, a realignment of the instrument may need to be performed (in most cases, this can not be done in the field). Once complete, click the *BEGIN Collection* button again to re-start the background checking process.
9. A new background file should be saved each day, and background levels checked between stations. During these between station checks, a new background scatter file should only be saved when additional deviation from the factory background can not be remedied by cleaning the mixing chamber and optical lenses with a pre-moistened wipe and/or thorough rinsing with de-ionized water.

Sample collection

1. Samples are collected and delivered to LISST operators in disposable plastic cups. Label two cups (duplicate sample) for each Niskin bottle and one cup for the surface sample (collected with a bucket).
2. When the rosette reaches deck and sample cups are being filled, expect to fall behind on analysis: maintain enough desk/bench space to place sample cups until analysis can be run.

Analyzing samples

1. Run all Niskin bottle samples collected from the water column at a station before running the surface sample collected using a bucket. This minimizes cross-contamination of the mixing chamber.
2. Connect-to and wake-up the LISST if not already done (as described above) and turn on magnetic stirrer.
3. Choose *File>Open Real-Time Session* (or press the  button to open the real-time session). Choose the most recent background file (saved during the “Checking Background” step) to use and specify output files in which to store the sample information for the run (sample IDs are used as data file names. eg. BM0540199-1.psd and .asc). A display similar to this will appear.



The left hand figure displays a bar chart showing the volume concentration in each of the 32 log spaced size classes. The right hand plot will be the Cumulative Concentration. The slider bar next to the button adjusts the acquisition rate. The slider bar controls the

data acquisition time. The Scale +/- Button adjusts the Particle Size Distribution scale (this can be set to 2.5 for easier real-time viewing).

4. Rinse the mixing chamber (as per instructions above) with de-ionized water.
5. Using sample water, perform two “small rinses” (described above), then close stop-clamp and fill mixing chamber so that optic lenses are completely submerged by the sample.
6. Allow any air bubbles resulting from filling the mixing chamber to escape then click *Start* in the LISST software.
7. Allow the LISST to collect 20 samples (scans) then click the *Stop* button. Stopping the LISST does not occur immediately and may take a few seconds to occur – do not proceed until the LISST has received the stop command.
8. Close the Real-Time Session window (data are automatically saved) and drain the sample water from the chamber.
9. Rinse the mixing chamber. You are now ready for the next sample.
10. Once all the samples for a given station are complete, run a analytical blank with DI water as if it were a sample.
11. After finishing with the instrument rinse the mixing chamber and leave filled with de-ionized water. Press the *Put LISST to Sleep* button (looks like a night-sky) or select *LISST>Put LISST to Sleep* to put the LISST back into its low power sleep mode.

Vessel-side In-water Continuous Monitoring SOP

1. Connect the LISST-100X to the PC and start the LISST-SOP program.
2. Open the Operating Modes window by choosing *LISST>Operating*



Modes or by pressing the  button on the toolbar. Select the *Operating Mode Tab* at the top of the window and choose “Fixed Sample Rate”. Select *Start Conditions* tab to configure when the instrument will begin sampling among five options: Depth, Time, External Mechanical Switch, External Digital Input, and Time Delay. Select the mode by clicking on the button next to its label and select the correct parameters as required.

3. Choose the *Stop Conditions* tab to select the conditions when sampling should stop. The available stop conditions are: Depth, Time, External Mechanical Switch, External Digital Input, Fixed number of samples, and Maximum Memory or Low Battery.
4. Select *Apply* or *OK* buttons to configure the instrument with the current settings. If the *Apply* button is pressed the program will return to the current window. Returning to the *Instrument Status* window will display a summary of the current settings. If the *OK* button is pressed, when the configuration is complete the user will

be prompted to open the *Terminal* window to start the program. To start the program and have it start looking for the Start conditions press the *Run* button on the *Terminal* window.

5. Once the program is confirmed to be running and waiting for the correct start conditions, the LISST-SOP program can be closed and the communications cable can be disconnected.
6. Replace the connector cap before deployment.
7. Deploy the configured LISST in water beside the vessel to carry out *in situ* monitoring of *in situ* particle size distribution in the water column.
8. When the start conditions are met and the instrument will start sampling. The green LED on the connector endcap will illuminate each time a sample is acquired. The instrument will continue collecting data until the pre-set stop conditions are met.
9. Stop a running program by re-connecting the communications cable, connecting to the LISST, and pressing the Stop button on

the Terminal window or the  button.

10. Press the Instrument Query Button  to display the instrument status including the number of samples saved.

11. Select *Offload* from the LISST menu or choose the  button from the toolbar. A list of files will appear. Choose the files to offload by clicking on them while hold down the CTRL key. The data is offloaded at 115K baud.

12. To process the data file choose *Open Raw Data file* or press the



button on the toolbar. Select the instrument serial number, raw data file to open, and background scatter file to use when processing the data. Press the *Process File* button to convert the raw file into processed size distributions.

13. Processed data files are stored as binary files with a PSD extension. The PSD files can be opened and viewed on the screen. To open a processed Particle Size Distribution file choose Open Particle Distribution File or press the button on the toolbar. The steps are the same as bench top operation.

