In Situ FIRe

Operation Manual 1.1.4

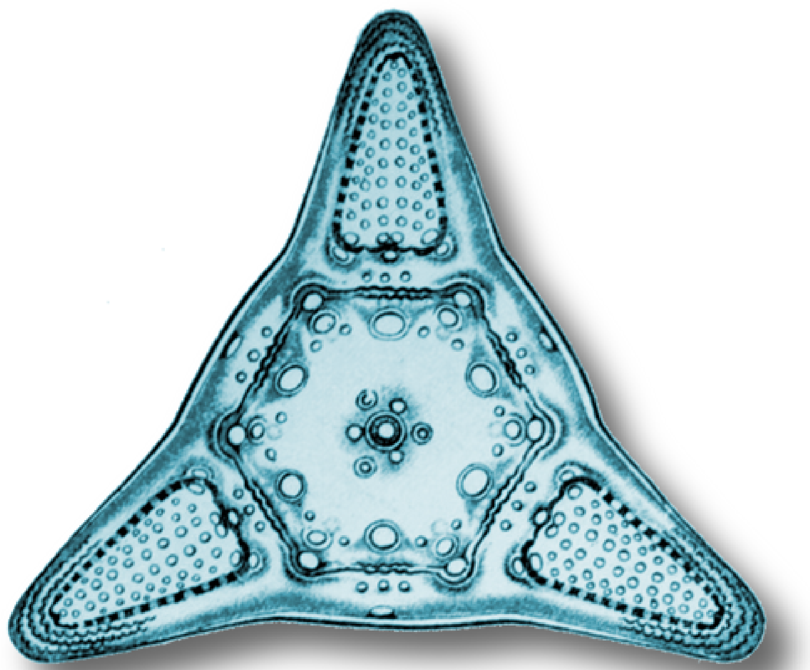

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Table of Contents

Quick Start

1. Run the program **FIReCom-<version>-b<build>-x86.exe** (e.g. FIReCom-1.1.0-b01-x86.exe) to install FIReCom on your personal computer. See Installing FIReCom for details on installation requirements. If FIReCom and the 1.1.1 *In Situ* FIRe USB driver have already installed on your computer, proceed to step 2.

2. If the computer being used has not been connected to *In Situ* FIRe before, it needs to complete installation of the *In Situ* FIRe USB driver for the instrument. Ensure that FIReCom is not running. Connect the power/USB/RS232 cable between the instrument's 8 pin male bulkhead connector and a USB port on the PC (the instrument does not have to be powered). The operating system will first complete installation of the native USB driver and then the VCP (virtual COM port) USB driver.

3. Select **Start->Programs->FIReCom->FIReCom** to run FIReCom.

4. Before connecting power to *In Situ* FIRe, ensure it has been powered off for at least **20 seconds** to allow the internal backup power to discharge. Rapidly cycling power can prevent successful restart.

5. Apply power to *In Situ* FIRe. This can be done using the 8 pin bulkhead connector and the power/USB/RS232 cable using a power supply (8-18 VDC) or battery (12 V). If using a power supply, it should be able to source at least 2 Amps. The *in situ* FIRe can also be powered from the 6pin bulkhead connector, using 18-72 VDC. See Accessories for more details.

6. When *In Situ* FIRe has completed start-up and initialization (60 seconds after connecting power source), FIReCom will automatically enter **Setup** mode and enable applicable buttons on the dashboard.

7. Select **Sensor->Operation Mode** to open the **Operation Mode** dialog. If this dialog fails to open or reports an error, then there is a problem with the connection. See Troubleshooting for help.

8. Review the FIRe Settings and modify if desired. Press **F1** key for help on any selected setting.

9. Select **Cancel** to continue with default settings or select **OK** to apply changes.

10. Fill the flow cell with a water sample, or submerge *In Situ* FIRe in a container of water.

11. Select the **Sample** button on the FIReCom dashboard to start acquiring sensor data in real time. FIReCom will display graphic plots of sensor data as they are received in real time from *In Situ FIRe*. Note that if no active fluorescence signal is detected, FIReCom will report errors because it is not able to properly calculate fitting parameters.

To switch from USB to serial communications, do the following:

1. Click the FIRe Settings button and note the **Serial Port** settings. The default is RS-232 at 38400 baud.

2. Disconnect the USB cable from the PC. Failure to disconnect the USB cable can prevent a successful serial connection to *In Situ* FIRe.

3. Ensure that your computer has an active Internet connection to allow the operating system to search for drivers.

4. Connect the RS232 cable directly to a 9 pin serial connector on your computer, or via the serial-USB converter supplied with *In Situ* FIRe. Note that other serial-USB converters may cause problems.

5. Select **Sensor -> Connect Serial** from the main menu.

6. Select the baud rate noted in step 1 above.

7. Select the COM port to which *In Situ* FIRe is phisically or virtually connected.

8. Press **Connect** to establish communication with *In Situ* FIRe.

CAUTION: *In Situ* FIRe is a complex instrument with numerous configuration options and settings. Before configuring and operating your *In Situ FIRe*, please ensure you understand the following basic operating principles:

- *In Situ* FIRe can be configured to collect any combination of STF (single turnover flash) and MTF (multiple turnover flash) profiles by creating sequences in FIReCom and uploading them to *In Situ* FIRe. Multiple sequences can be defined, but only the **Active** sequence will be executed. Ensure that the right sequence is active. See Principle of Operation and Flash Sequence Settings for more information.

- *In Situ* FIRe can be configured to collect (and output) *Raw* or *Processed* STF data products on board. See FIRe Settings for instructions.

- Raw data files logged internally by *In Situ* FIRe, or externally by FIReCom, can be post-processed as described in Processing Raw Data . MTF processed data is never processed on board *In Situ* FIRe, it must be done in post processing. Simple file playback for visual display is available by post processing with Post-Processing Options disabled.

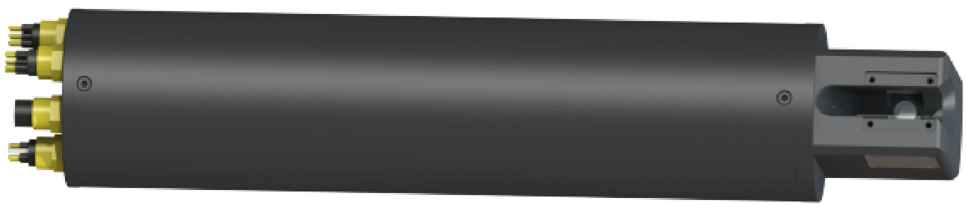
Overview

This chapter provides an overview of In Situ FIRe, how the sensor works, and physical and electrical specifications.

Topics in this chapter include:

About In Situ FIRe

In Situ FIRe (**F** luorescence **I** nduction and **Re** laxation) is a cost-effective solution for real-time chlorophyll analysis in coastal and freshwater environments, providing quick and continuous measurements.



Satlantic In Situ FIRe

Based on the research of Dr. Maxim Gorbunov and Dr. Paul Falkowski from Rutgers University, the FIRe technique uses active stimulation and highly resolved detection of the induction and subsequent relaxation of chlorophyll fluorescence yields on micro- and milli-second time scales.

Some of the many technical highlights of *In Situ* FIRe include:

- Exceptional sensitivity for measurements in low chlorophyll open ocean conditions.
- Real-time measurements of the excitation flash intensity to quantify absolute functional absorption cross-sections (σ PSII).
- Measurement of the reference excitation profile to provide real-time corrected fluorescence yields.
- A highly uniform LED light source that excites Chl a at 450 nm (680 nm detection).
- High ambient light rejection characteristics of the detector optics.
- Real-time processing of single turnover induction parameters (Fv/Fm, σ PSII).
- A standard integrated depth sensor and optional ambient PAR sensor.
- A flow cell accessory for dark-adapted measurements and pumped configurations.
- RS-232, RS-422, USB, and analog telemetry.

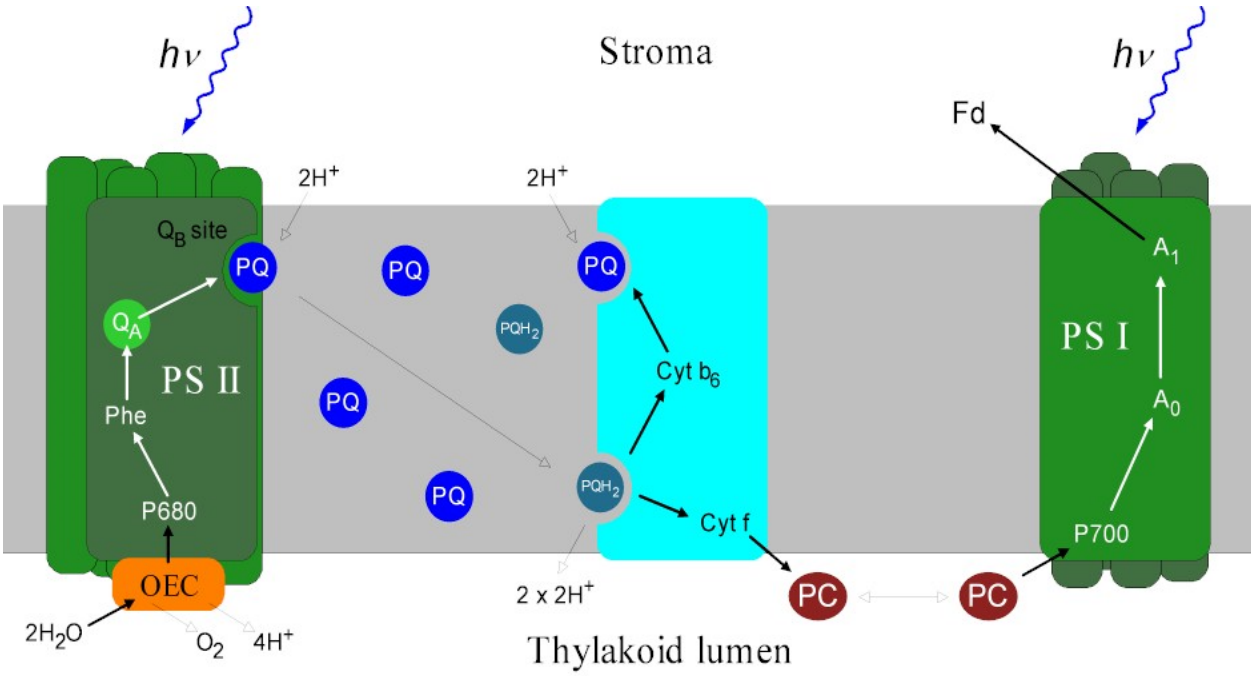
In Situ FIRe comes complete with FIReCom, a full-featured graphical application for Windows XP/Vista/7/8 and Mac OS X that provides easy access to all operational features of FIRe:

- configure sensor settings
- control sensor sampling

- visualize data in real time
- retrieve and process collected data

Principle of Operation

In Situ FIRe characterizes the photosynthetic physiology of oceanic and freshwater microalgae and bacteria. Measurements are made by illuminating the sample with an intense flash of light (approx. 50,000 µmol photons $m^{-2}s^{-1}$), sufficient to instantaneously saturate the photosystem II (PSII) reaction centers at the primary electron acceptor, $Q\ A$, (STF), or the plastoquinone pool, PQ, (MTF).



Either excitation type is then followed by a relaxation (order of some 100 ms), which allows the reduced reaction centers to reoxidize. The rate of reoxidation is measured via brief (1 µs) intermittent light flashes, and the fluorescence response to these flashes. These intermittent flashes are sufficiently weak in intensity and length to not induce a fluorescence induction, or actinic, effect.

The excitation phase is also called induction phase or flash phase, and abbreviations used in the literature are STI (Single Turnover Induction), STF (Single Turnover Flash), MTI (Multiple Turnover Induction), or MTF (Multiple Turnover Flash). The relaxation phase following the excitation is similarly abbreviated as STR (Single Turnover Relaxation) or STRP (Single Turnover Relaxation Phase). The corresponding terms for Multiple Turnover are MTR or MTRP. The complete excitation plus relaxation sequence is called a Single Turnover (ST) or Multiple Turnover (MT) profile.

Physiological Models

The fluorescence profiles as measured by the In-Situ FIRe are interpreted according to the model described by:

Z. S. Kolber, O.Prasil, and P. G. Falkowski, *Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols* , Biochimica et Biophysica Acta 1367 (1998) 88-106.

The profile analysis provides a quantitative characterization of the investigated sample via the functional cross section, the quantum yield, and other values (see below).

Terms and abbreviations

PS II
: Photosystem II

RC II
: Reaction Centers of PS II

Q_A
: Electron acceptor quinone, the primary electron acceptor of PS II . Q_A is the target saturation for STF protocols.

Q_{BX}
: Electron acceptor quinone, the secondary electron acceptor of PS II

PQ
: Plastoquinone, a subunit protein of PS II, another electron acceptor. The plastoquinone pool is the target or saturation for MTF protocols.

ST
: Single Turnover, a fluorescence induction protocol that ensures the reduction of all PSII RC’s instantaneously. This protocol is achieved through a single, 80 – 120 µs flash of light with an intensity of approximately 8,000 µmol photons $m^{-2}s^{-1}$.

MT
: Multiple Turnover, a fluorescence induction protocol that ensures sequential reduction of PS II at QA, QB, and PQ. This protocol is achieved through a frequency modulated flash lasting 500 – 800 ms at an integrated intensity of approximately 8,000 µmol photons $m^{-2}s^{-1}$.

Model quantifiers

I(t) – irradiance (µmol photons m^{-2} s^{-1})
: The FIRe illuminates the sample volume using LEDs. It measures the generated light intensity, and outputs it as a time resolved excitation profile.

$\sigma_{PS\ II}$ – functional absorption cross section of PS II ($Å^2$)
: The functional absorption cross section measures the ability of the photosynthetic apparatus to harvest light from the environment. Under illumination, reaction centers can absorb a photon to enter a chemically reduced state. These reduced centers are no longer able to absorb photons, and are effectively closed to further absorption.

C(t) – ratio of closed RC II

Theoretically, a dark-adapted sample initially has no closed reaction centers. Under illumination, reaction centers will absorb a photon, and become chemically reduced; this means they are closed to further reduction. C(t) lies between zero (none closed) and one (all closed).

p – connectivity factor

When reduced RC IIs are illuminated, they can pass the excitation to surrounding, non-reduced RC IIs. The connectivity factor measures the magnitude of this effect on a zero to one scale; at p=0, the effect is not present, at p=1, all light absorbed by closed centers is passed on until it reaches an open center. For a given absorption cross section, the higher the p-value means faster absorption.

F(t) – fluorescence yield

When a reduced RC II is illuminated, and does not pass the absorbed photon to a connected center (see connectivity factor), it will respond by emitting light at a wavelength longer than that absorbed (i.e. fluorescence). The fluorescence yield is defined as the measured fluorescence divided by the irradiance intensity. As a ratio of two light intensities, the fluorescence yield has no physical units.

$\mathbf{F_0}$ – minimum fluorescence yield after dark adaption

After dark adaptation, all PSII RC’s should be open.In this state, the fluorescence of the sample has its minimal value and is described by F_0 .

Fm – maximum fluorescence yield after dark adaption

After sufficient irradiation, all reaction centers are reduced and fluorescence is at its maximal value and is described by Fm. An irradiated sample reaches this state asymptotically.

$\mathbf{Fv = Fm - F_0}$ – variable fluorescence yield after dark adaption

This measure describes the difference between the maximum and minimum fluorescence yields.

$\mathbf{Fv / Fm = (Fm - F_0) / Fm}$ – photochemical efficiency of open reaction centers of PS II

Fv / Fm provides an estimate of the quantum efficiency of photosynthetic electron transport, or how effective the photosynthetic apparatus is at converting light energy into chemical reductant.

$\boldsymbol{\tau_1, \tau_2, \tau_3}$ – reoxidation time constants (s)

When illumination stops, the excited states of the reaction centers will eventually decay back to their ground state. Depending on the state of the closed center, the decay may proceed over one of three possible pathways, each with its own decay time constant.

$\boldsymbol{\alpha_1, \alpha_2, \alpha_3}$ – ratio of reoxidizing components

The closed centers reoxidize via one of three pathways, and the relative frequency states is measured by the α -values. The sum of the α -values must by definition be 1.

The physiological model as introduced by Kolber et. al. establishes a mathematical relation between the irradiance intensity and the measured fluorescence. At any time, two competing processes occur:

1. Reaction centers are reduced by incoming light. The rate of the overall reduction is quantified by the light intensity and by the functional absorption cross section.

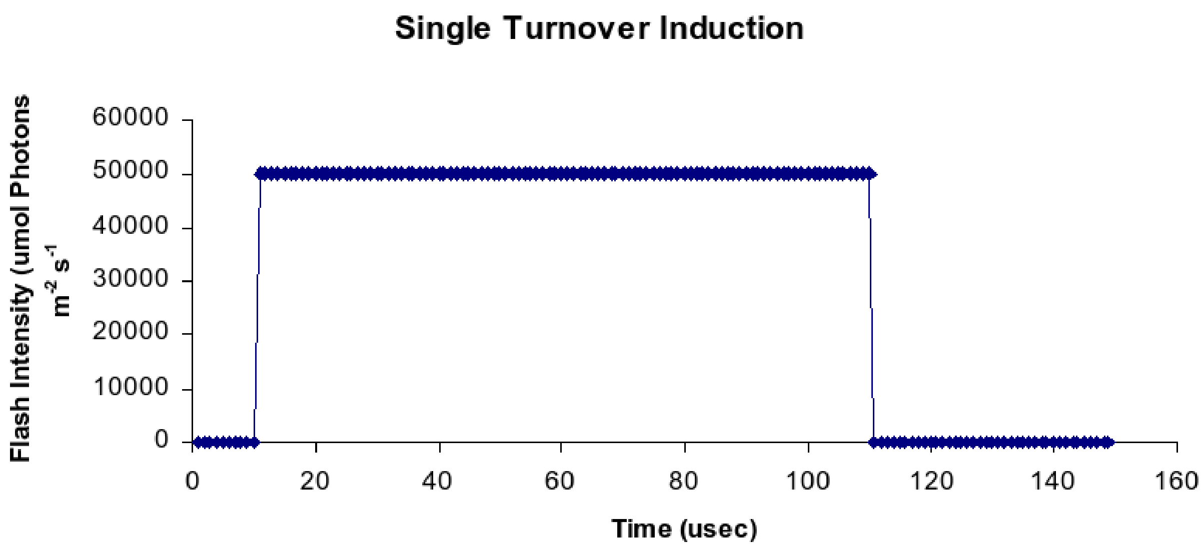
2. Reaction centers reoxidize back into their ground state. The rate of reoxidation is determined by three time constants.

Assuming that the dynamics of PS II are completely determined by these two processes, it is possible to calculate the physiological parameters characterizing the sample ($\sigma_{PS\ II}$, p, F_0, Fm, τ_1, τ_2, τ_3, α_1, α_2, α_3) from the measured fluorescence (F(t)) and irradiance (I(t)) profiles. Numerically, this is done by searching for those parameter values that most closely (in a least square sense) match the profile.

Single Turnover Induction

The purpose of the Single Turnover Induction (STI) is to supply the sample with sufficient irradiation to simultaneously reduce the PSII RC's at Q_A. Further, the STI period must be sufficiently short so that other chromophores are not affected and alter the fluorescence behavior of the sample. The duration of the STI must be shorter than the time scales for reoxidation.

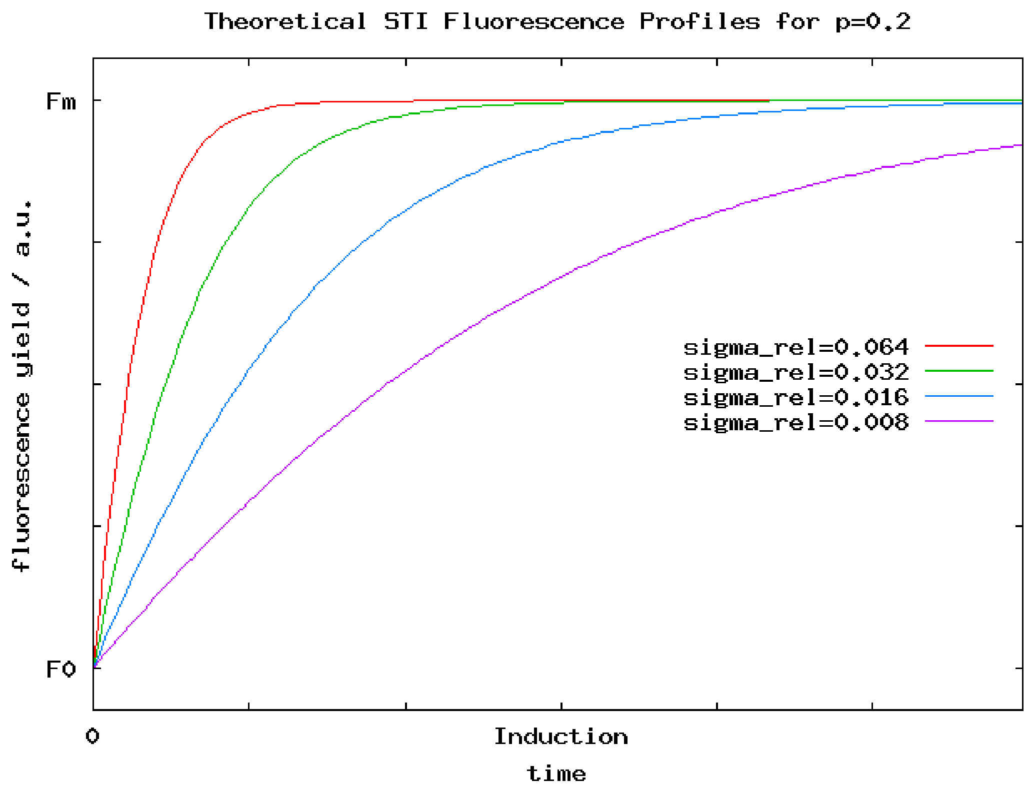
Ideally, a single turnover induction starts from a completely oxidized (dark adapted) state, where the minimum fluorescence F_o. Then, the sample is illuminated by the LEDs, causing the PSII RC's to reduce (close to further photons), and emit fluorescence. Optimally, the duration of a STF phase should be such that the measured fluorescence approaches a plateau. Typical STF durations are in the 80 to 120 µs range.



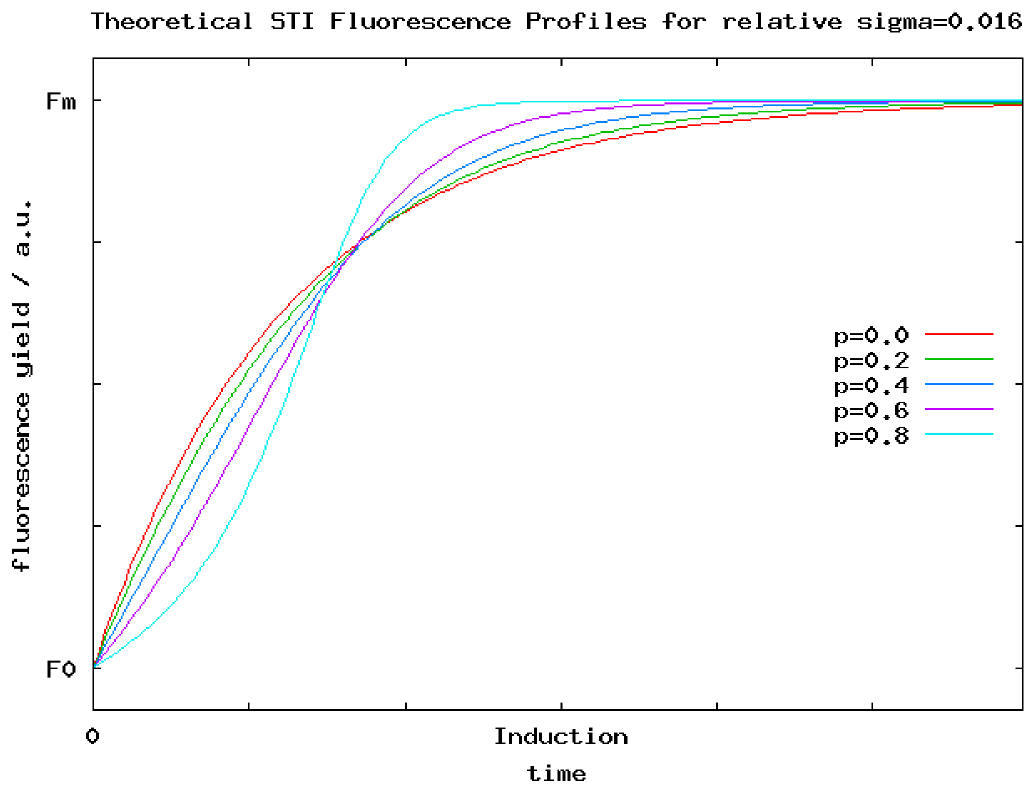
The magnitude of the fluorescence yield profile ranges from the background fluorescence (F_o), which is the height of the profile above the zero line to the maximum achieved fluorescence (F_m), which is asymptotically approached over time. The shape and curvature of the fluorescence yield profile determines the functional absorption cross section ($\sigma_{PS\ II}$) and the connectivity factor (p).

The RC's then absorb a proportion of the excitation light, and are reduced and closed to further photochemical pathway processes. Reaction centers that are closed will emit fluorescence upon further excitation. The relative amplitude of the fluorescence yield is a measure of the fraction of closed reaction centers, and the induction transient of the fluorescence yield represents the time course of the closed centers as well as the absorption cross section.

The effect of varying absorption cross section (σ), while other parameters (F_o, F_m, p) are kept fixed, is illustrated in the following plot. For higher the absorption cross section values, the observed fluorescence reaches F_m faster.



For a given absorption cross section, variations in the connectivity (p) change the shape of the fluorescence profile, as is illustrated in the next plot. With higher p values, the initial rise in the fluorescence is reduced, only to accelerate further on.



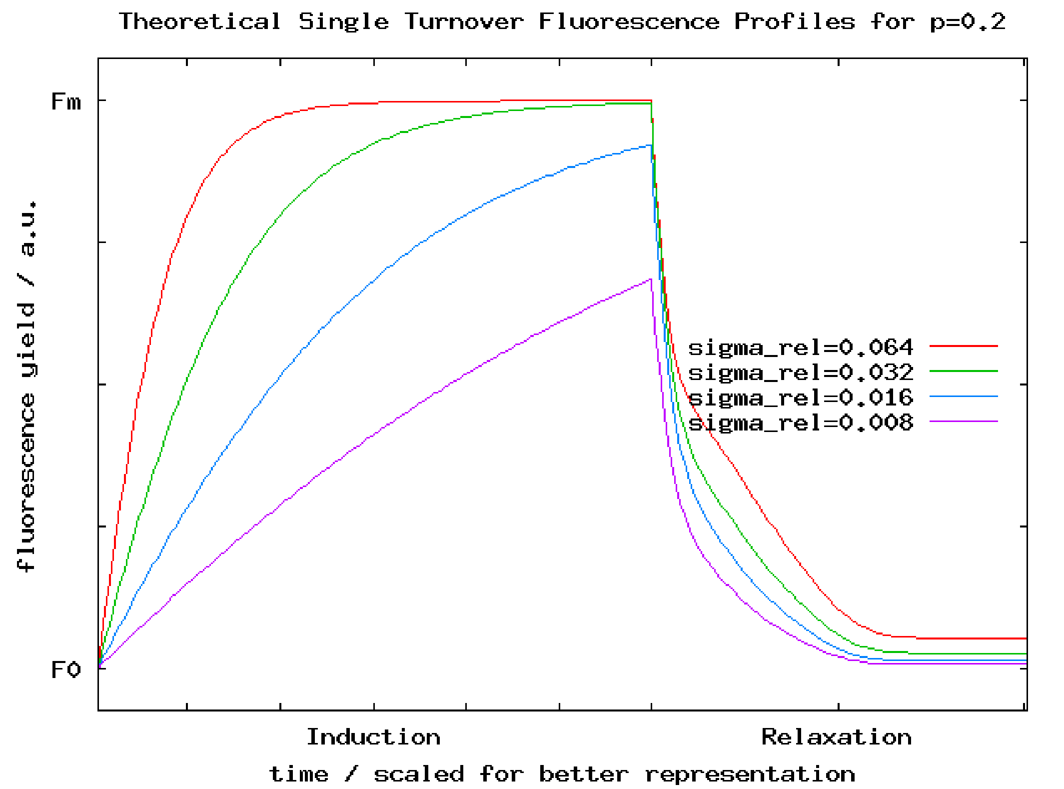
Multiple Turnover Induction

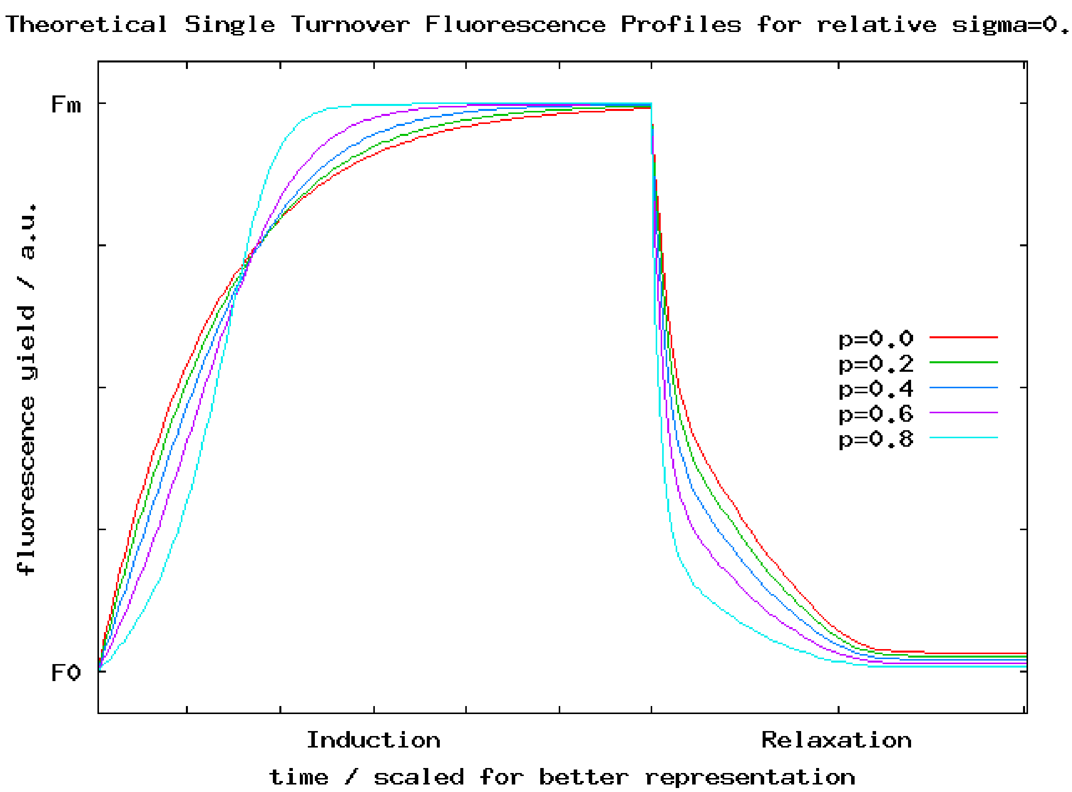
The purpose of the Multiple Turnover Induction is to supply the sample with an appropriate intensity and length of excitation to cause reduction on the time scale of multiple reoxidation time scales. After a sufficient time, excited reaction centers will reoxidize and then be re-reduced, potentially multiple times. This situation forces the photosynthetic electron transport system through multiple cycles that will reduce not only the primary electron acceptor (Q_A) (as in the Single Turnover Protocol), but also the secondary electron acceptor (Q_B), and the plastoquinone pool (PQ). This multiple turnover protocol operates on time scales longer than the single turnover protocols previously described (500 – 800 ms at an integrated flash intensity of approximately 8,000 $\mu mol\ photons\ m^{-2}s^{-1}$ versus 100 μsec at $50{,}000\ \mu mol\ photons\ m^{-2}s^{-1}$).

Relaxation

The relaxation for both ST and MT protocols follow the same biophysical model. Ideally, relaxation follows an induction that resulted in complete saturation, where all RC IIs are in a uniform, reduced, state. It is assumed that three independent pathways exist for the reoxidation of reduced reaction centers (see Kolber et.al., 1998).

Each pathway is characterized by a separate time constant (τ_1, τ_2, τ_3), and the respective contribution of these three pathways ($\alpha_1, \alpha_2, \alpha_3$).


Theoretical Single Turnover Fluorescence Profiles for p=0.2
Fm
F0
fluorescence yield / a.u.
sigma_rel=0.064
sigma_rel=0.032
sigma_rel=0.016
sigma_rel=0.008
Induction
Relaxation
time / scaled for better representation


Theoretical Single Turnover Fluorescence Profiles for relative sigma=0.
Fm
F0
fluorescence yield / a.u.
p=0.0
p=0.2
p=0.4
p=0.6
p=0.8
Induction
Relaxation
time / scaled for better representation

Major Components

Major components of *In Situ* FIRe include the optical head, the main housing, and the connector end-cap. The body and LED modules are constructed of anodized aluminum. The remainder of the optical head and the connector end-cap are constructed of acetal plastic.

in situ FIRe body.

FIRe Optical Head

The optical head contains the pump/probe LEDs, the reference photodiodes, the fluorescence detection optics, and the pressure sensor. The optical components are detailed below. The pressure sensor is detailed in Ancillary Sensors .

Pump/Probe (Excitation) LEDs

Pump and probe optical power is provided by four light emitting diodes (LEDs) mounted on each side of the optical head. The LEDs are thermally connected to anodized aluminum heat sinks, visible on the outside of the optical head. The standard LEDs are blue. The peak wavelength is typically 448 nm, but can be between 440-460 nm. The emission full-width half-maximum is 24 nm. The LEDs provide a highly uniform excitation intensity within the sample volume.

Reference Detectors

On each side of the sample volume, in the middle of the four LEDs is a reference detector that monitors the intensity of the opposite LEDs. These detectors are shielded to prevent detection of same-side LEDs, and spectrally filtered to minimize the effect of sunlight. The reference signal is collected simultaneously with the fluorescent signal and is used to correct for variations both within each flash, and to account for possible longer term variations in excitation.

Fluorescence Detector

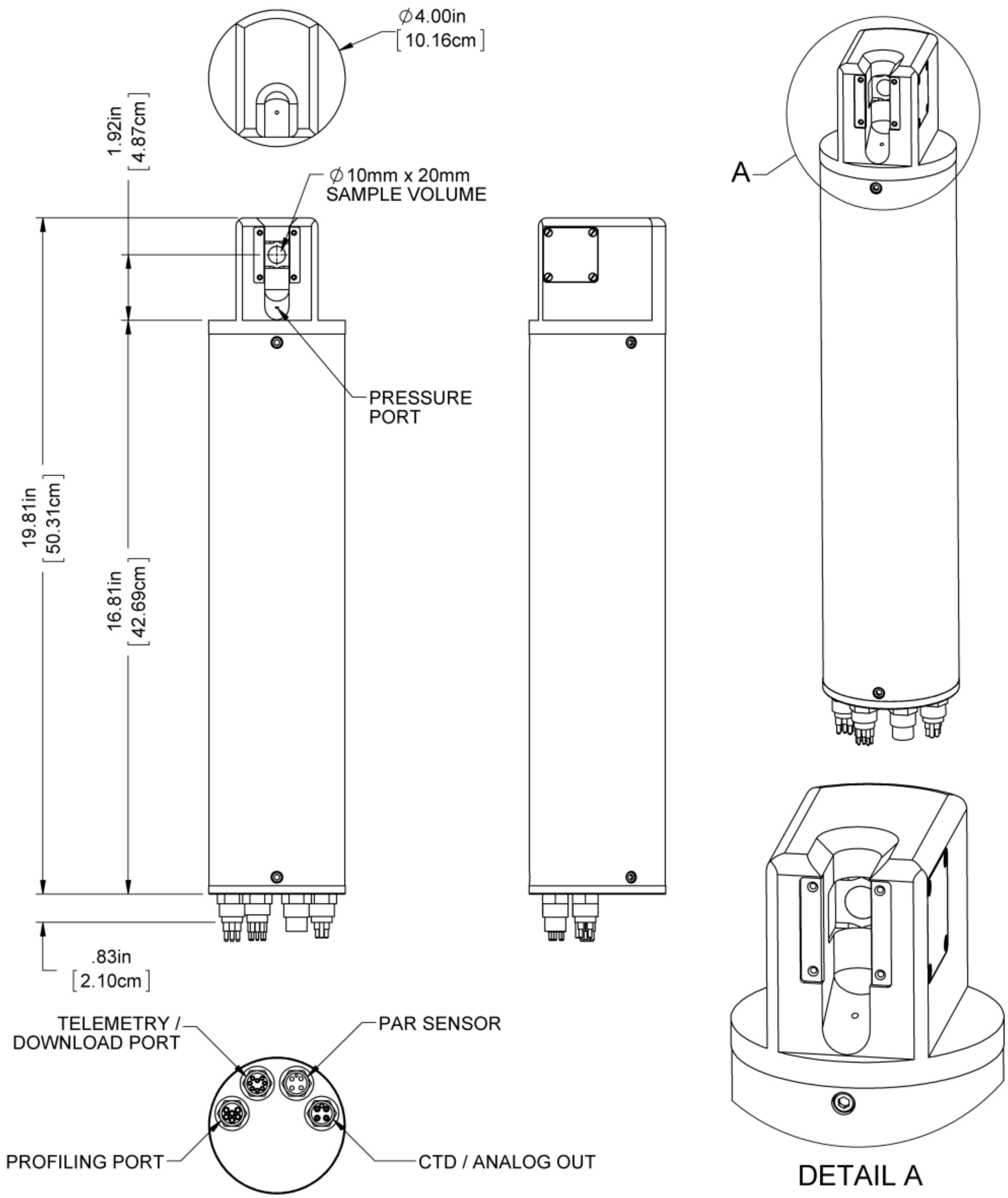
At the bottom of the sensing area, at right angles to the LEDs is a window that receives the fluorescent signal. Opposite this window is a removable stop that is attached to the head. The sample volume is a cylinder limited in radius by the detector optics and in height by the distance between the detector window and the stop. However, the field-of-view is always contained within the cylindrical sample volume.

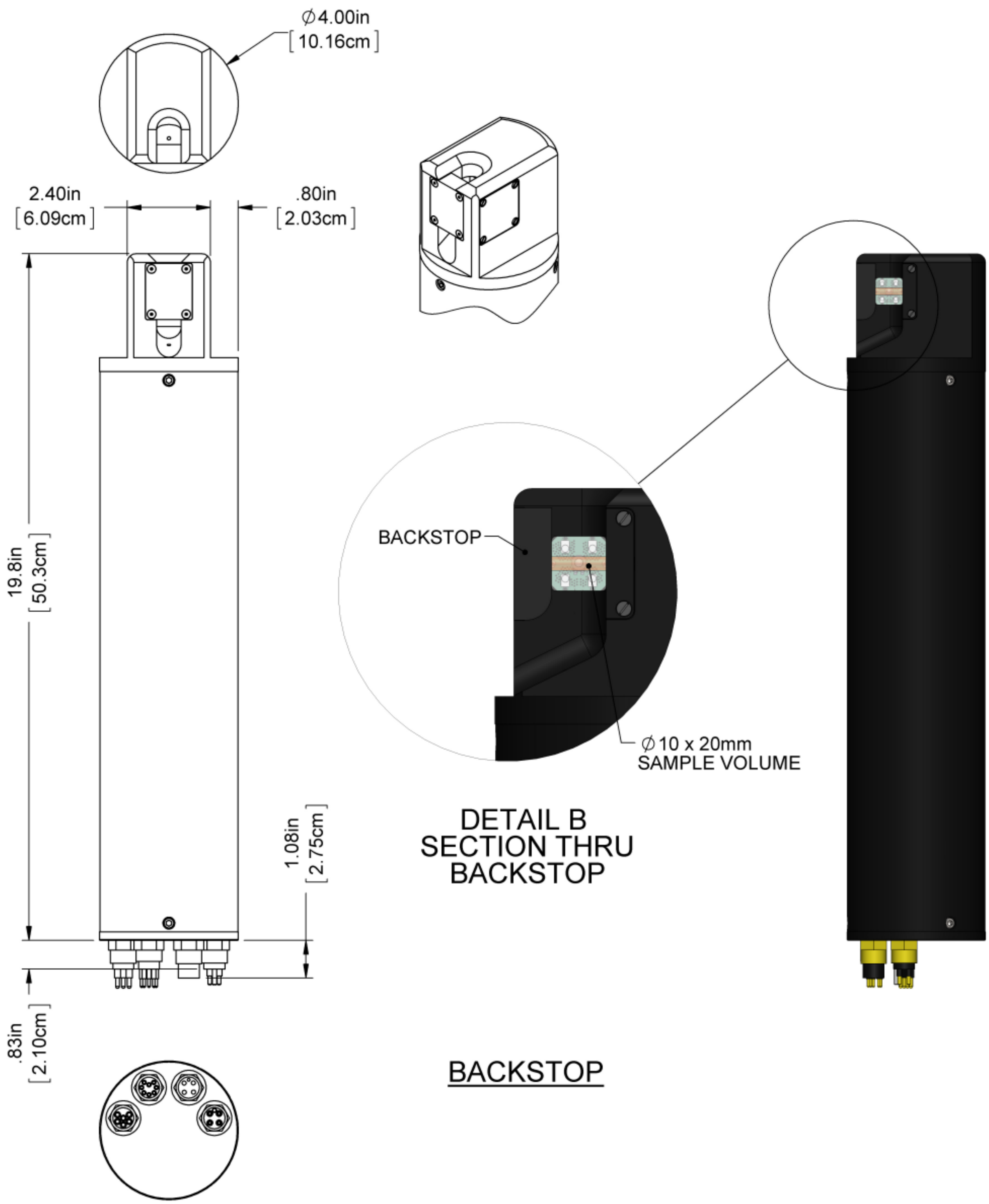
The fluorescent signal is measured through the window at the base of the sample volume by a high speed avalanche photodiode through a window at the base of the sample volume. The light is filtered with a bandpass filter centered at 678 nm with a full-width half-maximum bandwidth of 22 nm. The light is detected with a high speed avalanche photodiode. The sample volume is cylindrical, being constrained on the bottom by the detector window, on the top by the backstop or flow cell, and around the side by the field of view of the detector optics. These dimensions are 1 cm diameter by 2 cm, giving a sample volume of 1.57 cm3.

To maximize the fluorescence detection, the field-of-view or numerical aperture of the detector varies with radial position. At the center of the sample volume the field-of-view is maximum, and it decreases with radial distance from the center. In this way the sensitivity of the instrument is maximized while maintaining a sharp, well defined sample volume.

The APD is safe from damage in ambient sunlight. However, either the flow cell or the backstop should always be in place. If the backstop or flow cell was in in place and the detector window was pointed directly at the sun, damaged to the avalanche photodiode could result.

Instrument Drawings

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BACKSTOP
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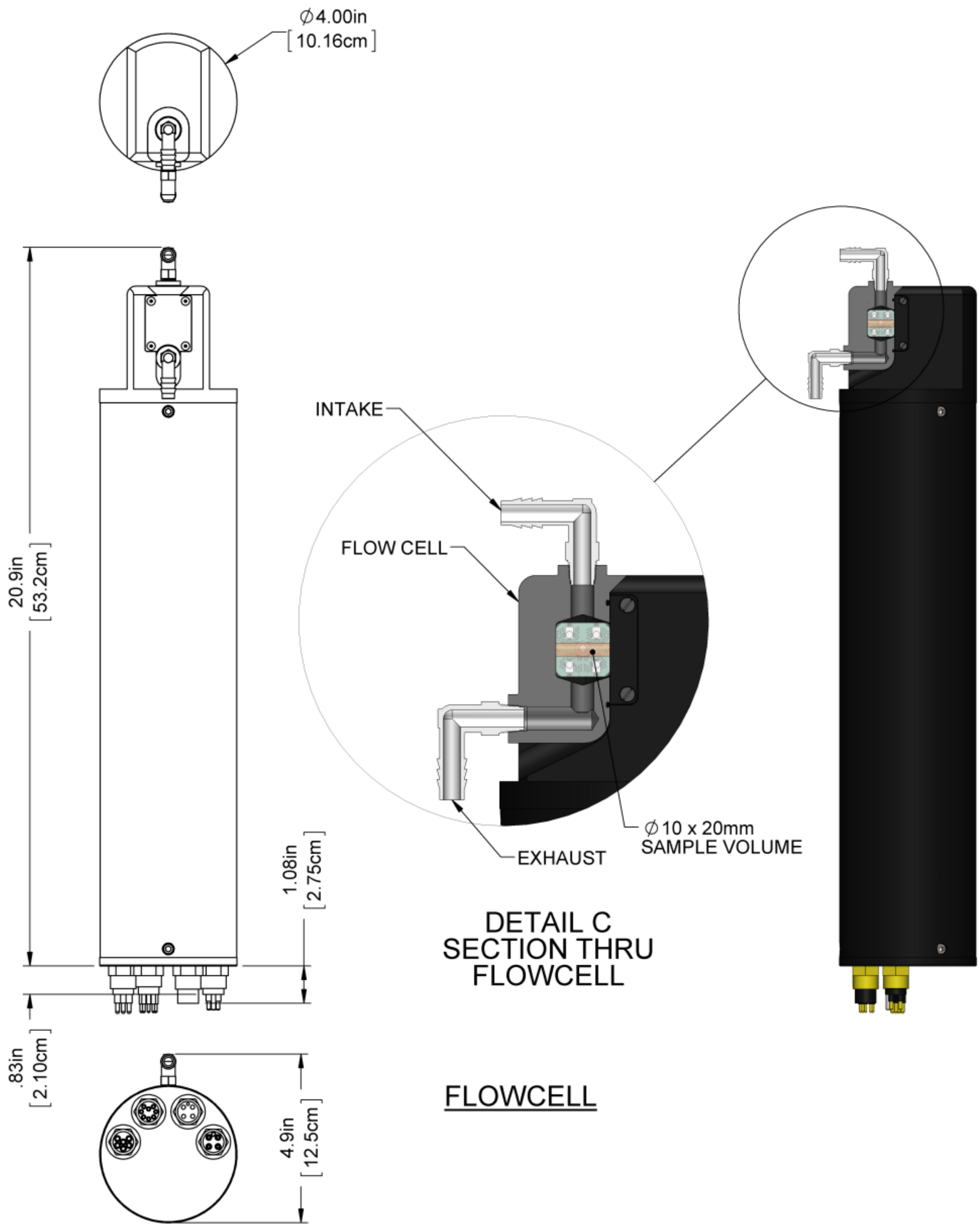
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INTAKE
FLOW CELL
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SAMPLE VOLUME
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[2.75cm]
DETAIL C
SECTION THRU
FLOWCELL
.83in
[2.10cm]
4.9in
[12.5cm]

FLOWCELL

External Interfaces

USB Interface

The Universal Serial Bus (USB) interface is used for both telemetry output and for FIRe configuration. Data is transmitted from *In Situ* FIRe via USB when the instrument is in *Acquisition Mode* , and FIRe configuration and maintenance can be performed from FIReCom when in *Setup Mode* .

RS-232 Interface

The RS-232 interface can be used for both telemetry output and for FIRe configuration. Although all configuration commands are available using a simple terminal emulator (such as Microsoft HyperTerminal), these commands are described in Command Reference.

It should be noted that a carriage return *and* linefeed are required from the terminal program. For instance, in Microsoft HyperTerminal, select *File -> Properties -> Settings -> ASCII Setup* and check the *Send line ends with line feeds* checkbox.

RS-422 Interface

The RS-422 interface can be used for both telemetry output and for FIRe configuration, similar to the RS-232 port. The RS-422 port is designed to be used with a profiling cable.

Analog Interface

FIRe outputs Fv/Fm measurements expressed as an analog voltage ranging from 0.0 V to 4.096 V. To convert voltage to Fv/Fm, divide it by 4.096.

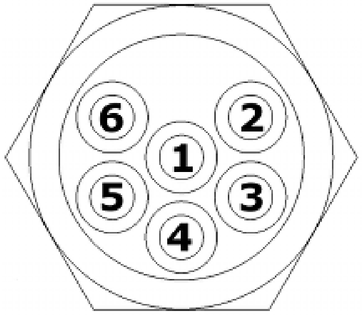
Interface End Cap

The interface end cap has four bulkhead connectors.

Connector End Cap

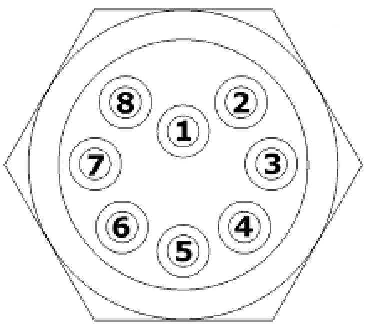
The four bulkhead connectors are, clockwise from left, an MCBH6M (power/RS-422), an MCBH8M (power/USB/RS-232), an MCBH4F (analog PAR), and an MCBH4M (CTD power in/analog out) manufactured by Subconn. The pin-out and function of each signal is described below. Please note that in this manual, the terms RS-232/422 imply EIA 232/422.