Processing LISST data

Opening Station Data Files

1. After LISST analysis for a station is complete, create a subfolder inside the station folder named "raw data" and copy the .psd files

into it. Create a second subfolder called “converted” and copy the .asc files into it.

2. If necessary, create a new copy of the Summary Small Particles template for the cruise, name accordingly (Format: YYYY-MM-DD_Summary Small Particles.xls) and place it in the daily root directory (refer to example directory structure provided if necessary). For each station sampled make copies of the “Template” worksheet which is located inside the file. To copy a worksheet, right click over the name of the worksheet at the bottom of your screen, select *Move or Copy*, in the next window ensure that *Create a Copy* is checked and click *OK*. Rename the duplicate worksheet to the name of the station being assessed (eg. BM001).
3. Update the *Date*, *Ship ID*, and *Station No.* fields at the top of the worksheet – sample and station IDs will auto-update accordingly throughout the worksheet.
4. Open all ASCII output files for the station in Excel. Open a new Excel window and select *File>Open*; ensure that “All files (*.*)” is selected in the *Files of Type* dropdown box then select all .asc files for the station (hold shift while clicking to select all) which should now be located in the “Converted” folder, then click *Open*.
5. A “Text Import Wizard” window will now appear. Under *Choose the file type that best describes your data* select *Delimited* and click *Next*, under *Delimiters* select *Space* and click *Finish*. This import step will need to be repeated for each file that was selected during the open process.
6. After all files have been imported you should have a separate Excel window open for each .asc file.

Recording SP_Sums macro

1. The macro you will now record automates the process of summing all small particle concentrations between 2.5 and 60nm, and flagging “above range” samples (false 0s) and outliers ($> \text{Mean} + 3 * \text{StDev}$).
2. Within one of the Excel windows you have just opened select cell A1.
3. Go to *Tools>Macro and select Record New Macro*.
4. Enter “SP_Sums” as the *Macro Name*, “k” next to *Ctrl +* under *Shortcut Key*, then select *Personal Macro Workbook* under the *Store Macro In* heading, and click *OK*.
5. Locate and select cell AR1 and type the following formula;

```
=IF(SUM(A1:AF1)>0, SUM(A1:S1), "over scale")
```

Press the enter key. This formula must be typed (not copy/pasted) in order for the macro to work properly.

6. Next, select cell AS1 and type the following formula into it;

```
=IF(AR1 = "over scale", AR1, IF(AR1 < (AVERAGE($AR$1:$AR$20) + STDEV($AR$1:$AR$20) * 3), AR1, "outlier"))
```

Press the enter key. As above, this formula must also be typed (not copy/pasted) in order for the macro to work properly. This formula will show “#DIV/0!” until step 6 is completed.

7. Select both the AR1 and AS1 cells (which should now have formulas in them), then click and hold the right mouse button over the bottom right corner of the cells (should be a small black box in that area) and drag the resulting box down to cell AR/AS20 then release the mouse button. Your formulas should now be auto-filled to all the cells between AR1 and AS20.
8. Select cells AS1 through AS20 and click *Edit>Copy*. Do not use hotkeys (Ctrl+c, Ctrl+v) for copy/paste operations while creating the macro as these actions will not be recorded.
9. With cells AS1 through AS20 still selected, click *Edit>Paste Special* and select *Values* when prompted, then click *OK*.
10. Finally, keeping cells AS1 through AS20 selected, once again click *Edit>Copy*.
11. The macro is now complete, click *Tools>Macro>Stop Recording* to save.
12. You will only need to record this macro once per computer – once it has been saved on your system, it will be accessible in any Excel worksheet you open and usable by the Ctrl + hotkey you specified.
13. When you eventually close the workbook you used to create the macro you will be asked to save the changes you made to “Personal.xls” – click *Yes* when prompted.

Calculating Column Sums and populating Summary Small Particles file

1. For each Excel workbook that you opened during the “Opening Station Data Files” stage (individual samples), execute the “SP_Sums” macro (hotkey is Ctrl+k, unless set otherwise) and paste the results under the correct sample ID heading in the Summary Small Particles worksheet you created for the current station. When you have finished pasting the results of the SP_Sums macro for a given sample into the Summary Small Particles worksheet, return to the sample data workbook and save (*File>Save As*) as an XLS file in the station root directory. After the sample file has been saved successfully, close the workbook and move on to the next sample.
2. Once all sample data has been entered into the Summary Small Particles file delete the columns in the worksheet that contain

unused sample IDs. Do this by selecting the entire column, right clicking, and selecting *Delete*.

3. Update the sample number and depth textbox in the bar chart (remove extra sample numbers if necessary).
4. If fluorescence analysis is being conducted, add the Fluorescence Intensity Ratio (FIR) results to the chart in the textboxes provided and move textboxes to a location above the pertinent sample IDs. Delete any extra textboxes.