MCBH6M 6-Pin Male Connector



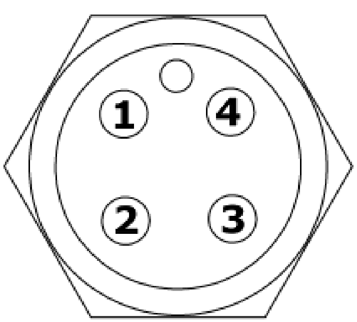
Pin	Name	Description
1	Vprofile	Power input, 18-72 VDC
2	GND	Power common
3	TX+	RS-422, data from In-Situ FIRe to PC, positive differential
4	TX-	TX- RS-422, data from In-Situ FIRe to PC, negative differential
5	RX+	RS-422, data from PC to In-Situ FIRe, positive differential
6	RX-	RS-422, data from PC to In-Situ FIRe, negative differential

MCBH8M 8-Pin Male Connector



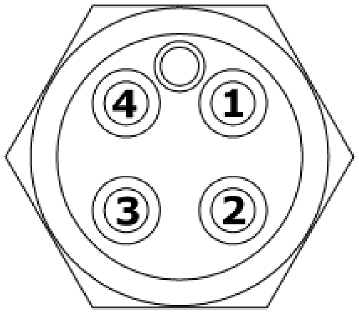
Pin	Name	Description
1	Vin	Power input, 8-18 VDC
2	GND	Power common
3	USB -	USB, negative differential
4	USB+	USB, positive differential
5	TX	RS-232, data from In-Situ FIRe to PC
6	RX	RX RS-232, data from PC to In-Situ FIRe
7	Vusb	USB 5V
8	GNDusb	USB common

MCBH4F 4-Pin Female Connector



Pin	Name	Description
1	GND	Power common, for PAR sensor
2	PARin	Analog input, from PAR sensor only
3	PARgnd	Analog ground, from PAR sensor only
4	Vout	Power output, 12 VDC for PAR sensor

MCBH4M 4-Pin Male Connector



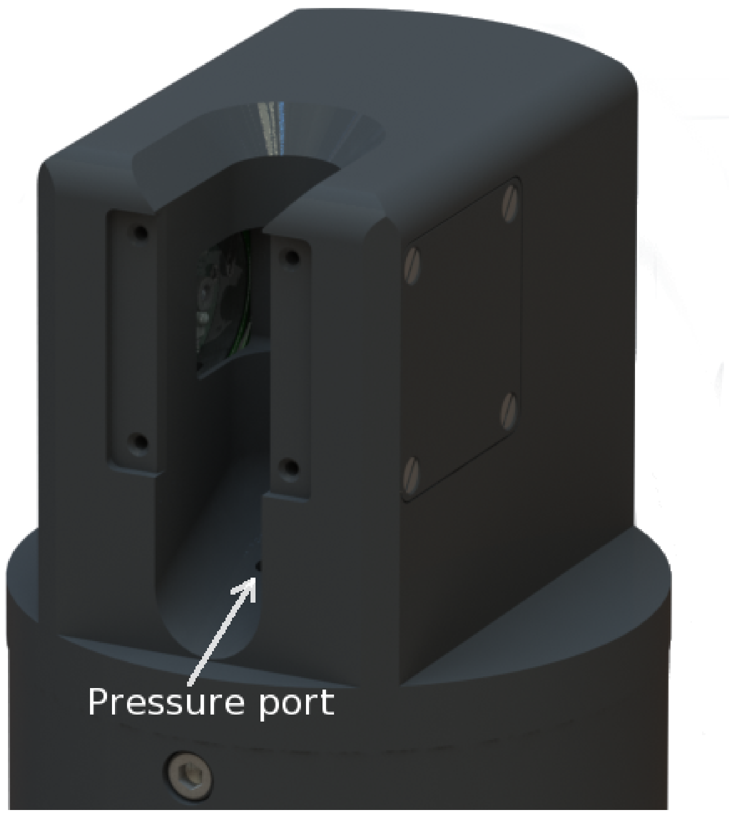
Pin	Name	Description
1	Vin	Power input, 8-18 VDC
2	GND	Power common
3	VA	Analog output voltage (0 – 4.096V) representing 4.096*(Fv/Fm)
4	GNDA	Analog output common

Ancillary Sensors

In Situ FIRe has an integrated ancillary pressure sensor. An optional external PAR sensor is also available. See Accessories for more informaton.

Pressure Sensor

A high quality pressure sensor is integrated in the *In Situ* FIRe optical head. The standard pressure sensor has a full scale of 300 bar and an accuracy of 0.05% (resolution approx. 15 cm). It is located in close proximity to the sample volume to minimize offsets. The pressure port is visible as a small hole near the sample volume. The sensing diaphragm is located 6.6 cm from the center of the sample volume. A compensating correction may be applied for deployments where the FIRe is mounted perpendicular to the water surface (vertical).



Pressure sensor inlet port on the FIRe optical head

To account for atmospheric pressure, FIReCom applies a constant tare of 10.1325 dBar to each pressure measurement received in the data stream emitted from FIRe.

Over time, the pressure sensor may exhibit some drift in its readings. The drift is a constant offset to the readings. The sensor can be reset as described in the Calibrate Pressure Sensor section.

Accessories

Flow Cell / Dark Adaptation Chamber

The removable flow cell accessory can be pressed into the optical head in order to seal and isolate the sample volume from the surrounding environment. With the flow cell in place, water samples can be pumped through the the resulting sample chamber at controlled rates. The maximum pressure for the flowcell is 140 kPa (20 psi).

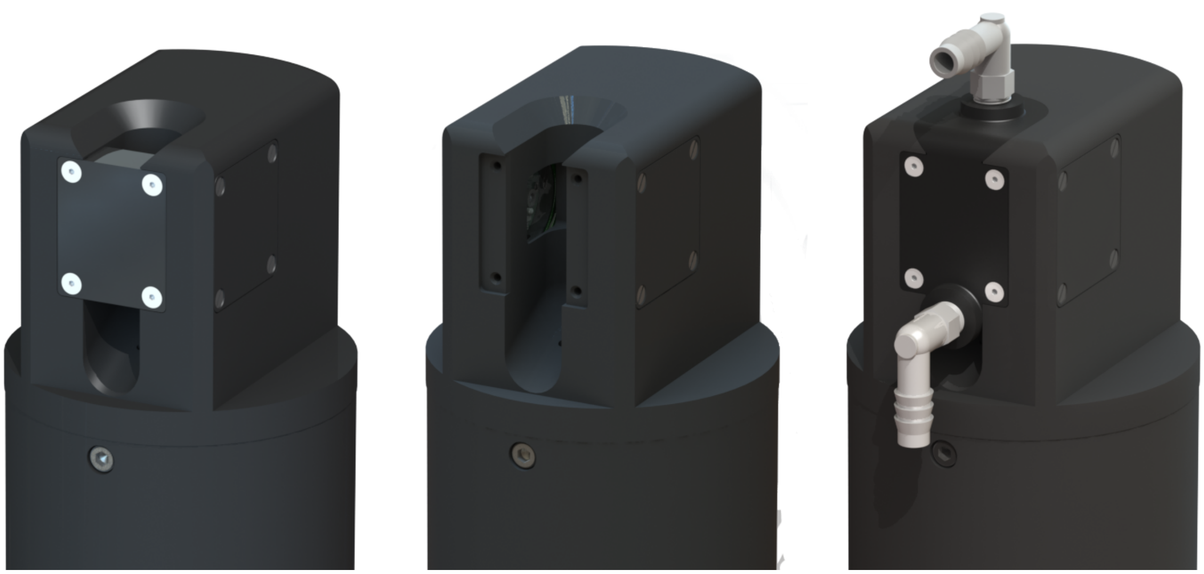
FIRe flow cell accessory

The flow cell accessory is particularly useful for:

- measuring dark-adapted samples
- preventing bio-fouling in long-term deployments
- underway flow-though operation aboard research vessels

To install the flow cell:

1. Remove the square backstop opposite the primary detector.
2. Check the the o-ring is properly seated in the groove that wraps the flow cell body.
3. Press the flow cell into place.
4. Secure the flow cell with provided retaining screws.
5. Pump some water through the cell to check for leaks.



In Situ *FIRe optical head fitted with, from left, backstop, no backstop, flow cell.*

If required, replacement retaining screws are 4-40, 5/16". One source is McMaster-Carr, part number 90585A203.

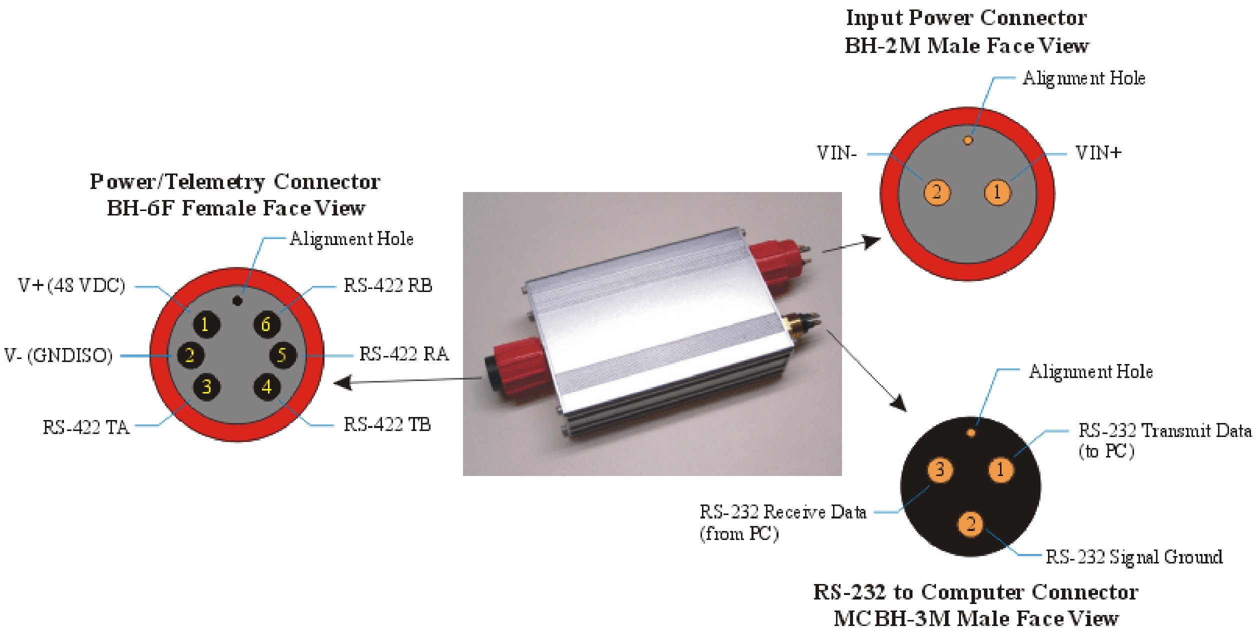
PAR Sensor (Optional)

In Situ FIRe has bulkhead electrical connections for a Satlantic Photosynthetically Available Radiation (PAR) Sensor. *In Situ* FIRe is pre-configured to accept an analog input from a logarithmic PAR sensor.

PAR data is collected for each STF/MTF flash and processed along with the fluorescence data. The PAR accessory allows you to relate the collected active fluorescence data with both depth (using the integrated pressure transducer) and available light. Further, these data can be used to compute higher-level parameters such as electron transport rates (ETR).

Profiling Hardware (Deck Unit and Cabling) (Optional)

The MDU-300 deck unit serves as both a nominal 48 Volt DC power source for the in-situ FIRe system and as an RS-422 to RS-232 level converter. The MDU-300 provides three connectors for data and power as detailed below:



MDU-300 pinout

In Situ FIRe has a power requirement of approximately 7 Watts. Assuming a pessimistic 75% conversion efficiency for the MDU-300, this system would require about 0.8 Amps from a 12 V battery. Using a large rechargeable 12 V battery, such as a 50 amp-hour gel-cell, will provide many hours of operation and will have sufficient power to run a laptop as well.

The **Profiling Cable** runs from the deck unit to the instrument body. It acts as a mechanical and electrical tether, providing a flexible, high strength connection between the vessel and the instrument and providing a channel to transport telemetry to the deck unit. The cable weighs approximately 740 g / 100 m (5 lbs / 1000 ft) in water. The breaking strength is nominally 725 kg (1600 lbs), and the minimum bend radius is 10 cm (4 in).

The **Supply Cable or Battery Cable** runs from the battery to the deck unit.

The **RS-232 Cable** runs from the deck unit to the computer.

Specifications

Physical

- Operating Temperature: 0-40 C
- Dimensions: 10.2 cm (4.0 in) diameter, 50.3 cm (19.8 in) long
- Construction: Anodized aluminum and Acetal
- Weight: 3.84 kg (8.45 lb)
- Depth Rating: 200 m

Optical

- Sample Volume: 1.57 cm3 (1 cm diameter, 2 cm high)
- Excitation Source:
    - Color: Royal Blue
    - Peak Wavelength: 447.5 nm typical (min 440 nm, max 460 nm)
    - Spectral Bandwidth: 24 nm typical (FWHM)
- Fluorescence Detection:
    - Center Wavelength: 678 nm
    - Spectral Bandwidth: 22 nm (FWHM)

Electrical

- Input Voltage: 8-18 VDC (using programming cable), 18-72 VDC (from MDU-300 over profiling cable)
- Power Consumption: 12 W at startup, 6.5 W standby, 7.5 W flashing (all measured at 12 V)

Data Storage and Communication

- Storage: 4 GB flash memory
- Serial Interfaces: RS-232 and RS-422, 9600-115200 baud

- Universal Serial Bus: USB 1.1 and USB 2.0 compatible

Ancillary Sensors

- Pressure
    - Range: 30 bar
    - Accuracy: 0.05%
    - Resolution: 15 cm
- PAR (Optional)
    - Range: 0-6500 umol photons m-2 s-1
    - Dimensions: 30.5 mm (1.2 in) diameter, 76.2 mm (3 in) long
    - Weight: 102 g (0.22 lb)
    - Depth Rating: 600 m

Flow Cell

- Pressure Rating: 140 kPa (20 psi)

Software

In Situ FIRe comes compete with FIReCom, a graphical software application for configuring and controlling FIRe, and for managing and processing data.

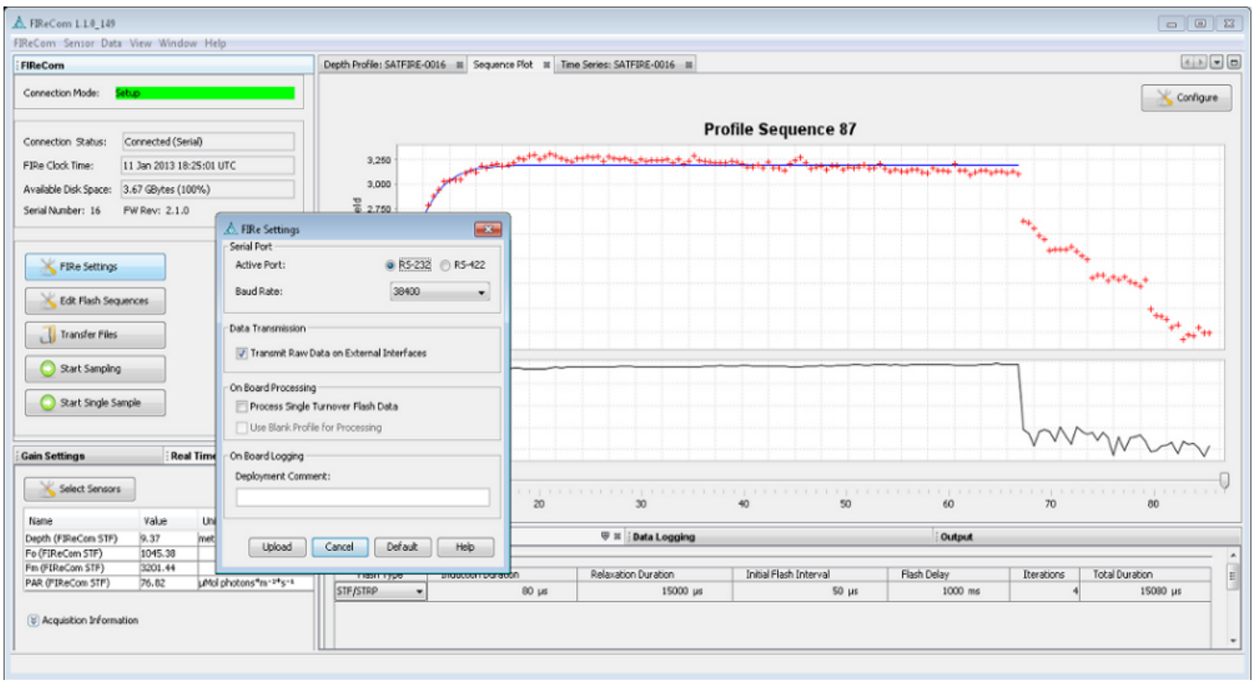
Topics in this chapter include:

FIReCom Overview

In Situ FIRe comes complete with FIReCom, an interactive graphic software application. Connect *In Situ* SeaFET to your computer via the supplied USB programming cable to enjoy these FIReCom features:

- Review and modify FIRe operational settings
- Schedule FIRe data collection activity
- Manage and retrieve logged FIRe data
- View FIRe data in real time
- Reprocess FIRe data and graph results

FIRe provides the tools necessary to configure and operate your *In Situ* FIRe for any deployment scenario. Manage on-board data storage, processing, and sampling modes. Set up real time data streaming via USB or serial port. Capture and plot data in real time for pre-deployment checks or interactive profiling casts. Re-process logged FIRe data for improved accuracy.



Installing FIReCom

For the latest FIReCom software updates, please visit http://satlantic.com/firecom .

Installing FIReCom on Windows

Supported Microsoft Windows systems: XP/Vista/7/8, 32 bit or 64 bit.

Minimum recommended computer hardware:

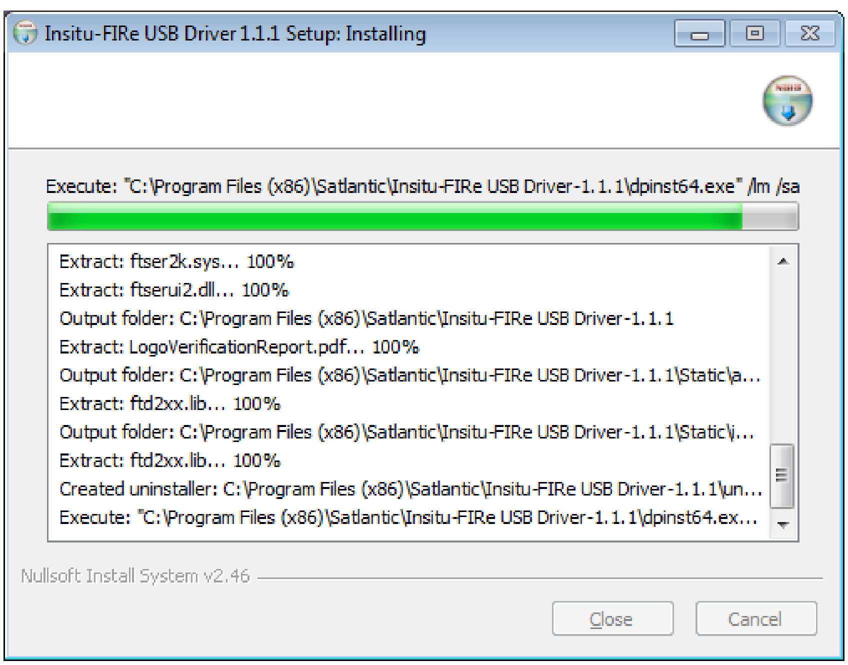
- Processor: 2.6 GHz Intel Pentium IV or equivalent
- Memory: 2 GB
- Disk space: 1 GB of free disk space

To install the software, run the FIReCom self extracting installer program found on your FIRe product CD-ROM or at http://satlantic.com/firecom. The installer is named in the form FIReCom-<version>-b<build>-x86.exe (e.g. FIReCom-0.1.0-b4-x86.exe).

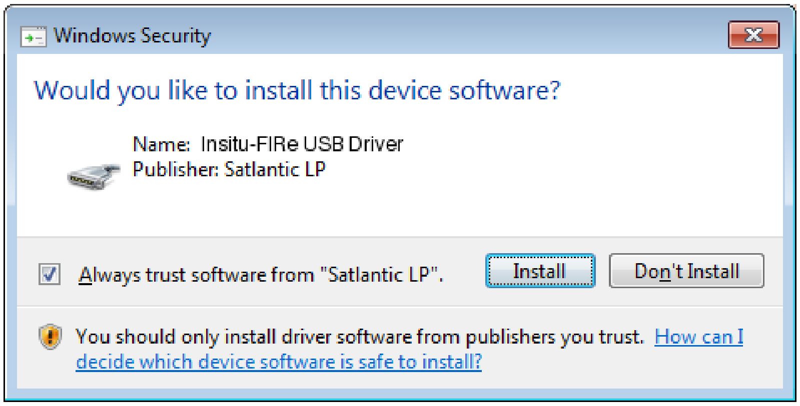
The installer program steps through the install process. The default option can be selected at each confirmation step.

Installing the In Situ FIRe USB Driver on Windows 7 or Vista

The last step in the installer is to install the *USB FTDI D2XX driver* . FIReCom requires this to be run at least once in order to connect via USB. On subsequent installs this step can be skipped. The installation of the *in situ* FIRe USB driver causes the following dialog to be displayed:



After the *in situ* FIRe USB Driver Setup dialog appears, a Windows Security dialog requests confirmation before proceeding to install the driver:



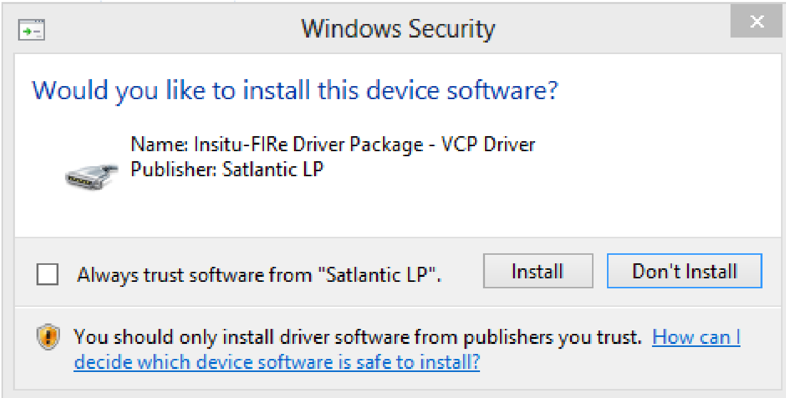
Check the *Always trust software from "Satlantic LP* " option. Press the Install button to confirm trust in software provided by Satlantic and to complete the USB-Serial driver installation.

Installing the driver multiple times should not cause any problems. If for any reason a problem occurs, please use MS Windows Device Manager to view the status of the *in situ* FIRe USB driver and correct any problems. For more information on troubleshooting this FTDI USB driver see D2XX driver (*http://www.ftdichip.com/Drivers/D2XX.htm*) page. FIReCom installs a customized USB driver currently based on the generic FTDI USB Driver version 2.08.14.

Installing the In Situ FIRe USB Driver on Windows 8

Installing the *USB FTDI D2XX driver* on Windows 8 is similar to that as on Windows 7. However, two Windows Security dialogs are displayed during the install process. The first one is shown below:

Press the *Install* button. A second Windows Security dialog, shown below, is then displayed.



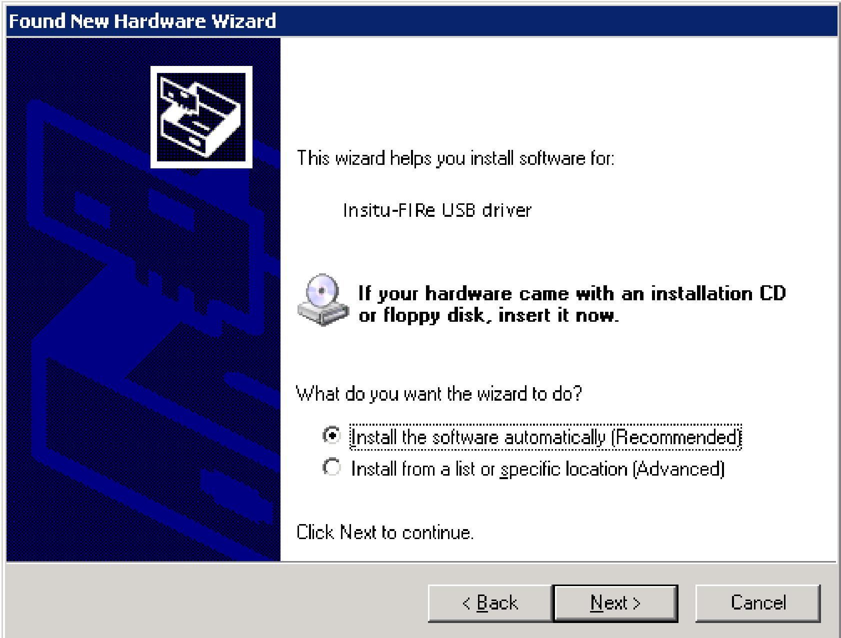
Press the *Install* button to finish the installation of the USB driver.

Installing *in situ* FIRe USB Driver on Windows XP

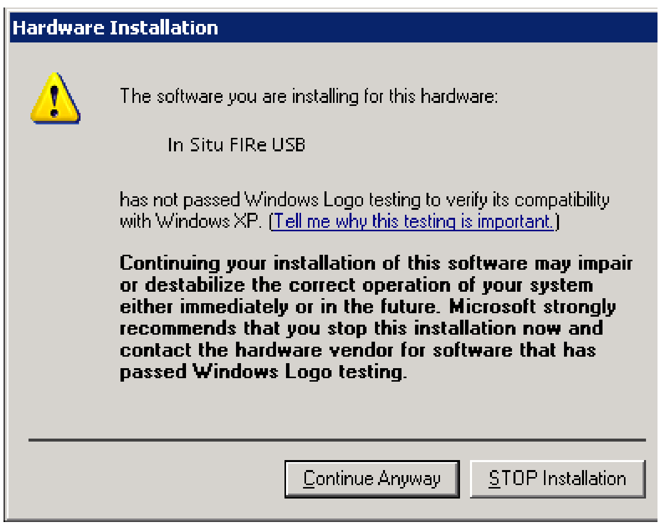
CAUTION *: The FIReCom program must not be running when completing the in situ* FIRe USB driver installation on Windows XP. If FIReCom is running while installing thee In Situ FIRe USB driver on Windows XP, the driver installation will fail.