Writing the daily report

1. Copy bar charts for each station from the daily Summary Small Particles spreadsheet into a Word document in the order they were collected.
2. Write a one-page report which includes station IDs, station locations, and comments regarding the days LISST analysis and fluorescence intensity ratios (Format: 2010-06-13_SHIPNAME_LISST.doc). An example daily report has been provided.

3.2 UV-Fluorescence Standard Operating Procedures

Standard Operating Procedure for the Quantech Fixed Wavelength Fluorometer

The Quantech Life Sciences Fluorometer has the ability to measure fluorescence at two emission wavelengths (340 and 450nm) during excitation by UV light at 280 nm.

The unit should be equipped with 2 sets of filters (one for use; one spare). There should be one narrow band excitation wavelength filter at 280nm (280NB), one narrow band emission filter at 340nm (340NB), and one narrow band emission filter at 450nm (450NB) in each set; there should also be one blocking filter.

Filter Placement

The positioning of the filters are as follows:

- When facing the instrument for operation with the cover open, the 280nm excitation filter fits into the filter slot at the 6 o'clock position.
- The emission filters (either 340nm or 450nm) fit into the filter slot at the 9 o'clock position.
- The blocking filter fits into the filter slot at the 12 o'clock position; it and the 280nm filters should remain in place (not be moved).

Startup

- At least 1 hour before the first sample is to be analyzed, the instrument is turned on (switch at the back of the unit near the power cord). The instrument will go through a start up sequence and display a 15 minute countdown.
- After 15 minutes has elapsed, the display will show ← Menu →. Push the <Right Arrow> once until the display reads ← Advanced Functions →. Push the <Enter> key. <Date and Time> will appear. Press the <Right Arrow> key until <UV Lamp Options> is displayed. Press <Enter>. The display will read <UV Lamp Installed?> with <No> underneath. Press the <Up/Yes> key to turn the UV lamp on and press <Enter>. You now must wait **20 minutes** for the UV bulb to warm up. ← Menu → should once again be displayed.
- After at least 20 minutes, press the <Right Arrow> to select ← Advanced functions → (Press <Enter>). Press the <Right Arrow> until <Manually Set Gain and PMT Voltages>. Press <Enter>. The display will show <Turn off Auto Gain?> Press the <UP/Yes Arrow> and press <Enter>. Press the <Up/Yes Arrow> until Gain= 100 is displayed and press <Enter>.
- ← Menu → will be displayed. Press the <Right Arrow> to select ←Advanced Functions→ (Press <Enter>) and <Right Arrow> until <View Diagnostic Information> is on the display. When ←Raw PMT.... Hardware→ is displayed, press the <Left Arrow> key to select <Raw fluorescence>, and press the <Right Arrow> to select <UV Lamp>. Press the <UP/Yes> key to select the gain at 100. The raw fluorescence value should start fluctuating.

Drawing Samples for Analysis

Note: SAMPLES FOR THE FLUORESCENCE INTENSITY RATIO SHOULD ONLY BE TAKEN FROM BOTTLES FIRED IN AREAS OF ELEVATED FLUORESCENCE IN THE CTD TRACE.

- A 20 ml sample is acidified with 20 μ L of 10% HCl in a 20 mL scintillation vial equipped with a foil or Teflon lined cap and stored at 4°C.
- Samples are drawn from the same cups as used for the LISST with the following exception – pour the contents of sample cup #2 into sample cup #1 (after the LISST sample from cup #2 has been successfully analyzed). This creates an integrated sample from the one niskin bottle/sample depth.
- Draw duplicates (labeled “sampleID”-1 and “sampleID”-2) from the integrated cup. Acidify the sample. (NB – It is easier to add the acid to the vial first and then the sample to ensure adequate mixing).
- Place approximately 3.5 mL of sample into a methacrylate disposable cuvette and allow the samples to approach room temperature; a cold sample will effect the fluorescence measurement. Refrigerate the remaining sample for storage, or discard if not to be archived.

Sample Analysis

- Place the cuvette into the holder of the Quantech fluorometer. Make sure the 340nm emission filter is in the proper filter holder position (9 o'clock). Close the lid.
- Wait a moment (count to five) and glance at the display. As the reading is not stable, glance at the reading and record the first number you see in the back of the fluorometry notebook under a column labelled "340". Repeat this procedure twice more for a total of three (3) times (for example, sample ID BM550107-2 had 340 nm readings of 55, 63, 44).
- Open the cover of the fluorometer and replace the 340nm filter with the 450nm filter. Close the cover, count to five, and read the display three times in the same way as for the 340nm wavelength (for example: sample ID BM550107-2 had 450 nm readings 27, 23, 33).
- Average the three 340nm readings (eg, $55+63+44=162 / 3= 54$) and the three 450 readings (eg, $27+23+33=83 / 3= 27.7$)
- Obtain the fluorescence intensity ratio (FIR) by dividing the 340 nm by the 450 nm average (eg, $54 / 27.7 = 1.95$)
- Repeat this procedure for the next samples.

Reporting Data

The FIR data are to be included with the LISST particle data in the daily report. FIR should be recorded as a number on the LISST particle graph above the corresponding LISST sample in the histogram.