After the driver installation steps are completed, and before FIReCom is run, the driver must be activated by connecting the *in situ* FIRe to one of the computer's available USB ports. Connect the FIRe to a USB port, then select the *No, not this time* and *Install the software automatically [Recomended]* options on the following *Found New Hardware Wizard* dialogs:

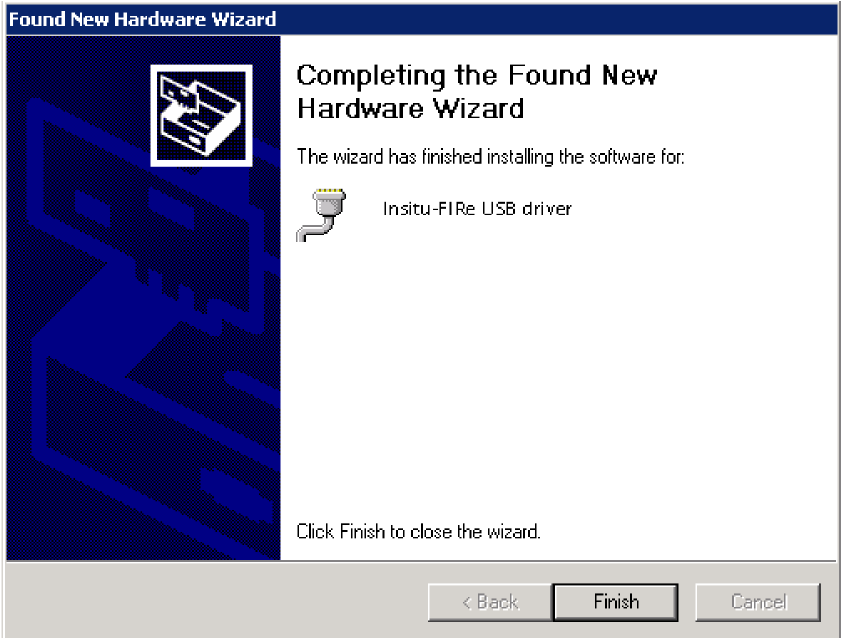
Press the *Next* button to proceed:



After the second dialog, another dialog is displayed to warn that the USB driver publisher can't be verified. Select the *Continue Anyway* button to safely install.



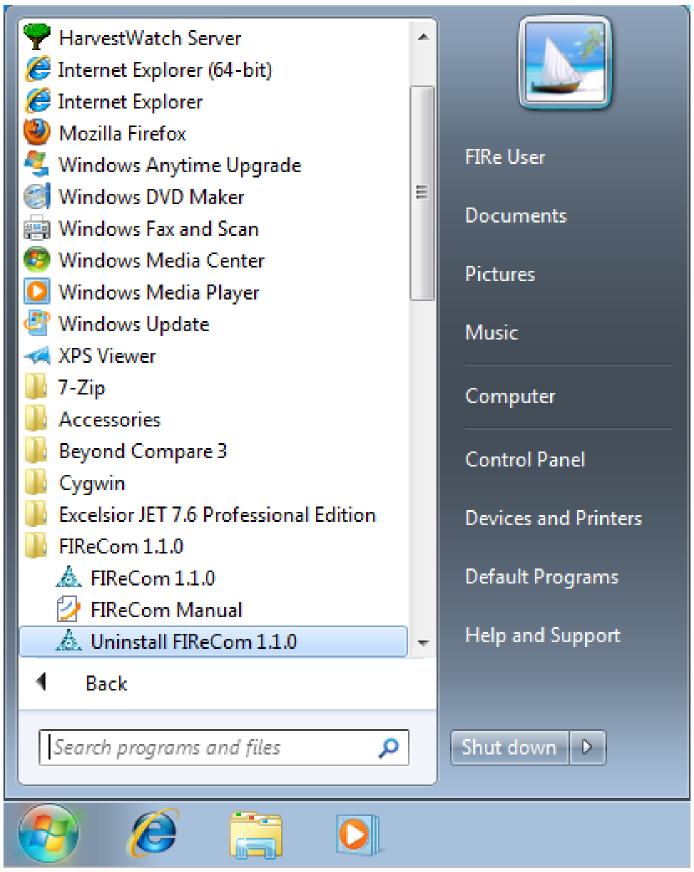
Press Finish button to exit the Found New Hardware Wizard.


Found New Hardware Wizard
Completing the Found New Hardware Wizard
The wizard has finished installing the software for:
Insitu-FIRe USB driver
Click Finish to close the wizard.
< Back
Finish
Cancel

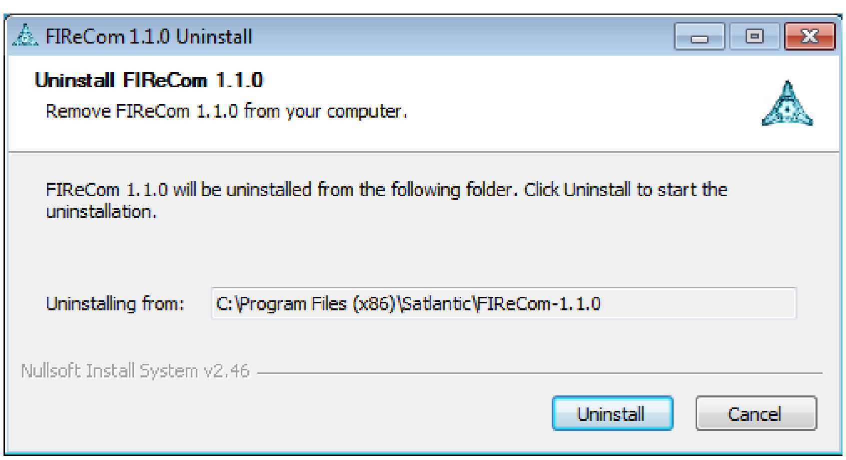
Uninstalling FIReCom

Uninstalling FIReCom on Windows 7

To uninstall the FIReCom software, click on Start -> All Programs -> FIReCom-<version> -> Uninstall FIReCom-<version> as shown in the following screenshot from a Windows 7 computer.

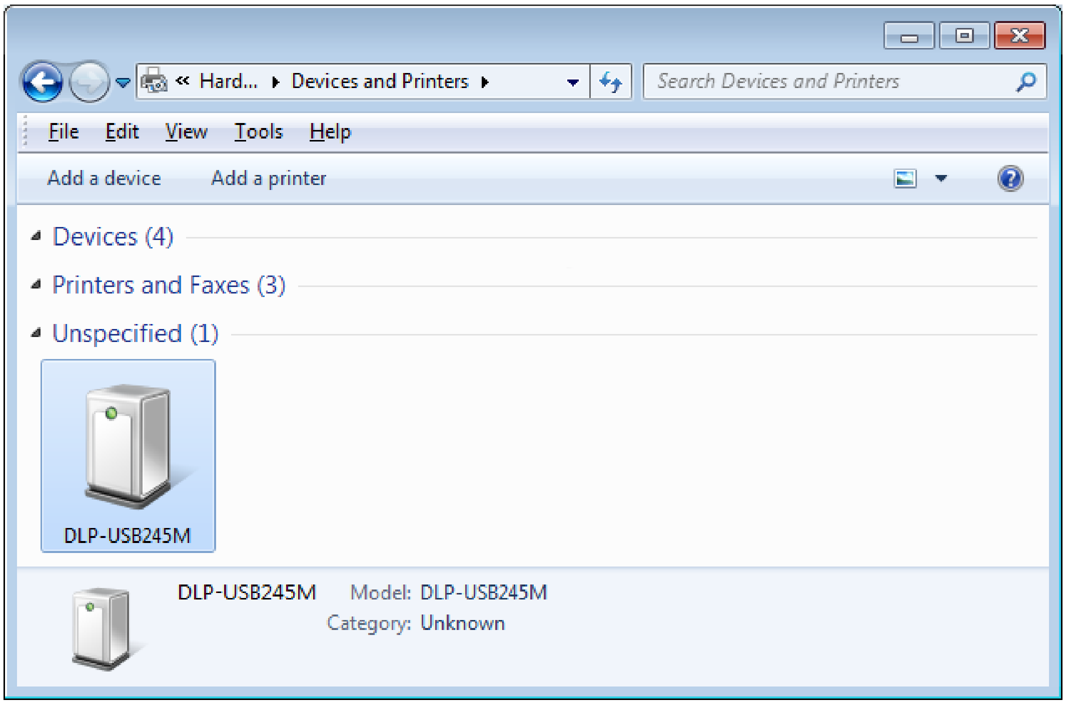


Press the *Uninstall* button on the Uninstaller to proceed with the uninstall of FIReCom.

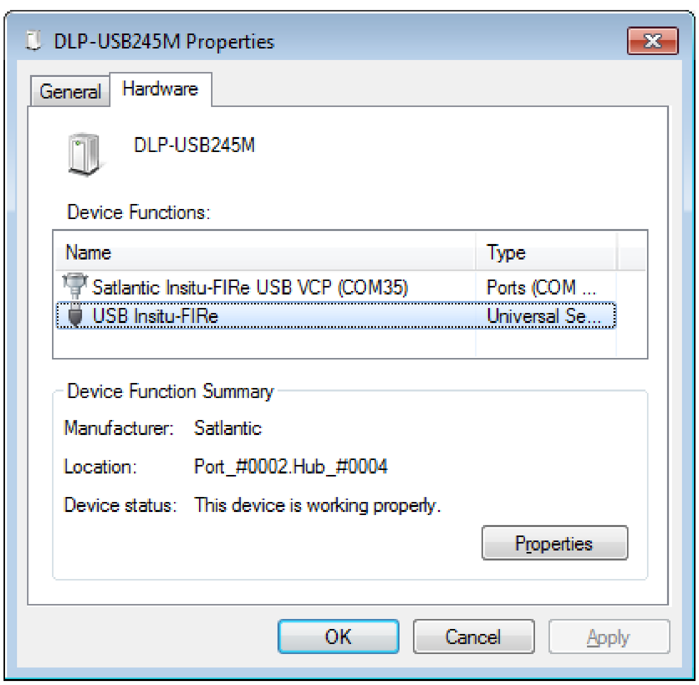


Uninstalling the In Situ FIRe USB Driver on Windows 7

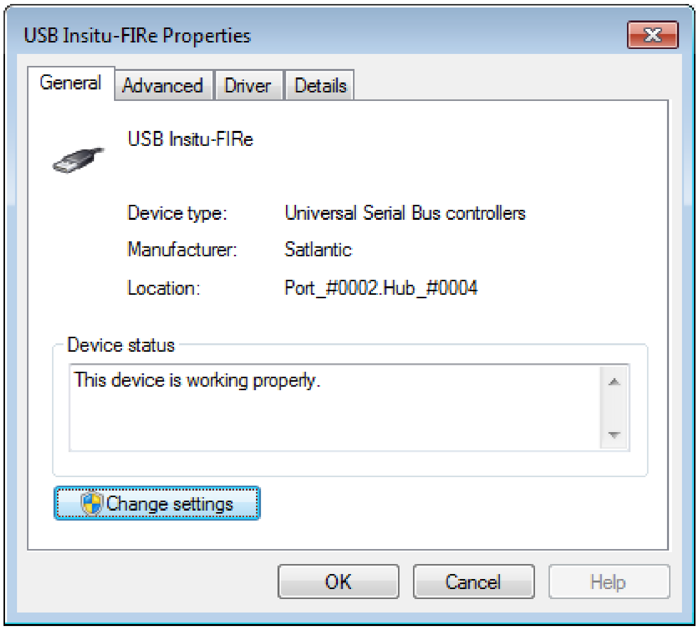
The FIRe instrument must to be plugged into an USB port on the computer in order to uninstall the *USB FTDI D2XX* driver. To begin the uninstall process bring up the *Devices and Printers* window from the *Control Panel* as shown in the following diagram.



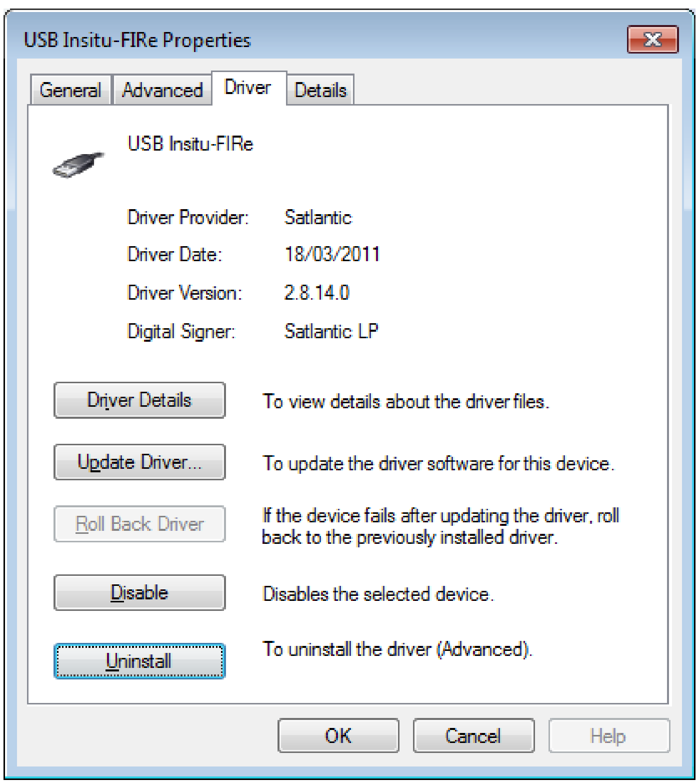
Right click on *DLP-USB245M* and click on the *Properties* menu item to display the DLP-USB245M Properties dialog. Click on the *Hardware* tab.



Click on the *Properties* button.



Click on the *Driver* tab.

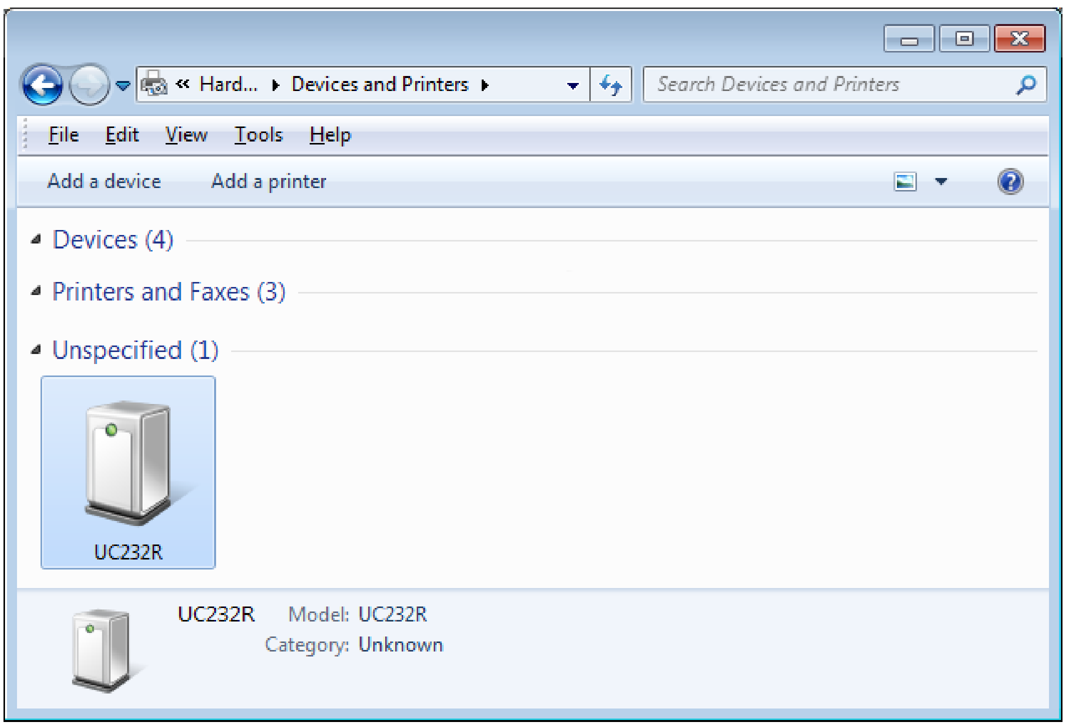


Click on the *Uninstall* button.

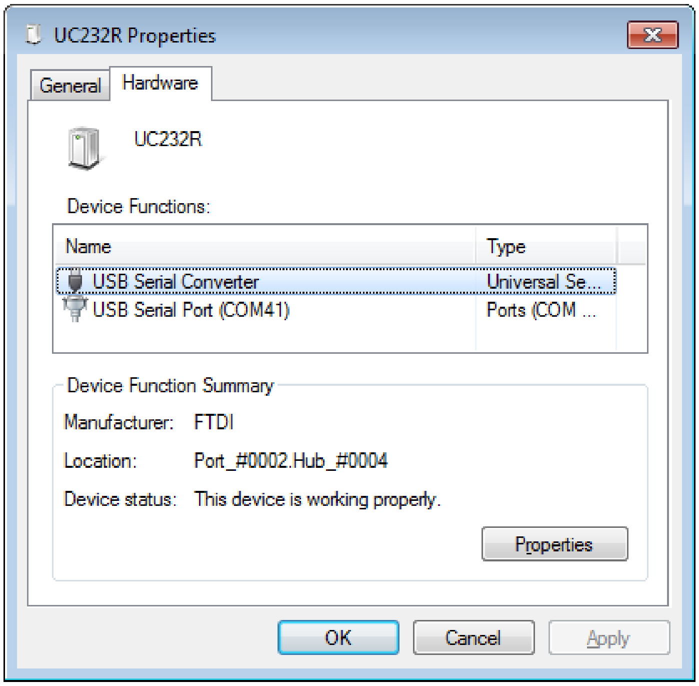
Click on the check box to delete the driver software and then on the *OK* button to finish with the uninstall of the *USB FTDI D2XX* driver.

Uninstalling the FTDI USB Serial Converter Driver on Windows 7

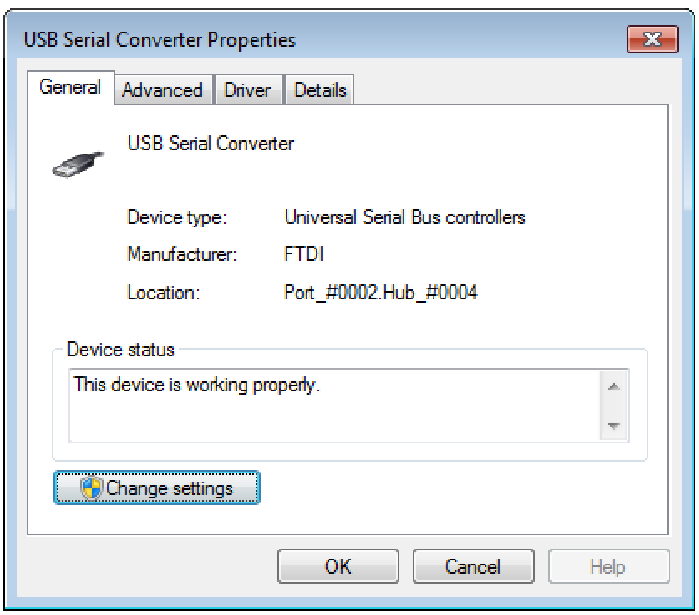
Make sure that the *FTDI USB Serial Converter* driver is plugged into an USB port on the computer before beginning the uninstall proccess. Bring up the *Devices and Printers* window from the *Control Panel* as shown in the following diagram.



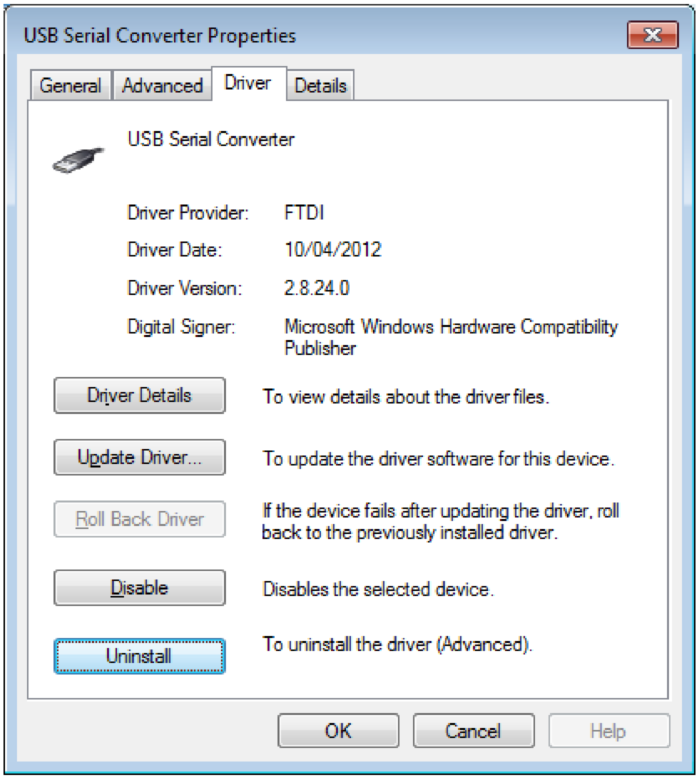
Right click on *UC232R* and click on the *Properties* menu item to display the UC232R Properties dialog. Click on the *Hardware* tab.



Click on the *Properties* button.



Click on the *Driver* tab.

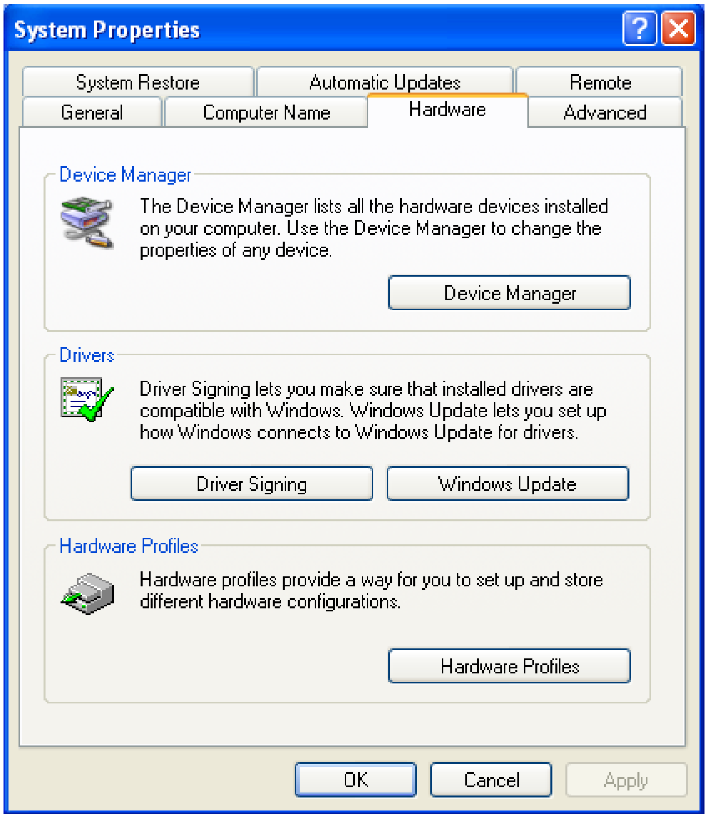


Click on the *Uninstall* button.

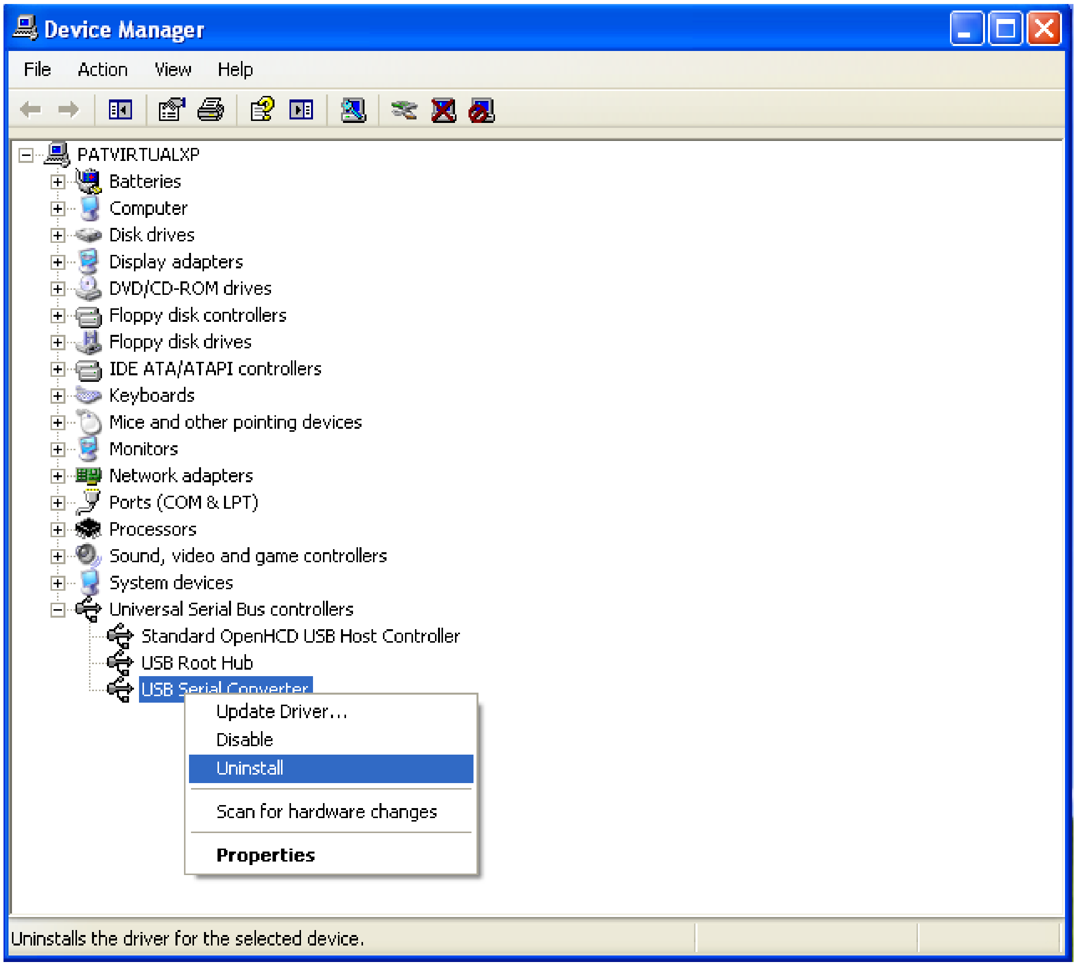
Click on the check box to delete the driver software and then on the *OK* button to finish with the uninstall of the *FTDI USB Serial Converter* driver.

Uninstalling the FTDI USB Serial Converter Driver on Windows XP

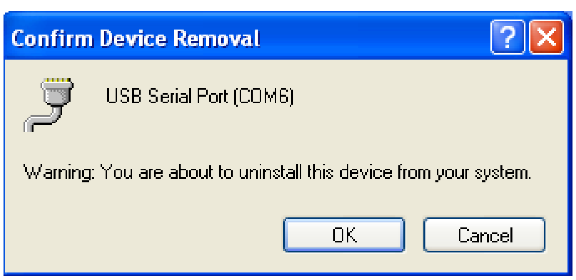
As on Windows 7, make sure that the *FTDI USB Serial Converter* driver is plugged into an USB port on the computer before beginning the uninstall proccess. Bring up the *System Properties* window by clicking on *System* from the *Control Panel*. Click on the *Hardware* tab as shown in the following diagram.


System Properties
System Restore
Automatic Updates
Remote
General
Computer Name
Hardware
Advanced
Device Manager
The Device Manager lists all the hardware devices installed on your computer. Use the Device Manager to change the properties of any device.
Device Manager
Drivers
Driver Signing lets you make sure that installed drivers are compatible with Windows. Windows Update lets you set up how Windows connects to Windows Update for drivers.
Driver Signing
Windows Update
Hardware Profiles
Hardware profiles provide a way for you to set up and store different hardware configurations.
Hardware Profiles
OK
Cancel
Apply

Click on the *Device Manager* button.



Right click on the USB Serial Port and then click on *Uninstall*.



Click on the *OK* button to confirm and proceed with the uninstall of the *FTDI USB Serial Converter* driver.

Navigating FIReCom

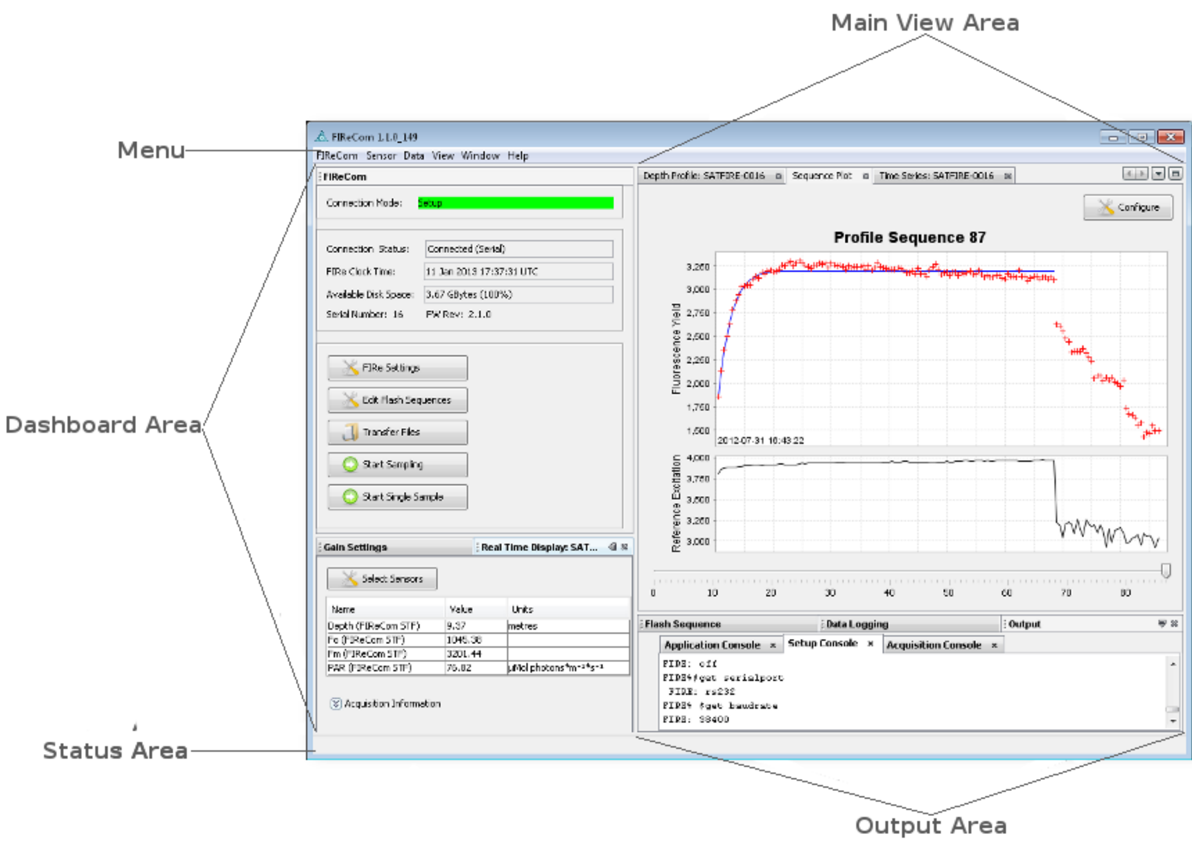
FIReCom has provides a **Menu** for navigating all available actions, and a flexible multiple-window interface for displaying key information relevant to the operating mode of the sensor and software.

The **Dashboard Area** always displays the Dashboard for connecting to *In Situ* FIRe. Other windows such as the Data Processing Dashboard can also reside in the dashboard area.

The **Main View Area** is where larger windows such as the Time Series Graph and the Profile Sequence Plot appear when acquiring or processing data.

The **Output Area** shows diagnostic console outputs from the connected FIRe and of the software application itself.

The **Status Area** along the bottom of the main window shows information messages and progress reports as the software executes various actions.



Menu

The following is a summary of all actions available on the FIReCom main menu:
FIReCom menu lists actions for setting software preferences.

- **Message Logging** - edit Message Logging Settings for logging events, warnings, and errors specific to the software.
- **Preferences** - set FIReCom Preferences for display, serial connection, and data directory.
- **Exit** - halt and exit FIReCom

Sensor menu lists actions for connecting to FIRe and configuring it for operation:

- **Connect/Disconnect Serial** - initiate or terminate a serial connection to *In Situ* FIRe. See Connecting to FIRe for more information.
- **Start Sampling** - start or stop a continuous Real Time Data Acquisition on the connected *In Situ* FIRe. Also starts Real Time Data Display and allows Logging Real Time Data by FIReCom.
- **Start Single Sample** - start acquisition of a single profile sequence on the connected *In Situ* FIRe.
- **FIRe Settings** - review and modify basic settings on the connected *In Situ* FIRe. See FIRe Settings for more information.
- **Edit Flash Sequences** configure STF and MTF Flash Sequence Settings on the connected *In Situ FIRe.*
- **Flash Iteration Settings** - configure Flash Iteration Settings on the connected *In Situ FIRe.*
- **Transfer Files** - use the *File Manager* to assist in Transferring Files from the connected *In Situ* FIRe.
- **Create Blank Profile -** compensate for background fluorescence and the sensor noise floor by Creating Blank Profiles .
- **Ancillary Calibration** - upload new PAR Sensor Calibration coefficients to the connected *In Situ* FIRe.
- **Sync Time -** synchronize the *In Situ* FIRe internal clock with UTC time from the local computer. See Clock Synchronization for more information.
- **Summary Report** - generate a Sensor Summary Report showing status and settings of the connected *In Situ FIRe.*
- **Advanced** sub-menu lists actions typically used only as directed by Satlantic for remote diagnostics and repairs.
    - **Command Terminal** - open a terminal input to the connected *In Situ* FIRe to issue direct commands as documented in the Command Line Interface .
    - **On Board Processing** - set expert parameters for On Board Processing performed by the connected *In Situ* FIRe.

- o **Upload FIRe Instrument XML File** - upload an Instrument XML metadata file to the connected *In Situ* FIRe. See Application Files for more information.
- o **Upload FIRe Sequence File** - upload a flash sequence file to the connected *In Situ* FIRe. See Application Files for more information.
- o **Upload FIRe Firmware Patch File** - upload a firmware patch file to the connected *In Situ* FIRe. See Application Files for more information.
- o **Calibrate Pressure Sensor** - apply a Pressure Sensor Calibration offset to connected *In Situ* FIRe.

Data menu lists operations for processing and replaying logged FIRe data.

- **Process Raw Data** - select raw data files to process as described in Processing Raw Data .
- **Replay Processed Data** - select processed data files to display. See Replay Processed Data for more information.
- **Post Processing Options** - modify Post Processing Options that govern processing raw data in FIReCom.
- **Advanced Post Processing Settings** - expert parameters for FIReCom post processing. See Advanced Post Processing Settings for more information.

View

- **Graphs**
  - o **Sequence Graph** - view the sequence of fluorescence yield profiles and reference excitation profiles on the Real Time Data Display
  - o **Time Series Graph** - view the time series graph on the Real Time Data Display.
  - o **Depth Profile Graph** - view the depth profile graph on the Real Time Data Display
  - o **Blank Profile Graph** - view the blank profile graph. See Creating Blank Profiles for more information.
- **Output** - actions in this menu show tabbed consoles in the *Output* area.
  - o **Setup Console** - show all output generated by *In Situ* FIRe as it executes commands in *Setup* mode.
  - o **Acquisition Console** - show the sensor data as it is transmitted by *In Situ* FIRe while in *Acquisition* mode. The Acquisition Console does not log acquired sensor data directly.

- **Application Console** - show a log of all the actions performed by FIReCom, and any errors or warnings when communicating with *In Situ* FIRe. See Message Logging section for more information.

- **Current Flash Sequence** - view the active flash sequence on the connected *In Situ* FIRe.
- **Gain Settings** - view the Gain Settings panel.
- **Real Time Data** - view the *Real Time Display* panel for Monitoring Real Time Data Acquisition .
- **Data Logging** - view the *Data Logging* panel for Logging Real Time Data .
- **Acquisition Monitor** - view the *Data Acquisition Monitor* to show error statistics when Monitoring Real Time Data Acquisition

Window - each of the following actions operates on the currently selected sub-window or panel of the FIReCom application:

- **Maximize Window** - enlarge the selected panel to fit the top-level FIReCom window.
- **Close Window** - hide the selected panel or window.
- **Undock Window** - detach the selected panel from the main application and show it in its own window.
- **Close All Documents** - hide all non-mandatory panels and sub-windows.
- **Reset Windows** - reset application window layout to the default.

Help - actions for accessing documentation and software version:

- **Help Contents** - show the user manual in the *Help* viewer with a navigable list of contents.
- **Troubleshooting** - show the *Troubleshooting* section of the manual
- **Satlantic.com** - visit the Satlantic web site.
- **User Manual** - open the printable user manual. Requires PDF reader software installed on the local computer.
- **About** - show software version information.

Customizing FIReCom Layout

FIReCom provides a flexible multiple-window interface for customizing the layout of most components. Windows can be viewed, dragged, tabbed, docked, and stretched to best suit the job at hand within the available screen size.
To **move a window** to an alternate location:

1. Click and hold the top bar on the window.
2. Drag it within its own area, or to a different area.
3. Move the window around near the desired location until an orange rectangle frame appears.
4. If the orange rectangle fully covers an existing window, the dragged window will be tabbed within it.
5. If the orange rectangle partly covers and existing window, the existing window will be resized to make room for the dragged window to locate adjacent.
6. Release the mouse button to place the window in its new location.

To **resize a window** :

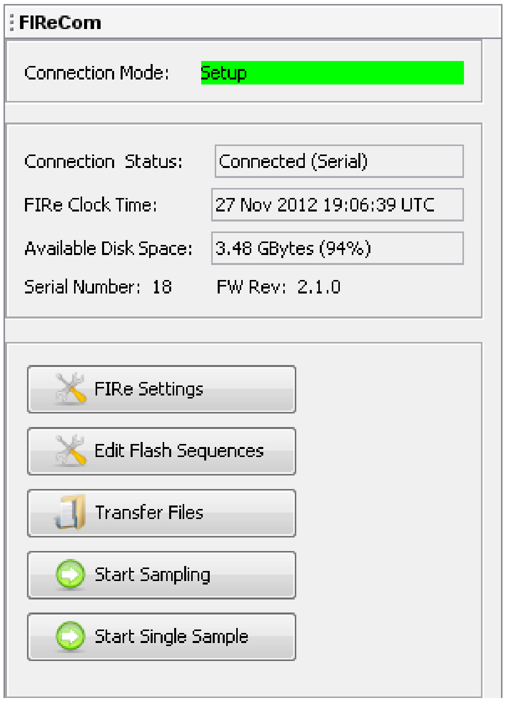
1. Roll the mouse pointer over an edge of the internal window until the pointer changes to a double-ended arrow.
2. Click, drag, and release to set the new window size

To **undock a window** :

1. Click and hold the top bar on the window.
2. Drag it outside the area of the main window.
3. Release to undock the window into its own main window.
4. Alternately, select *Window->Dock Window* on the menu.
5. Select *Window->Dock Window* on the menu to return the window to its internally docked location

FIReCom Dashboard

The dashboard panel in the upper left area of the main FIReCom window displays the identity and status of the connected *In Situ* FIRe. It also provides a menu of buttons linked to the most commonly accessed functions for configuring and operating *In Situ* FIRe.



The following information fields are populated when communication with *In Situ* FIRe has been established.

- **Connection Mode** indicates if *In Situ* FIRe is awaiting commands in *Setup* mode, or if it is collecting data samples in *Acquisition* mode, or if it is in *Transition* between these two modes. *Disconnected* indicates that there is no active connection to *In Situ* FIRe.
- **Connection Status** indicates port connection status and whether the port is serial or USB.
- **FIRe Clock Time** shows the UTC time reported by the internal clock on *In Situ* FIRe.
- **Available Disk Space** indicates the amount of available on-board data storage space on *In Situ* FIRe.
- **Serial Number** is the serial number of *In Situ* FIRe.
- **FW Rev** indicates the revision number of the operating firmware installed on *Is Situ* FIRe.

The bottom half of the dashboard panel lists a menu of buttons for commonly accessed functions affecting *In Situ* FIRe.

- **FIRe Settings** invokes the FIRe Settings dialog for changing basic operating parameters of *In Situ* FIRe.
- **Edit Flash Sequences** starts the Flash Sequence Settings editor to configure the sampling sequence executed by *In Situ* FIRe.
- **Transfer Files** starts the *File Manager* for Transferring Files from *In Situ* FIRe.
- **Start Sampling** commands *In Situ* FIRe to commence executing the active flash sequence until commanded to stop.
- **Start Single Sample** commands *In Situ* FIRe to execute a single iteration of the active flash sequence and then return to *Setup* mode.

Connecting to FIRe

FIReCom can connect to *In Situ* FIRe via one of the three following External Interfaces :

1. USB (Universal Serial Bus) via MCBH8M connector
2. Serial RS-232 via MCBH8M connector
3. Serial RS-422 via MCBH6M connector

Each of these interfaces supports configuration, command, file transfer, and data acquisition from FIReCom.

USB Connect

USB is the preferred connection method for using FIReCom for pre-deployment configuration and test of *In Situ* FIRe. The USB interface povides significantly higher data transfer rates than serial connection and is therefore best for Transferring Files from *In Situ* FIRe after long deployments.

1. Ensure that the FIReCom software and USB driver are correctly installed and activated on your computer as described in Installing FIReCom .
2. Connect the supplied USB programming cable to the MCBH8M bulkhead connector.
3. Connect the cable power leads to a 10 V - 18 V DC power supply. *In Situ* FIRe will be fully started after approximately thirty seconds.
4. Run FIReCom software.
5. Insert the Type A USB connector end of the cable into an available Type A USB socket on the computer running FIReCom.
6. Watch the FIReCom Dashboard for indication that that the connection mode is in the *Transition* state as FIReCom awaits response from *In Situ* FIRe. Mode will change to *Setup* when *In Situ* FIRe is ready for commands.

Serial Connect

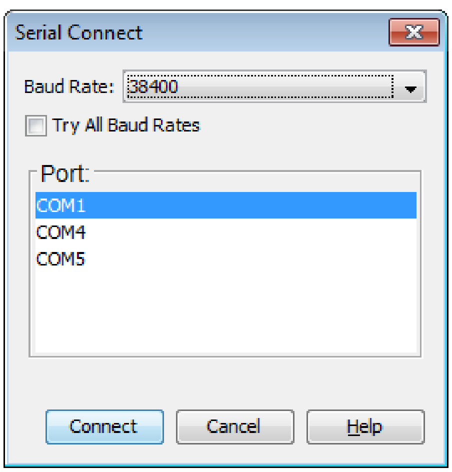
In Situ FIRe provides two serial ports:

1. The RS-232 port on the MCBH8M bulkhead connector is suitable for bi-directional serial connection to FIReCom or other data logging equipment over distances of 15 meters (50 feet) or less.
2. The RS-422 port on the MCBH6M bulkhead connector is suitable for bi-directional serial connection to FIReCom or other data logging equipment where cable length exceeds 15 meters (50 feet).

Only one of these two serial ports can be active at any one time. *In Situ* FIRe is normally pre-configured with the RS-232 port as the active port, transmitting at 38400 baud. To change the active serial port, connect from FIReCom on the current active serial port or via USB as described above, then adjust the FIRe Settings for active serial port and restart *In Situ* FIRe to apply.

To connect FIReCom to *In Situ* FIRe via serial port:

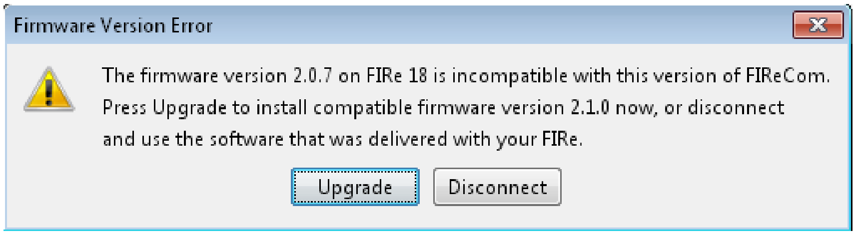
1. Connect the wet-mateable serial cable to the active serial port: either MCBH8M (RS-232) or MCBH6M (RS-422). Ensure connectors are free of dirt and male pins are lightly lubricated with DC 111 silicone grease before mating.
2. Apply power to *In Situ* FIRe via programming cable, battery pack, or deck unit (see Accessories). Allow thirty seconds for *In Situ* FIRe to start.
3. Connect the FIReCom host computer serial port to the deck unit or directly to the cable as required. Note the operating system designation (example: COM2) of the physical serial port.
4. Select *Sensor -> Connect Serial* on the FIReCom main menu to show the *Serial Connect* dialog.
5. Select the **Baud Rate** that is configured on *In Situ* FIRe, or select **Try All Baud Rates** to attempt connection on all possible baud rates.
6. Select the **Port** noted in step 3 above.
7. Press **Connect** to initiate the connection attempt. The FIReCom Dashboard should first indicate connection mode is in the *Transition* an then indicate *Setup* when *In Situ* FIRe is ready for commands.



Firmware Compatibility

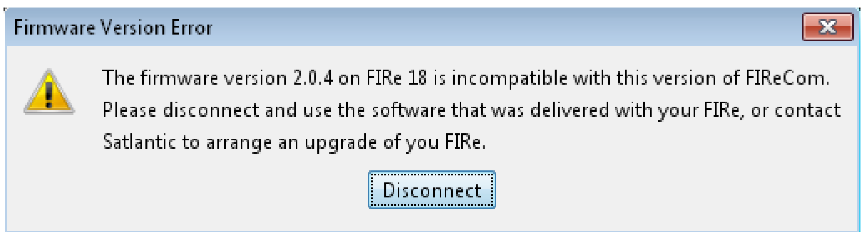
When a connection is successfully establised, FIReCom identifies the firmware revision running on *In Situ* FIRe and determines whether it is compatible with the software version being used.

If the detected firmware revision is incompatible, FIReCom will offer to apply a firmware upgrade:



Press the *Upgrade* button to confirm and proceed. If you do not wish to upgrade the firmware on your *In Situ* FIRe at this time, press *Disconnect.* You can continue to use the older version of FIReCom that shipped with your *In Situ* FIRe.

FIReCom is unable to upgrade firmware on some older version of *In Situ* FIRe. The only option at this point is to *Disconnect* and revert to the version of FIReCom that shipped with your *In Situ* FIRe:

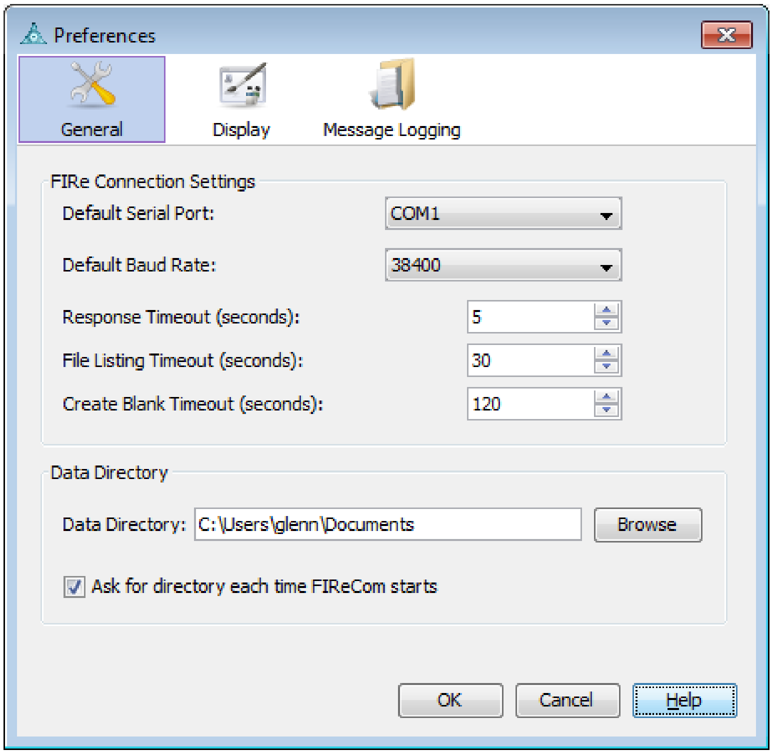


Please Contact Satlantic to arrange a Return Materials Authorization if you wish to upgrade your *In Situ* FIRe to use the latest firmware and software.

FIReCom Preferences

To review and modify FIReCom software preferences, select *FIReCom -> Preferences* on the main menu.

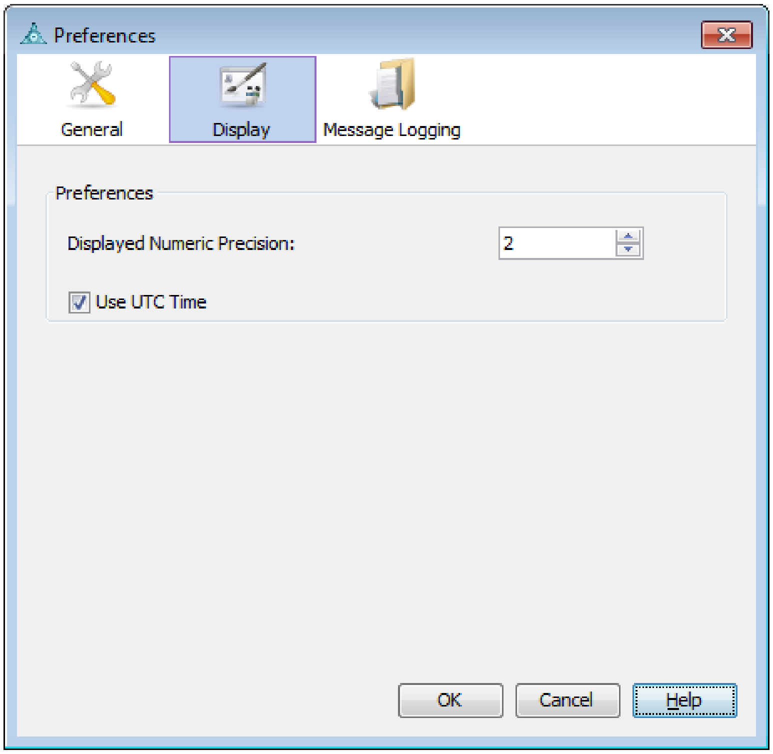
The *Preferences* dialog manages settings for the behavior of the FIReCom software. Preferences edited in this dialog affect software behavior only. This dialog does not alter the settings on *In Situ* FIRe.



The following software application general preferences may be edited:

- **Default Serial Port** sets the default serial communications port shown in the *Serial Connect* dialog. See Connecting to FIRe for more information.
- **Default Baud Rate** sets the default baud shown in the *Serial Connect* dialog. See Connecting to FIRe for more information.
- **Response Timeout** sets the number of seconds that FIReCom will wait for a response from a FIRe. This value can be increased if low baud rates or communication delays cause disconnections.
- **File Listing Timeout** sets the number of seconds that FIReCom will wait for a response from a FIRe for a file listing.
- **Maximum Retries** sets the number of times FIReCom will send a command or attempt connection to *In Situ* FIRe before giving up if no response is received.
- **Data Directory** identifies the local directory to which logged data will be saved.

- **Ask for directory each time FIReCom starts** causes FIReCom to prompt you to specify the the data directory every time it starts. This may be useful where multiple *In Situ* FIRe instruments are alternately accessed from one computer and you wish to keep data files for each separated.



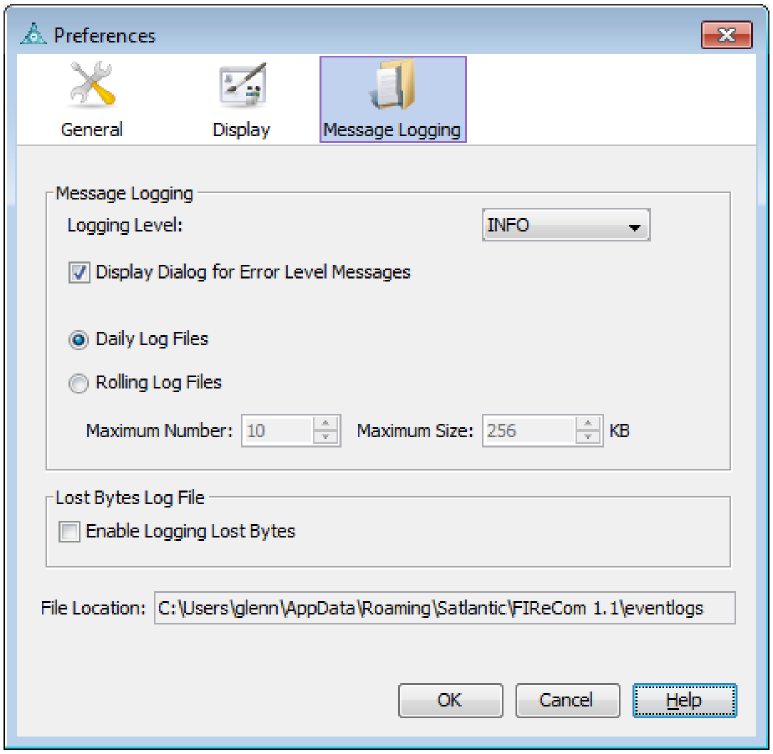
The following software application display preferences may be edited:

- **Displayed Numeric Precision** sets the number of decimal places to show for all floating point values displayed by FIReCom.

- **Use UTC Time** instructs FIReCom to display times in the UTC (Universal Coordinated Time, formerly Greenwich Mean Time) time zone. Otherwise, the local time zone of the host computer is used. *In Situ* FIRe always records time in UTC regardless of this software interface setting,

Message Logging Preferences

FIReCom maintains an application message log file that records diagnostic information that can assist with sensor troubleshooting. By default, the message log captures a modest amount of diagnostic information. The Preferences dialog's *Message Logging* panel configures how much diagnostic information is recorded, and where.



The **Logging Level** drop-down box allows you to select the verbosity of the FIReCom application logs. The following log levels can be selected. All log levels are cumulative, i.e. INFO level also logs WARN and ERROR.

- **TRACE** is the most detailed and verbose level of logging. This generates very large log files and should only be used as directed by Satlantic to assist in remote troubleshooting.
- **DEBUG** generates a high level of logging detail and is mainly used as directed by Satlantic to assist in remote troubleshooting.
- **INFO** is the default log level showing information (status), warnings, and errors.
- **WARN** limits log messages to warnings and errors.
- **ERROR** only logs errors.

Enable the **Display Dialog for Error Level Messages** option to cause a pop-up message to be prominently displayed whenever an ERROR or SEVERE message is logged. Such messages are written to the log file regardless of this setting.

The message log file does not grow unbounded. If the **Daily Log Files** option is selected, a new log file is created each day. Otherwise, when the **Rolling Log Files** option is selected, the **Maximum Size** setting determines the size at which an active log file is saved and a new one started. Once the the **Maximum Number** of saved log files is reached, the oldest file is deleted whenever a new one is started.

The **File Location** field shows where event log files are being store on the local file system.

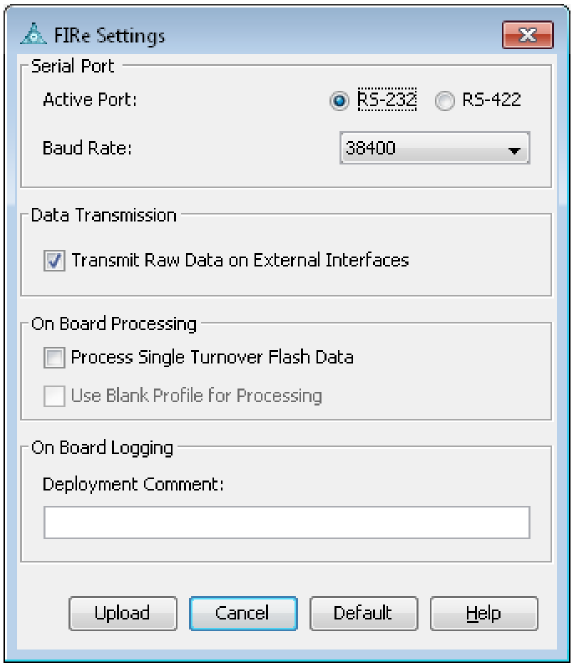
Settings

This chapter describes In Situ FIRe device settings and how to manage them via FIReCom software.

Topics in this chapter include:

FIRe Settings

To review and modify general FIRe settings, connect to *In Situ* FIRe and select *Sensor -> FIRe Settings* from the main menu to invoke the *FIRe Settings* dialog. Modify settings as desired and press the *Upload* button to apply them the connected *In Situ* FIRe.



Serial Port Settings

In Situ FIRe has two bi-directional serial ports (as described in External Interfaces) , either one of which can be used for both receiving commands and outputting data frames. Select either **RS-232** or **RS-422** to set the active serial communications port on the instrument. Select the desired **Baud Rate** of the active serial port the list of supported baud rates: 9600, 19200, 38400, 57600, 115200. Lower baud rates are recommended for longer serial cables, particularly for deep profiling applications over RS-422 on the MCBH6M bulkhead connector.

If the *Serial Port* settings have been changed when the **Upload** button is pressed, FIReCom displays a message dialog to inform you that a reboot of *In Situ* FIRe is required. When this message has been confirmed, FIReCom reboots *In Situ* FIRe to apply the new serial port settings.ls

Data Transmission Settings

In Situ FIRe transmits raw (unprocessed) data through its USB and serial port interfaces when actively sampling. To suppress the transmission of raw data, de-select the **Transmit Raw Data on External Interfaces** option.

If **Process Single Turnover Flash Data** is selected below, *In Situ* FIRe will transmit processed data regardless of whether or not raw data transmission is enabled.

On-Board Processing Settings

In Situ FIRe is capable of transmitting processed single turnover flash (STF) data. This can be useful for remote deployments where an external controller may be required to periodically communicate calculated fluorescence measurements. On-board processing is available for STF data only. Processed results for multiple turnover flash (MTF) data must be calculated off-line by the FIReCom

Select the **Process Single Turnover Flash Data** option to configure *In Situ* FIRe to log and transmit processed data frames in addition to raw data frames. Check the **Use Blank for Processing** sub-option to configure *In Situ* FIRe to apply a blank profile when processing STF data.

On-Board Logging

In Situ FIRe logs all raw data samples and processed data to its large-capacity internal storage area. The data log files generated by *In Situ* FIRe can be prepended with a contextual **Deployment Comment** specified here.

Flash Sequence Settings

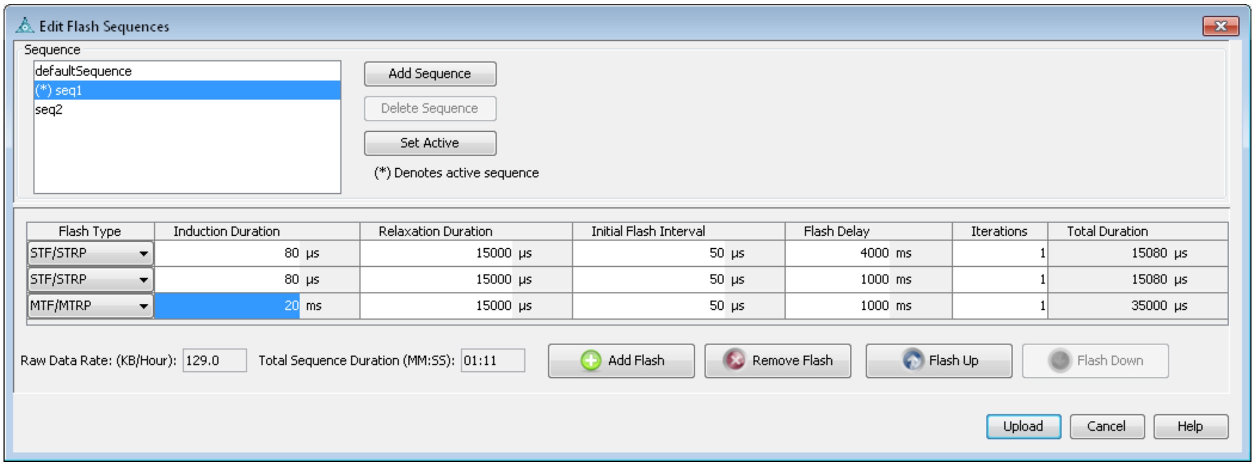
In Situ FIRe can be programmed to excite and measure the sample volume using any sequence of flash/relaxation phases. Each such phase in the sequence can be either an STF/STRP (single turnover flash / single turnover relaxation phase) or an MTF/MTRP (multiple turnover flash / multiple turnover relaxation phase).

Two constraints on sequences are imposed:

1. a sequence must contain at least one flash/relaxation phase, and
2. the first phase in the sequence may only be STF/STRP.

Many different sequences can be defined on *In Situ* FIRe, but only one may be active (enabled) at any one time.

To review and/or modify flash sequence settings, ensure that *In Situ* FIRe is connected and in *Setup* mode. Select *Sensor -> Edit Flash Sequences* from the main menu to show the flash sequence editor dialog:



The top panel of the *Edit Flash Sequences* dialog lists the name of each flash sequence that is defined on the connected *In Situ* FIRe. The sequence preceded by an asterisk (*) is the *active sequence.* This is the sequence that will be executed by *In Situ* FIRe when its next begins sampling.

To add a new sequence, press the *Add Sequence* button and provide a name for the new sequence when prompted.

To delete a sequence, select the name of the sequence on the list so that it is highlighted. Then press *Delete Sequence* to delete the selected sequence.

To change the active sequence, select the sequence to enable and press the *Set Active* button.

The table on the bottom pane on the *Edit Flash Sequences* dialog shows a the attributes of the sequence that is highlighted in the *Sequence* list above. Each row on this table represents an induction/relaxation event. These events will be executed by *In Situ* FIRe in the order they are listed.

Cells in the first six columns of the table are editable:

- **Flash Type** : select the desired type of induction/relaxation event as either STF/STRP or MTF/MTRP.
- **Induction Duration** sets the duration of the induction phase in microseconds.
- **Relaxation Duration** sets the duration of the induction phase in microseconds.
- **Initial Flash Interval** sets the number of microseconds to idle between the first two flashes within the relaxation phase of the flash. This initial interval is used to calculate the intervals between subsequent flashes within a profile
- **Flash Delay** sets the number of milliseconds to pause after each execution of this event.
- **Iterations** sets the number of times this event should be executed before advancing to the next event in the sequence. Note that this can be overridden by selecting *Gain-dependent Iterations* in the *Flash Iteration Settings* dialog.
- **Total Duration** is a read-only field that shows the total number of microseconds spent in the induction and relaxation phases, not including flash delay. It is expressed as (*Induction Duration + Relaxation Duration) x Iterations* .

To add a new flash event to the sequence, press the *Add Flash* button and then adjust the flash event attributes as desired. Press the *Flash Up* or *Flash Down* button to change the position of the selected flash event relative to others in the sequence. To delete a flash event, select the event so that it is highlighted, then press the *Remove Flash* button.

See *Flash Timing Explained* for a more in-depth explanation of how *In Situ* FIRe executes induction and relaxation flashes.

Flash Timing Explained

Single Turnover Induction

The length of the single turnover induction is user-specified. For the induction phase, the fluorescence signal passes through a low-pass filter and is sampled at instantaneous points in time with a 1 µs repetition period. Preceding the induction phase, 14 dark samples are recorded. The first three dark samples are discarded and the remaining points are included in the data frame. During pre-processing a dark correction is performed by subtracting the average dark value from each of the subsequent induction points.

Multiple Turnover Induction

For multiple turnover induction, approximately 5 µs LED flashes are generated with a period of 44 µs. The fluorescence signal passes through a low-pass filter and is sampled at instantaneous points in time with a 2 µs period. For each flash, 12 samples are taken, 9 before the LED flash (dark samples) and 3 during the flash (light samples). The first and last dark samples are discarded and the remaining darks are averaged. Only the middle light sample is used; it corresponds to when the flash has reached its peak value. The dark average and light value are included in the data frame. No subtraction occurs until dark correction during pre-processing. In pre-processing, the dark average for each flash is subtracted from the associated light value.

The number of samples from a multiple turnover induction can be larger than the available high-speed memory used to capture the samples. In these cases the flashes are subsampled (i.e. every second or third flash, as required).

Relaxation

Before relaxation timing starts, there is a fixed delay of 14 µs. After that, the intermittent flashes begin to measure reoxidation. The period of the flashes begins at the user-defined initial flash interval or period. The period increases by 3-times after 600 µs, 15-times after 2400 µs, and 100-times after 11400 µs (if required). The changing period allows the system to best capture the rapid decay of the signal at the beginning of relaxation while reducing the amount of data at the tail end of the signal where there is little change. Within each flash period, a dark reading is taken, followed by a reading with the LEDs on. Each reading is an integration over 1.8 µs. When the LED is on, it is for 1.1 µs in the middle of the sample time. Both the dark and light value are included in the raw frame. No subtraction occurs until dark correction during pre-processing. In that case, the average of all dark points is subtracted from each light point.

Reference Signal

For each of the final data points generated above, a similar reference data point is also generated. The reference signal measures the intensity of the LEDs with two photo diodes mounted in the middle of the LEDs and pointing at the LEDs on the opposite side of the sample volume. The signals from the two photo detectors are summed and processed in the same way as the fluoresce signal. They are sampled at exactly the same time as the fluorescence data. The resulting reference data first normalized to the maximum value and these data are then used to scale the fluorescent signal. In this way the fluorescence data accounts for any short and long term variations in LED intensity.

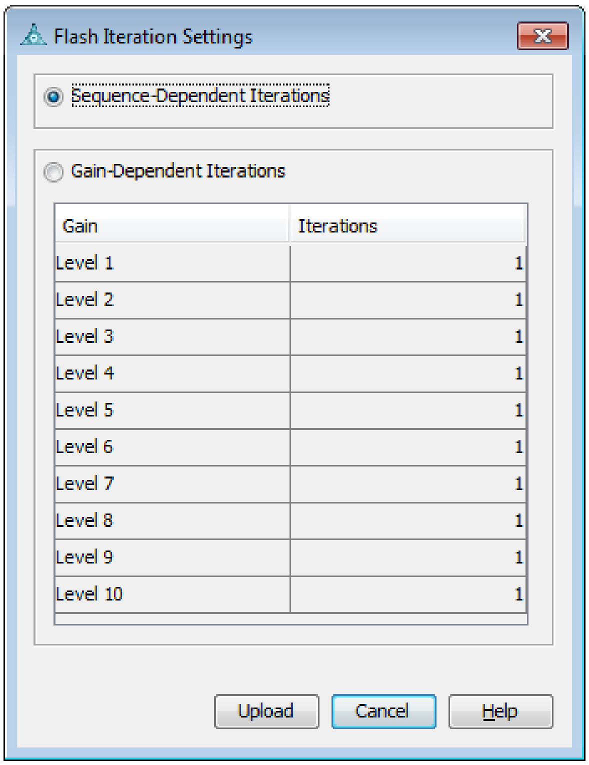
Autogain

When autogain is selected, the system uses pre-set STF parameters. It starts at a gain of 5 and increases or decreases the gain as required until it finds an optimum gain value, defined as the gain that generates an Fm value closest to 4000 counts. FIRe then switches to the user-defined parameters and begins the user-defined flash sequence.

Flash Iteration Settings

In Situ FIRe can be configured to average multiple flash iterations for a given sample in order to reduce signal noise.

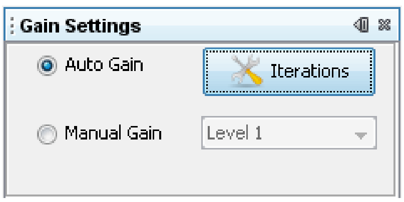
Select *Sensor -> Flash Iterations Settings* from the main menu to set the number of iterations to be taken during the collection of a profile. The number of iterations is either dependent on the Flash Sequence Settings or on the gain level. In the latter case, the number of iterations can be specified for each gain level in the *Gain-Dependent Iterations* table.



Gain Settings

Gain is a measure of the ability of *In Situ* FIRe to amplify the fluorescent signal collected from a sample. To accommodate a wide range of Chl-a concentrations in natural phytoplankton and laboratory cultures, the electronic gain of the detector unit is adjustable over a range of four orders of magnitude. The gain can be varied using 10 preset values, nominally referred to as Level 1 to Level 10. The actual electronic gain is an exponential function of these gain levels.

To modify gain settings, ensure that your FIRe is connected and in *Setup* mode, then select *View -> Gain settings* from the FIReCom menu to display the *Gain Settings* panel in the lower portion of the dashboard. All changes made to gain settings in this panel are immediately applied to the connected FIRe.



Auto Gain Setting

In Situ FIRe can be configured to use collected profiles at the start of each sampling sequence to determine an optimal gain for a specific sample. It does this by sampling, examining the results, adjusting the gain and re-sampling. This process is continued until an optimal gain is determined.

When the *Auto Gain* option is selected, *In Situ* FIRe applies pre-set STF parameters. It starts at a gain of 5 and increases or decreases the gain as required until it finds an optimum gain value, defined as the gain that generates an Fm value closest to 4000 counts. FIRe then switches to the user-defined parameters and begins the user-defined flash sequence.

In Situ FIRe is normally configured to sample over several iterations as specified for each flash event per the sequences defined in the *FIRe Settings* dialog. Alternately, the number of iterations can be set to be gain-dependent instead of sequence dependent. Press the *Iterations* button to invoke the *Flash Iteration Settings* dialog to explicitly set a specific number of iterations for each gain level.

Manual Gain Setting

To explicitly set the sensor gain to a specific level, select the *Manual Gain* option then select the desired level. Gain can be set to Level 1 (minimum gain) to Level 10 (maximum gain).

Relative Gain Levels

The gain settings in FIReCom are expressed as levels. Use the table below to derive relative gains from a given level setting:

Gain SettingRelative Gain (dB)Relative Gain

Level 1	0.0	1.00
Level 2	4.4	2.78
Level 3	8.9	7.74
Level 4	13.3	21.6
Level 5	17.8	60.0
Level 6	22.2	167
Level 7	26.7	464
Level 8	31.1	1292
Level 9	35.6	3594
Level 10	40.0	10000

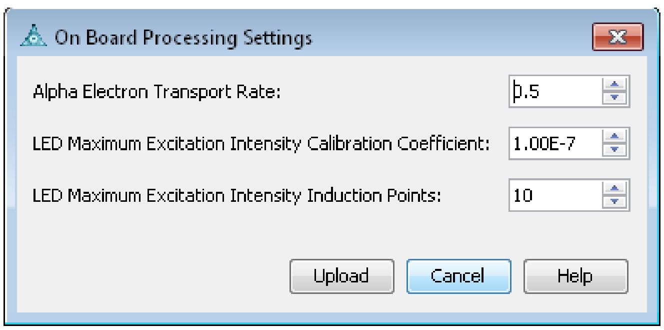
On Board Processing

In Situ FIRe analyzes measured fluorescence profiles according to the the model described by Z. S. Kolber, O.Prasil, and P. G. Falkowski, *Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols* , Biochimica et Biophysica Acta 1367 (1998) 88-106.

The on-board profile analysis provides a quantitative characterization of the investigated sample via the functional cross section, the quantum yield, and other values.

The following *In Situ* FIRe on-board processing settings are intended for advanced expert users only. Modification of these options can drastically change the output of on-board processing routines. It is not recommended they be changed unless fully understood.

To modify expert parameters of the on-board processing algorithm, ensure that *In Situ* FIRe is connected and in setup mode. Then select *Sensor -> Advanced -> On Board Processing* to show the settings dialog. Enter the desired parameters then press *Upload* to apply.

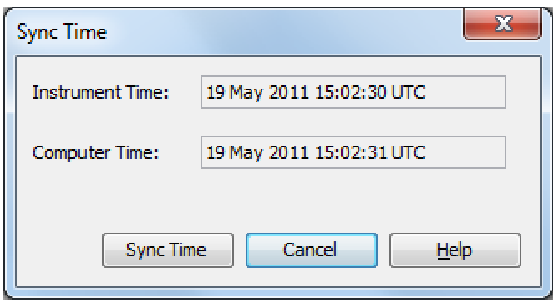


Clock Synchronization

In Situ FIRe has an internal system clock that is used for time-stamping logged data samples and diagnostic messaged. This clock can drift slightly over time depending on environmental conditions. It is recommended to synchronize the internal clock between deployments when connected with FIReCom.

To check the internal clock time and to synchronize it with the local computer, ensure that *In Situ* FIRe is connected and in setup mode. Select *Sensor -> Sync Time* from the main menu. The *Sync Time* dialog that appears shows the *Instrument Time* of the internal clock and the *Computer Time* of the local computer. FIReCom always displays both times in UTC (Universal Coordinated Time) regardless of the time zone settings on the local computer.

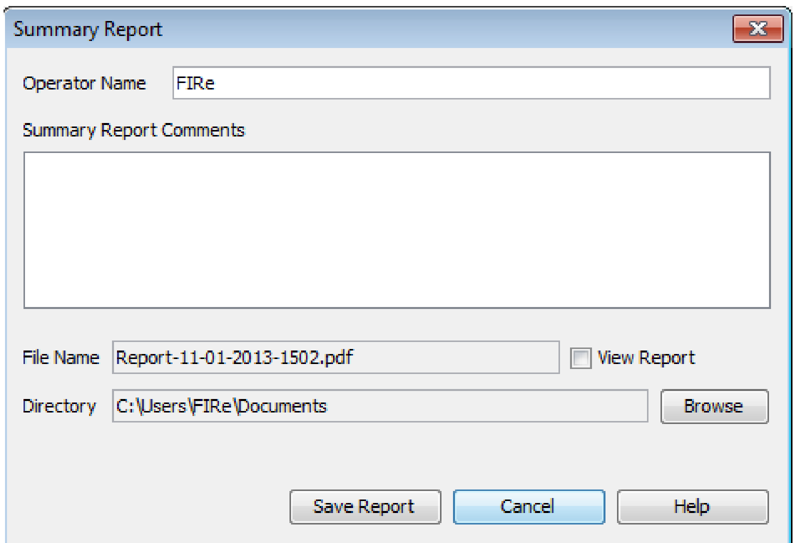
Press the *Sync Time* button to set the internal clock on *In Situ* FIRe to match the time on the local computer.



Sensor Summary Report

FIReCom can generate a *Summary Report* of settings and diagnostics retrieved from the connected *In Situ FIRe* . It is strongly recommended to create a summary report before and after deployment to serve as a contextual record for data collected during the deployment.

To generate a summary report, ensure *In Situ* FIRe is connected and in setup mode. Select *Sensor -> Summary Report* from the main menu to show the report creation dialog. Provide any relevant contextual information in the *Summary Report Comments* section. Check the *View Report* option if a PDF reader is installed and you wish to view the report immediately after it is created. Press *Save Report* to create and save the summary report.



Deployment

This chapter describes various deployment scenarios and how to configure and operate In Situ FIRe for each.

Topics in this chapter include:

- Deployment Procedures
- Creating Blank Profiles
- Pressure Sensor Calibration
- Real Time Data Acquisition
- Real Time Data Display
- Logging Real Time Data
- Monitoring Real Time Data Acquisition
- PAR Sensor Calibration
- Application Files

Deployment Procedures

Profiling Mode

For most profiling applications, the preferred method of connecting *In Situ* FIRe to deck-side power and data communications is via the optional 6-conductor RS-422 profiling cable and MDU-300 deck unit as described in *Accessories* . The RS-422 communications protocol allows the use of a long deployment cable through the entire range of the FIRe pressure rating (200 m). The USB connection is limited by cable length (1.5 meters) and is inappropriate for a profiling situation. The RS-232 connection can be used for profiling but this telemetry protocol limits cable length to approximately 50 m.

Prior to operating the FIRe in a profiling application, run the FIReCom software on a computer and connect it to the instrument via the supplied USB programming cable. After FIReCom reports connection to the FIRe, open the *FIRe Settings* dialog and set the following:

- Enable *Transmit Raw Data on External Interfaces* check box
- In *SerialPort* section, select *Active Port* to match the selected connection (RS-232 or RS-422).
- For long cable RS-422 profiling, recommended baud rate is **9600**
- Save a *Summary Report* to record and review device status and settings

After applying above settings, save a *Summary Report* to record and review device status and settings. Then remove USB and power. Leave *In Situ* FIRe powered off for at least 20 seconds and then restart the instrument. Connect the RS-422 cable to FIRe and connect the deck unit to a computer running FIReCom software. Wait 60 seconds after re-powering *In Situ* FIRe then use FIReCom to establish a data connection. Observe data as it is emitted in real time to confirm that *In Situ* FIRe is configured correctly.

In Situ FIRe can be mounted on a profiling cage or lowering frame. Be sure to protect the the exterior housing from damage due to metal clamps or brackets. Damage to the anodized surface of the instrument can result in corrosion problems. Ensure that dummy plugs are installed and tightly sealed on unused connectors to prevent in-water shorting.

The FIRe should be rinsed thoroughly with fresh water after retrieval to avoid corrosion.

Moored Deployment

In Situ FIRe is capable of logging data internally, but it does not have self-scheduling ability with a low-power sleep mode. It therefore relies on external scheduled power control for long-term deployment where periodic sampling bursts separated by power-saving dormant periods are required.

The 8 pin male connector on the FIRe is used to power the instrument with an 8-18 Volt DC power source. The ideal scheduled power source is data logger such as a Satlantic STOR-X that can control power cycling to the FIRe. *In Situ* FIRe requires a 60 second warm-up time before it can begin logging and transmitting data. It is important to configure the scheduled power source so that this 60 second delay is taken into account before any external data collection takes place.

The 8 pin male connector also provides bi-directional RS-232 serial communication. If connecting the FIRe to a serial data logger, the 8 pin male connector can be used exclusively for both power and communications. *In Situ* FIRe will report the serial data frames as outlined in the Data Format Reference section of this manual.

In Situ FIRe has an analog interface available on the 4 pin male connector. If you choose to use this interface, a second cable will have to be connected to the FIRe. This analog output provides an analog voltage output signal equal to 4.095*Fv/Fm. This feature can be used if your data logger has an ADC that can accept this voltage range. Refer to your data logger's manual and software for calibration of the ADC.

Prior to operating *In Situ* FIRe in a moored application, run FIReCom software on a computer and connect it to the instrument via the supplied USB programming cable. After FIReCom reports connection to *In Situ* FIRe, open the *FIRe Settings* dialog and set the following:

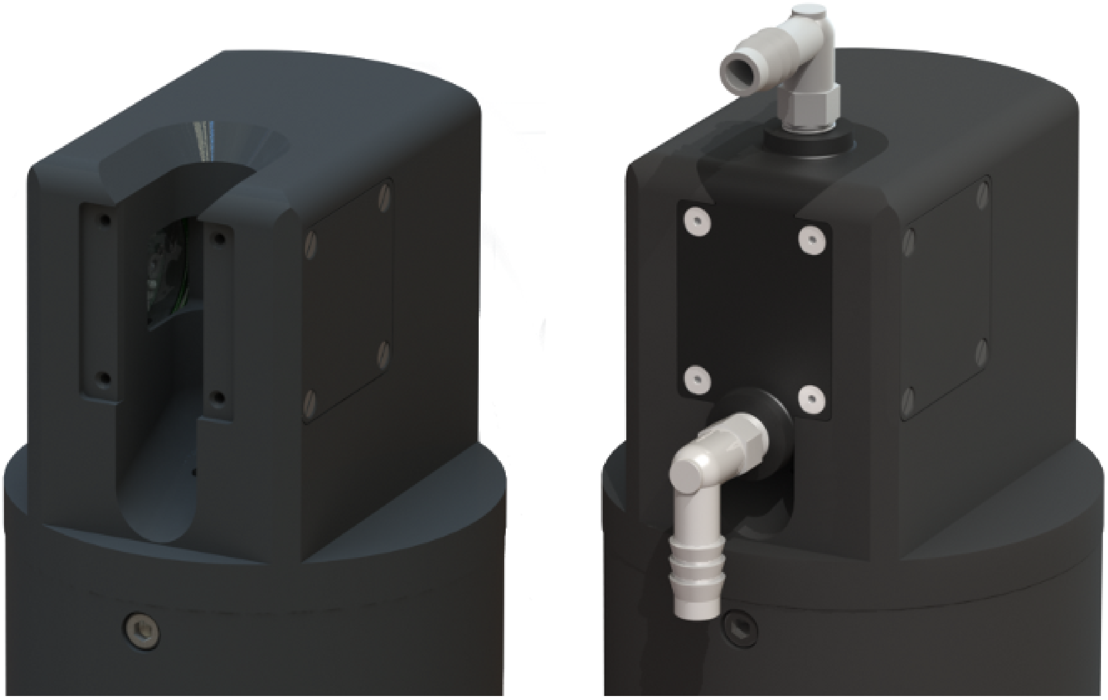
- Enable *Transmit Raw Data on External Interfaces* check box

Save a *Summary Report* to record and review device status and settings. Then disconnect and apply power to the device to test internal data logging and/or telemetry transmission to an external logger. When mounting *In Situ* FIRe, be sure to protect the exterior housing from damage due to metal clamps or brackets. Damage to the anodized surface of the instrument can result in corrosion problems. Ensure that dummy plugs are installed and tightly sealed on unused connectors to prevent in-water shorting.

Flow Cell

The flow cell is meant for gravity-fed flow through applications at low pressures (up to 20 psi). The flow cell and backstop are interchangeable. The same screws are used to secure both items in place. Flow rate should be considered; if flow is too low the sample may become photo-quenched or damaged and if the flow rate is too high then the proper excitation dynamics may not be observed. If we assume 0.5 s between flashes and a 1 cm wide sample volume, then flow rates of 1 to 1.5 meters/minute are fine, allowing the sample volume to be replaced between flashes.

The flow cell is installed by sliding or wiggling it into place slowly. As it moves into place, watch that the o-ring does not buckle and get pinched. This can happen easily with a new o-ring, but after an o-ring has been seating in the flow cell groove for a time, it should not be an issue. After the flow cell is fully seated, the four holding screws must be installed to prevent leaks.



When filling the flow cell, it should be noted that air will get trapped in the top of the flow cell if the instrument is horizontal. The FIRe should be tipped vertically to remove the air pocket. If the air pocket is not removed, it will change the optics of the sample chamber.

When taking blank measurements with the flow cell, no flow is necessary. Vinyl caps are provided to cover the ends of the barbed elbows to eliminate stray light from the sample volume.

To install a new o-ring on the flow cell, it should first be softened and stretched slightly by hand. Wetting the o-ring grove and the o-ring help to keep it seated. When in place the flow cell can be installed on the FIRe. Leaving the flow cell in place for a period of time will allow the o-ring to assume the new shape and will prevent it from falling off the flow cell.

CAUTION: Do not grease the o-ring as the grease may fluoresce.

Blanks

Offsets in the measured fluorescent signal will cause errors in the calculated Fv/Fm errors, due a change in the value of Fm. Offsets will always be present due to the electronic noise floor as the fluorescent signal is amplified. There may also be contributions from dissolved fluorescing entities (e.g. CDOM) that may be present in the solution. The effect off this offset is noticeable at gains of about 5 and is very significant at the highest gains.

A blank measurement is most accurate when using a filtered sample of the seawater (or media) in which you are working. This will capture any dissolved fluorescing entities (e.g. CDOM) that may be present in the solution. Care should be taken with the filtering process, so as to not rupture the cells, releasing additional chlorophyll into the blank. If access to filtered seawater or culture media is restricted, and the samples are known to have negligible CDOM, ultra-pure de-ionized water may be used as a substitute.

The first step is to make sure the windows are completely free of grease or oils that can fluoresce. We recommend collecting some data (either as a regular data collection or as a blank collection) first with ultra-pure de-ionized water and a gain of 10. Fill the flow cell with the water, tip the FIRe vertically to remove trapped air and continue filling. Place caps over the elbow-fittings to eliminate stray light. Note the number of counts at the end of the induction. Then remove the flow cell, clean the windows again and repeat the process. Keep repeating the process as long as the counts are decreasing.

Next, replace the de-ionized water with the prepared blank medium. Again, be sure to remove any trapped air from the flow cell. The FIReCom interface for collecting blanks is described in the Creating Blank Profiles section. The blank signal should be flat through the induction with no visible rise at the beginning of the induction. If there is a rise, it indicates that there are still living cells in the sample and it should be filtered further.

When processing data, a complete set of blank files should be available (i.e. one for each gain). If onboard processing is used, for instance if an analog output is used, then the blanks must be collected before deployment. Otherwise the water samples could be collected along with the FIRe deployment and used to generate blank data afterwards. New blank files should be generated any time it is believed that the water properties have changed. In a moored application, this may mean collecting water samples periodically. In a profiling mode this may mean collecting water samples at different depths.

Light and Dark Adapted Measurements

The method by which the samples are supplied to *In Situ* FIRe allows different physiological paremeters to be measured. This section is not meant to cover all of the possibilities in detail, but to inform the user about different considerations when using the FIRe.

Dark adapted measurements, to measure Fo, Fm, along with Fv/Fm and other calculated values, can be made in a couple different ways. The first way would be to make measurements at night. The second way is to use the flow cell. The flow cell and barbed fittings are lightproof. In a moored application, a small pump could pull water into the sample volume and allow it to become dark adapted before turning on the FIRe and making a measurement. In the lab, if a lightproof storage container and tubing are used, then the samples can be gravity fed through the system. In all cases, an extended dark adaptation may be necessary.

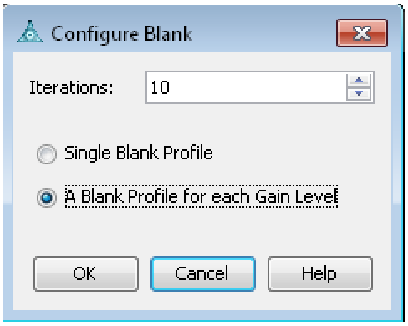
For natural light adapted measurements, the FIRe can be used *in situ* during the day to obtain Fo', Fm' and Fv'/Fm' and other values, as well as steady state fluorescence F' (or Fs). Care should be taken to ensure the cells are illuminated at the ambient PAR prior to measurement. If the instrument is profiling alone and there is no shading by the ship, then this condition should be satisfied. If the FIRe is mounted on a CTD rosette, then it should be mounted on the top to minimize shading by the rosette. You should fully consider the details of the short-term history of the water before the measurement to ensure the results are as expected.

Creating Blank Profiles

In Situ FIRe is able to create blank profile samples that can be applied during processing to account for any offsets in the fluorescence measurement caused by both background fluorescence in the sample and the FIRe noise floor. Blanks are necessary to obtain correct Fv/Fm values and are most important at higher gain levels. A more detailed description of what a Blank sample is and why a blank sample might be required is described in the Deployment Procedures section.

To create a blank profile, ensure that *In Situ* FIRe is connected and in setup mode. Select *Sensor -> Create Blank Profile* from the main menu. In the *Configure Blank* dialog, enter the number of **Iterations** to be used during the blank samples collection. The induction duration, relaxation duration, initial flash interval, and flash delay of the first STF defined in the active flash sequence are used to configure the blank. Sequences are explained in the Flash Sequence Settings section.

The dialog offers a choice between collecting a single blank profile or collecting a blank at each of the ten gain settings. The currently set gain setting is used when collecting a single blank profile. The configuration of the gain settings is described in the Gain Settings section.



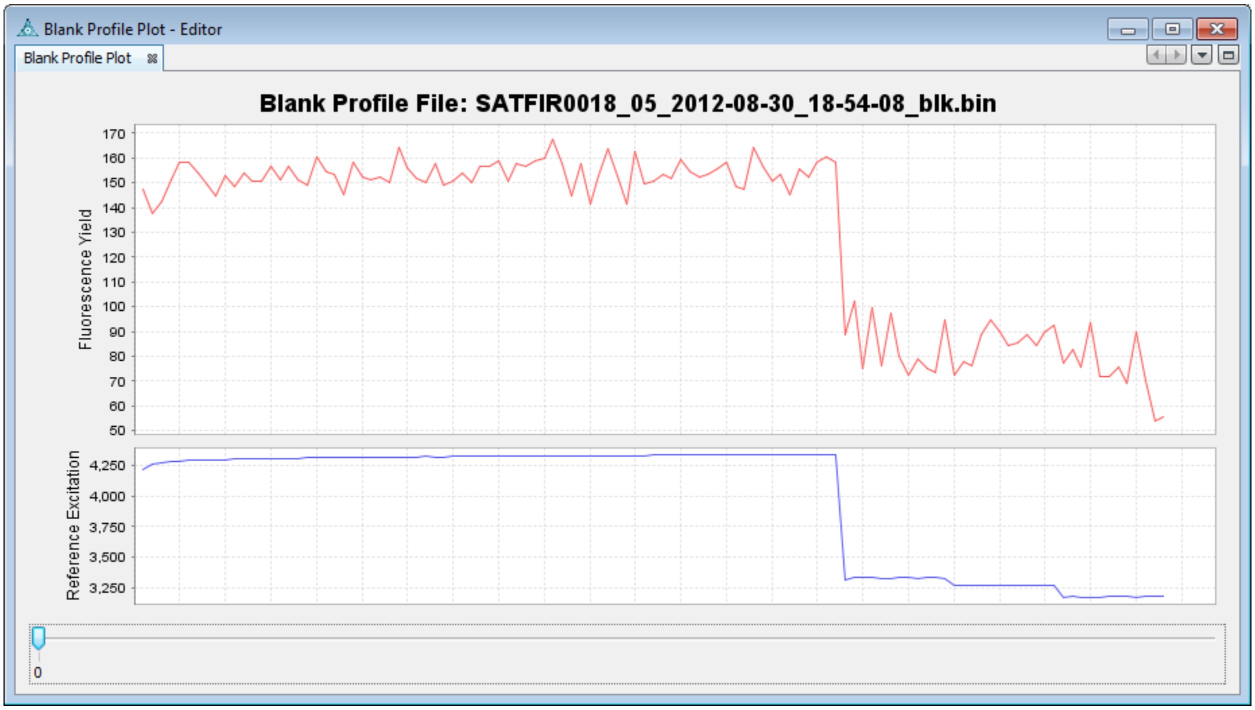
The *Configure Blank* dialog offers a choice between collecting a **Single Blank Profile** or collecting **A Blank Profile for each Gain Level** . The currently-set gain setting is used when collecting a single blank profile. The configuration of the gain settings is described in the Gain Settings section.

A blank measurement is most accurate when using a filtered sample of the seawater (or media) in which you are working. This will capture any dissolved fluorescing entities (e.g. CDOM) that may be present in the solution. Care should be taken with the filtering process, so as to not rupture the cells, releasing additional chlorophyll into the blank. If access to filtered seawater or culture media is restricted, and the samples are known to have negligible CDOM, ultra-pure de-ionized water may be used as a substitute.

Blank Profile File Display

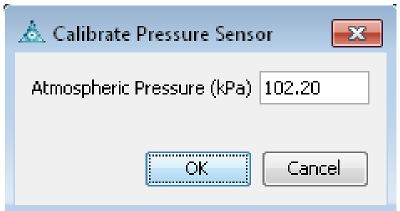
The *Create Blank Profile* action commands *In Situ* FIRe to create a blank profile file. FIReCom then retrieves the new blank profile file and displays it in the *Blank Profile Plot* window. When collecting a blank profile for each gain level, all ten are plotted and can be reviewed and verified one at a time.

Saved blank profile files can be applied when processing raw data on *In Situ* FIRe or when processing logged raw data with FIReCom.


Blank Profile Plot - Editor
Blank Profile Plot
Blank Profile File: SATFIR0018_05_2012-08-30_18-54-08_blk.bin
Fluorescence Yield
170
160
150
140
130
120
110
100
90
80
70
60
50
Reference Excitation
4,250
4,000
3,750
3,500
3,250
0

Pressure Sensor Calibration

The offset value of the *In Situ* FIRe internal pressure sensor calibration can drift. This offset can be corrected with an independently measured local atmospheric pressure reading in kPa. Connect to FIRe via FIReCom, then select *Sensor -> Advanced -> Calibrate Pressure Sensor* from the main menu. Enter the local atmospheric pressure measurement in kPa then press *OK* to apply the correction.



Real Time Data Acquisition

FIReCom can be used to display data collected in real-time by the connected *In Situ* FIRe. This real-time data display can assist in setting up and verifying *In Situ FIRe* before a long-term deployment, or to provide real-time feedback when interactively sampling.

Configuring *In Situ* FIRe for Acquisition

In Situ FIRe can collect data for two distinct types of profiles:

1. MTF (Multiple Turnover Flash) and
2. STF (Single Turnover Flash).

These two flash types are described in the Princple of Operation section above. FIReCom allows you to configure the order in which STF and MTF profiles occur. By default, FIReCom is configured with a simple STF, MTF repetitive cycle or sequence. You can adjust the number of profiles, profile types and the settings for each profile within acquisition sequences via the *Flash Sequence Settings* dialog.

Starting an Acquisition

The main purpose of *In Situ* FIRe is to measure fluorescence.

- When *In Situ* FIRe is powered up it will automatically go into acquisition mode. The way in which it will process frame data will depend upon how the instrument is configured.
- When a FIRe is powered and FIReCom is running the FIRe can be put into Setup mode by connecting to USB or by connecting to a serial port and pressing the connect button. From Setup mode an acquisition with real time display can be started by pressing the *Sample* button on the FIReCom Dashboard . A description of the real time display acquisition is provided below.

When starting an acquisition within FIReCom, the Real Time Display allows monitoring of the generated data. Before deployment it is advisable to confirm that the generated data are within the expected range.

Selecting Sensors for Displays

Each *Configure Display* dialog allows you to select which raw frame and processed frame sensor values will be displayed within the *Real Time Display* , *Time Series Graph* and *Depth Profile Graph* windows. The sensors selected for each display are stored locally and are maintained even when FIReCom is restarted. Separate selected sensor lists are maintained for the *Real Time Display* , *Time Series Graph* and *Depth Profile Graph* windows.

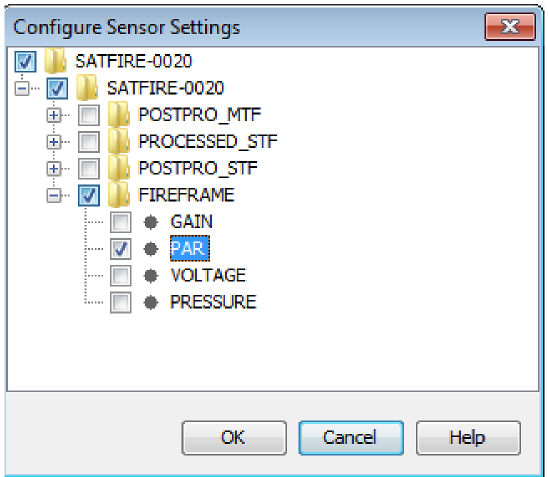


Illustration: Configuration for acquisition, time series, and depth profile sensor displays.

Each *Configure Sensor Settings* dialog displays the frame definitions contained in the FIRe instrument package file. Sensors can be selected for display using a mouse by either clicking the check box next to a sensor or clicking the check box next to a frame definition. For the *Time Series Graph* and *Depth Profile Graph* , a limited number of sensors should be selected so that the graphs created for each selected sensor are not too small.

The following table describes which sensors are available for each FIRe frame definition. Detailed sensor descriptions are specified in the Data Format Reference section of this manual.

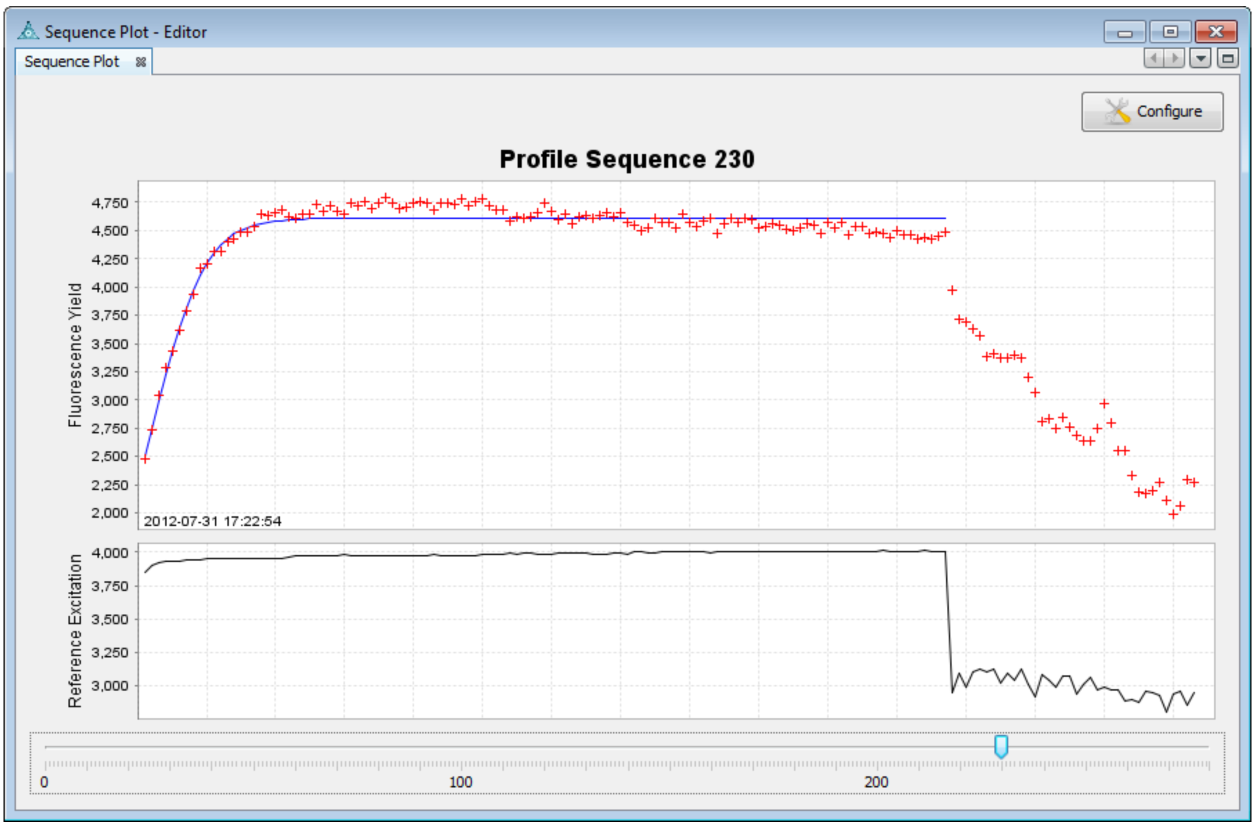
FIRe Raw Frame	PAR, Voltage, Pressure
FIRe Processed STF Frame	F_o, F_m, F_v, F_v/F_m, p, σ, abs σ, LED, ETR, PAR, Voltage, Pressure
Post Processing STF Frame	F_o, F_m, F_v, F_v/F_m, p, σ, abs σ, LED, ETR, α[3], τ[3] PAR, Voltage, Pressure
Post Processing MTF Frame	F_o, F_m, F_v, F_v/F_m, LED, ETR, α[3], τ[3], PAR, Voltage, Pressure

Real Time Data Display

FIReCom can display sensor data values from raw and processed STF data frames transmitted in real-time by a connected *In Situ* FIRe. The same FIReCom windows can also display raw and processed STF and MTF data frames when data files retrieved from *In Situ* FIRe are re-processed.

Sequence Plot

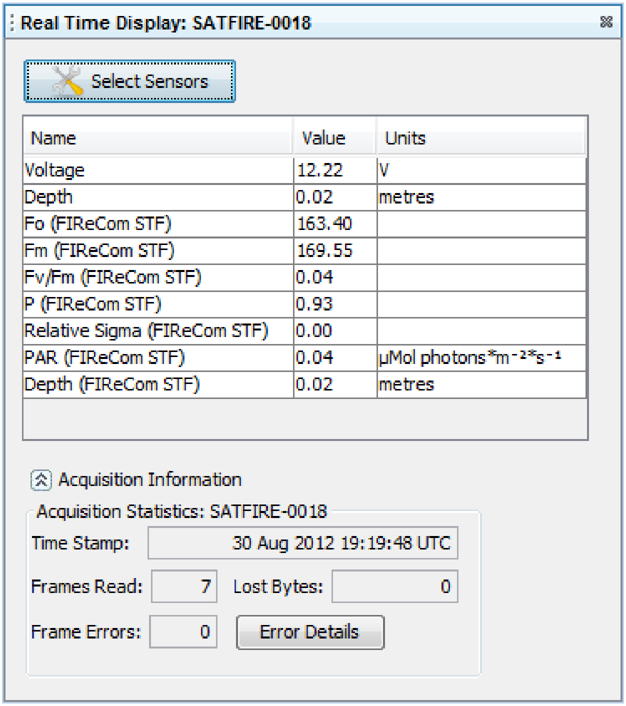
To view a sequence of fluorescence yield profiles and reference excitation profiles, select *View -> Graphs -> Sequence Graph* from the main menu to show the The *Sequence Plot* window in the top right quadrant of the application. This graph plots dark-corrected sensor counts representing the voltage output directly proportional to the amount of light reaching the avalanche photodiode.



Real Time Display

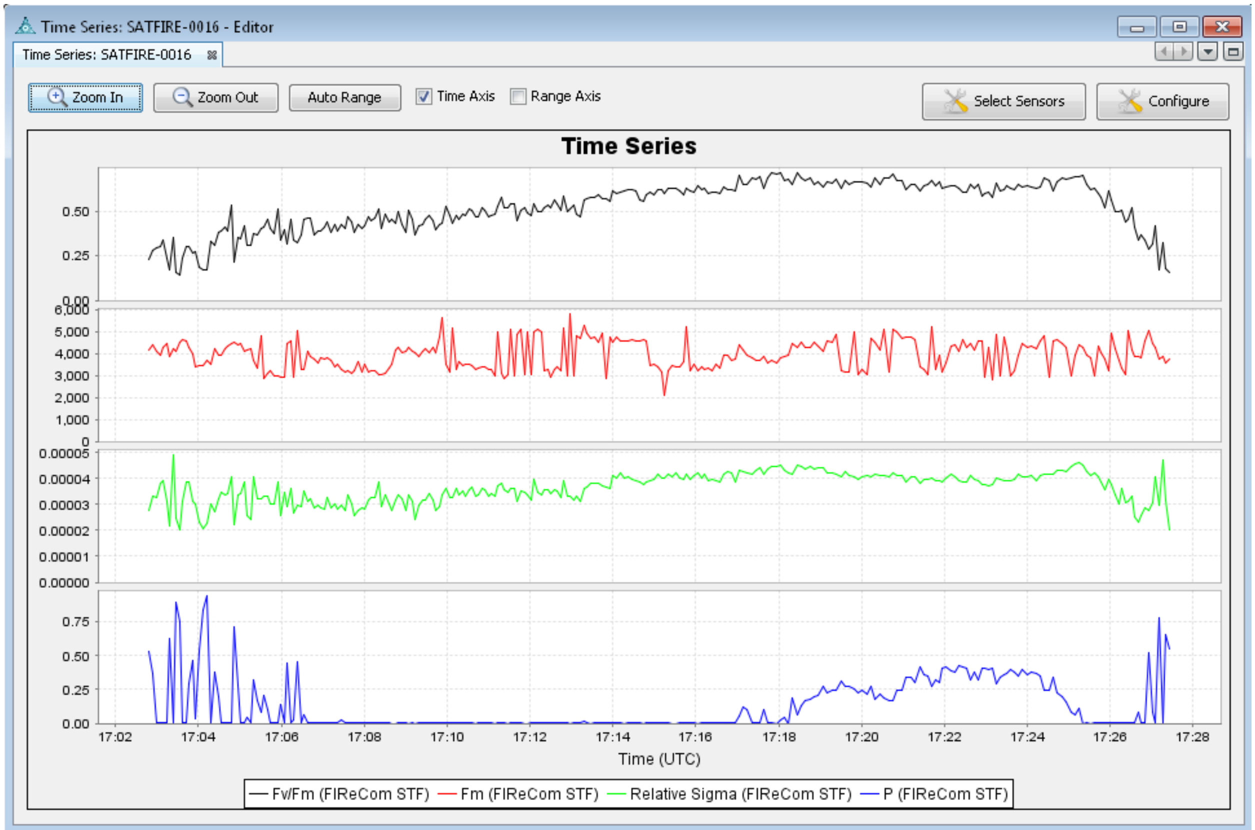
To see an updated tabular view of the most recently acquired sensor values:

1. select *View -> Real Time Data* to show the The *Real Time Display* panel
2. press the *Select Sensors* button to select which sensor values should be displayed



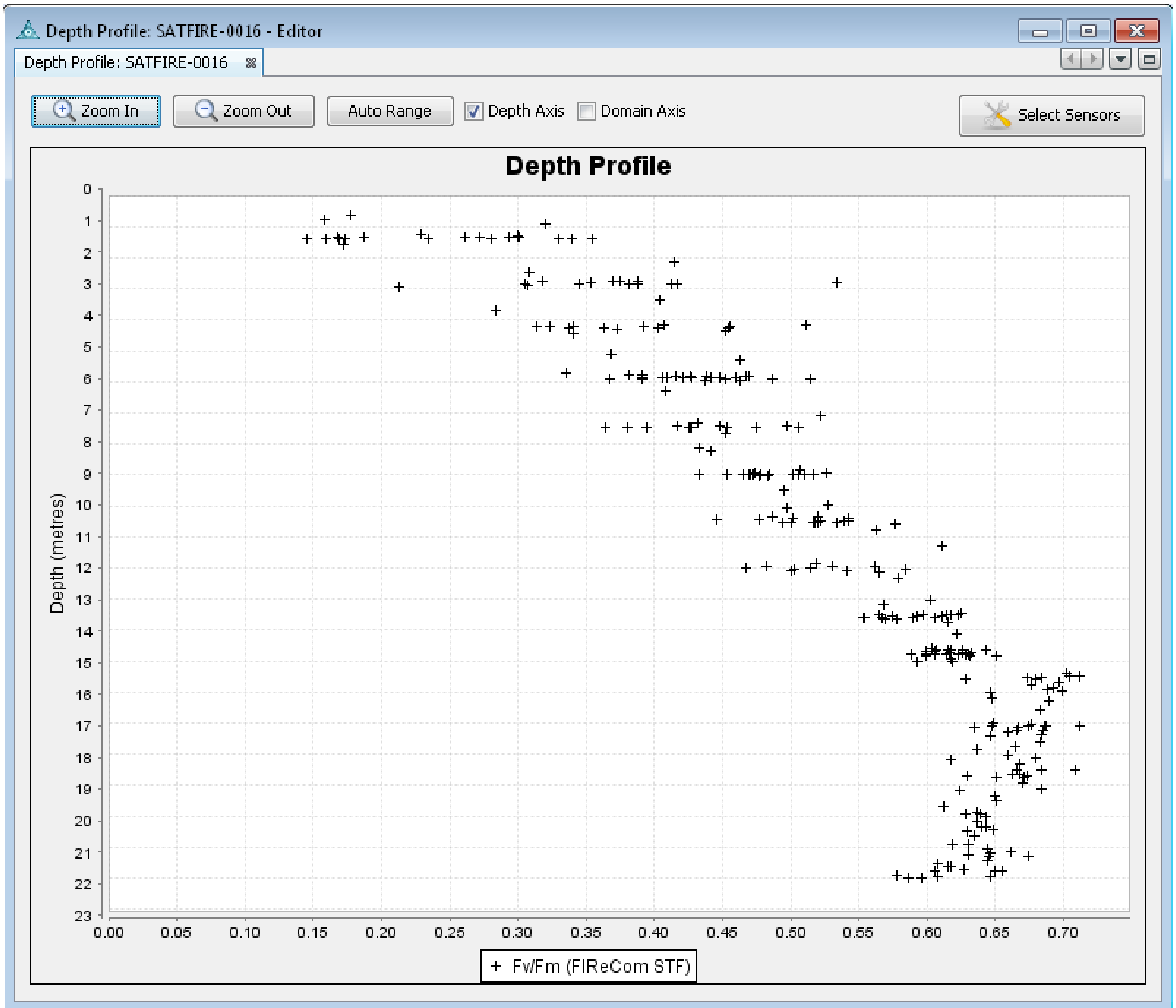
Time Series Graph

Select *View -> Graphs -> Time Series Graph* from the main menu to display selected sensor data values plotted vs time. Press the *Select Sensors* button to select which sensor values should be plotted in the time series graph.



Depth Profile Graph

Select *View -> Graphs -> Time Series Graph* from the main menu to show sensor data values plotted vs depth. Press the *Select Sensors* button to select which sensor values should be plotted in the depth profile graph.



Zooming and Panning Graphs

The *Time Series Graph* and the *Depth Profile Graph* provide several mechanisms for zooming and panning the displayed data:

- Press the *Zoom In* or *Zoom Out* buttons to zoom on the plotted data around the date center point.
- Press the *Auto Range* button to reset the graph so that the whole data range is visible.
- Check the *Axis* options to zoom on one or both axes. If neither is checked, the zoom buttons are disabled.
- Click on an individual sub-graph to limit domain/range zooming to that graph only. Click outside the individual sub-graphs to restore domain/range zooming to all graphs.
- Press and hold the left mouse button while dragging the mouse pointer from left to right within a graph. Release to zoom in on the selected area.

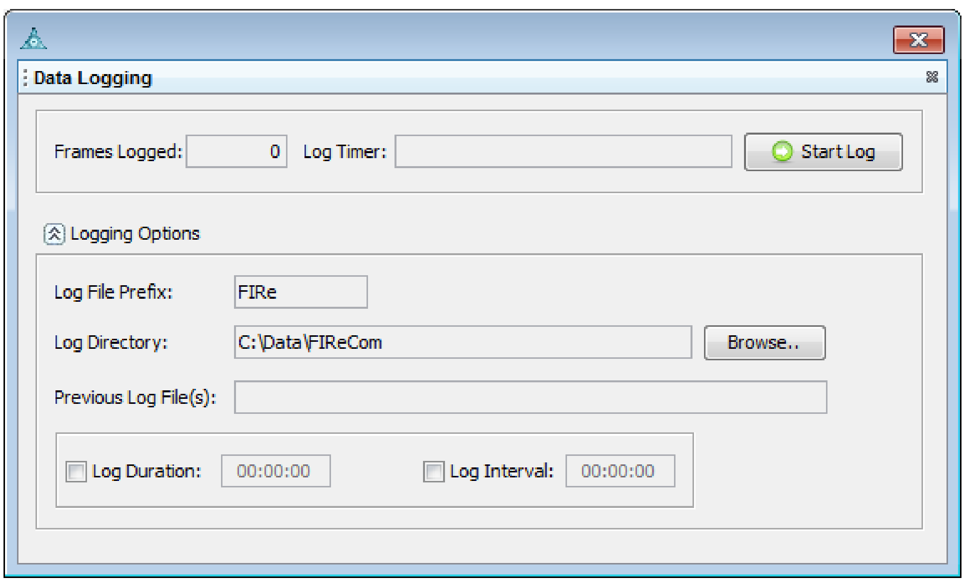
- Press and hold the left mouse button while dragging the mouse pointer from right to left to zoom out.
- Right-click over the graph to select from a menu of zoom operations.
- To pan the view, place the pointer on the graph, then press and hold Ctl (Windows) or Alt (Mac) while holding the left mouse button. Drag the mouse to the desired view then release the mouse button.

Logging Real Time Data

In Situ FIRe has ample internal storage to log months or even years of sensor data, which is ideal for autonomous deployment. For interactive tethered operation such as a profiling cast or pre-deployment test, FIReCom can be commanded to log a copy of the sensor data as it is transmitted to the host computer in real time.

To log real time data to the local computer:

1. Ensure that *In Situ* FIRe is connected and ready in *Setup* mode as described in Connecting to FIRe .
2. Select *View -> Data Logging* on the main menu to show the *Data Logging* panel.
3. Press the *Start Sampling* button on the FIReCom Dashboard to initiate a Real Time Data Acquisition .
4. Press the *Start Log* button on the *Data Logging* panel to begin logging acquired data to a local log file.
5. When the desired amount of data is logged, press the *Stop Log* button to stop logging data and close the active log file.



For every data logging action, FIReCom creates two separate log files:

1. A file with suffix *.raw* containing raw, unprocessed data
2. A file with suffix *.pro* containing STF data processed on-board *In Situ* FIRe.

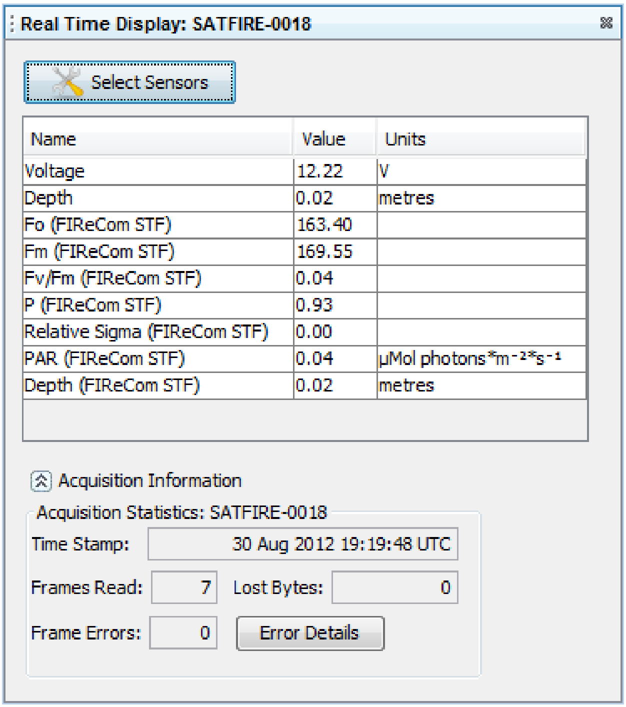
Additional logging options are available to control how much data to log, and how to store it on the local computer. Press the *Logging Options* expander icon to set the following options:

- **Log File Prefix** specifies an optional text prefix to be prepended to name of each log file saved.
- **Log Directory** sets the directory on the local computer to which log files should be saved.
- **Log Duration** , when selected, causes logging to automatically stop after the specified duration in hours:minutes:seconds.
- **Log Interval** , when selected, causes FIReCom to pause for hours:minutes:seconds specified between closing the last log file and opening a new one.

Monitoring Real Time Data Acquisition

FIReCom displays data acquisition statistics when acquiring data in real time from a connected *In Situ* FIRe, or when processing and replaying data from previously logged files. Overall acquisition statistics are displayed in the *Real Time Display* panel. Cumulative statistics for each of the data frame types are displayed in a table on the *Data Acquisition Monitor* panel.

To display overall statistics for the active acquisition, select *View -> Real Time Display* from the main menu. Then press the *Acquisition Information* icon to expand the statistics panel below.



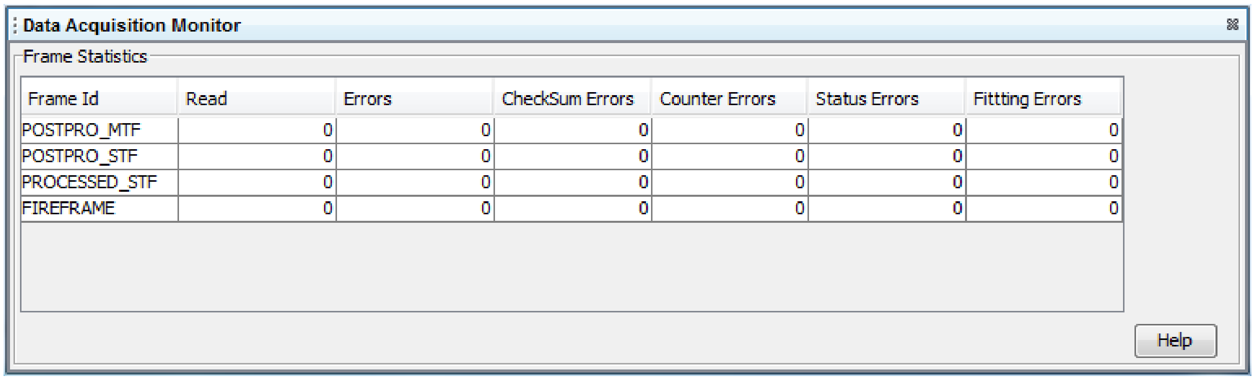
The following fields are updated with each frame of data acquired:

- **Acquisition Statistics** title indicates the serial number of *In Situ* FIRe from which data is being acquired.
- **Time Stamp** shows the date and time (UTC) of the most recently acquired data frame. For a real-time acquisition direct from *In Situ* FIRe, this time is generated by the local computer clock. For acquisition from a previously logged data file, the time stamp is retrieved from the data file.
- **Frames Read** indicates the total number of complete data frames successfully acquired.
- **Lost Bytes** is a running count of the total number of nonassignable bytes received between complete data frames. This number will can be expected to increment at the start of an acquisition if a partial frame is received before the first complete frame is identified. If lost bytes are consistently reported between all frames acquired, the most likely cause is a mismatch between the *Instrument*

Package File that describes the data frames, and the data itself. Lost bytes can also indicate signal corruption due to faulty connectors or electromagnetic interference.

- **Frame Errors** shows a cumulative count of errors detected in the sensor data itself. An error can be counted for any number of reasons including a failed check sum, frame counters appearing out of sequence, status errors, or mathematical errors when converting to physical units.

Press the *Error Details* button to show the *Data Acquisition Monitor* , which displays a summary of data acquisition statistics for each of the data frame types being acquired.

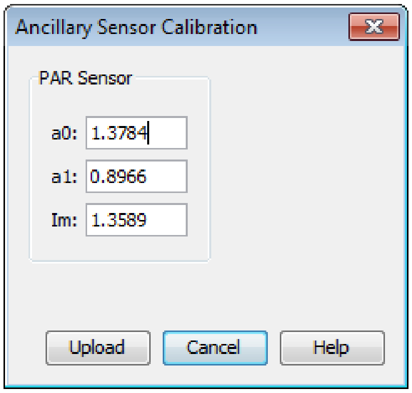


Each row of the table on the *Data Acquisition Monitor* panel provides cumulative acquisition statistics for a single data frame type.

- **Frame Id** is the unique identifier of the data frame type as defined in the *Instrument Package File* .
- **Read** is the number of valid frames of the listed frame type that have been read during an acquisition.
- **Errors** is the number of frames that contained one or more errors.
- **Checksum Errors** is the number of frames that contained check sum errors, typically discarded.
- **Counter Errors** is the number of frames that contained counter errors, typically not discarded.
- **Status Errors** is the number of frames that contained status errors, typically discarded.
- **Fitting Errors** is the number of frames that contained fitting errors, typically discarded.

PAR Sensor Calibration

The optional PAR sensor is factory-calibrated at Satlantic and pre-configured for *In situ* FIRe. In the event that your PAR sensor is recalibrated or replaced, connect to *In Situ* FIRe via FIReCom and select the *Sensor -> Ancillary Calibration* menu to input the PAR sensor calibration coefficients provided by Satlantic. Press *Upload* to apply the coefficients.



Application Files

FIReCom and *In Situ* FIRe use several different application files to define sensor data formats, flash profile sequences and application configurations.

Instrument XML File

Your *In Situ* FIRe is pre-configured with an *Instrument XML* (eXtensible Markup Language) file that describes the data frame and sensor definitions for your specific instrument. When FIReCom connects to *In Situ FIRe* , it retrieves this instrument file so that it can correctly acquire and display data.

You should not normally need to manage or modify the Instrument XML file on your *In Situ* FIRe. In the event that you are directed by Satlantic to install a new instrument XML file, select *Sensor -> Advanced -> Upload FIRe Instrument XML File* to upload the spplied XML file to *In Situ* FIRe.

Flash Sequence File

Your *In Situ* FIRe is pre-configured with a default flash sequence file. You can define addtional flash sequences via the Flash Sequence Settings dialog. The *Sensor -> Advanced -> Upload FIRe Sequence File* function is mainly used to upload a custom sequence file if provided by Satlantic staff.

Upload Firmware Patch File

This function is used to upload a firmware update or patch to *In Situ* FIRe. Only use this function if directed to do so by Satlantic support staff.

Application File Locations

Application files are downloaded by FIReCom and stored locally. The folder used to store application files is platform specific. See the table below for default locations.

System	Application Folder
Windows XP	C:\Documents and Settings\%USERNAME%\My Documents\Satlantic\FIReCom X.X
Windows Vista	C:\Users\%USERNAME%\My Documents\Satlantic\FIReCom X.X
Windows 7	C:\Users\%USERNAME%\My Documents\Satlantic\FIReCom X.X
Windows 8	C:\Users\%USERNAME%\My Documents\Satlantic\FIReCom X.X

FIReCom also stores its application log in this directory. See section Message Logging for more information on these log files.

Data Recovery and Processing

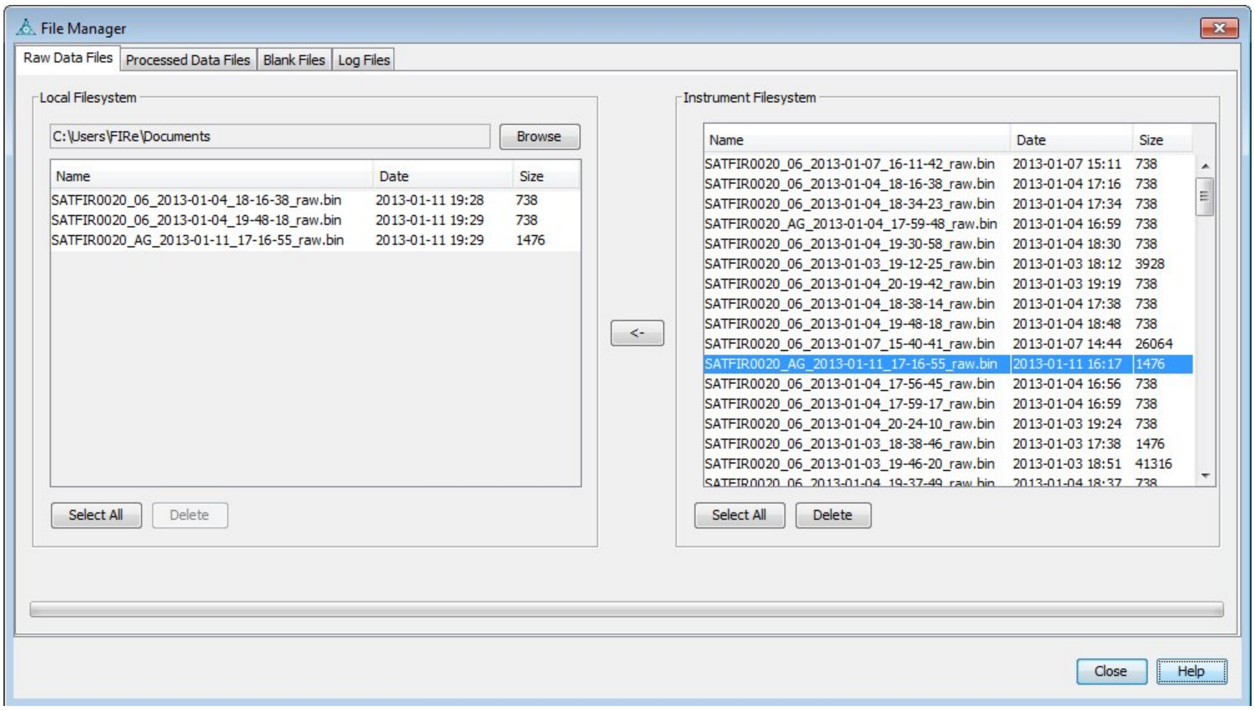
This chapter describes post-deployment activities for retrieving internally logged data and processing it.

Topics in this chapter include:

Transferring Files

In Situ FIRe stores raw data files, processed data files, blank profiles, and message logs on its internal drive. The FIReCom *File Manager* provides an easy way to manage these files on the device. The file manager shows four tabbed panes, each for listing and transferring a specific type of file:

- Raw data files
- Processed data files
- Blank files
- Log files



To retrieve files from *In Situ FIRe:*

1. Ensure that *In Situ FIRe* is connected and in *Setup* mode.
2. Press the *Transfer Files* button on the FIReCom Dashboard.
3. Select the tab listing the type of file to transfer (exampled: *Raw Data Files*)
4. Click to select desired files on the *Instrument filesystem* list, or press *Select all* .
5. Click the left-arrow to begin transferring selected files.
6. Optional: after confirming successful transfer to the local computer, press the *Delete* button to remove the files from FIRe to free up internal storage space.

Local File Locations

FIReCom uses files to define frame definitions, profile sequence and application configurations. These files are downloaded by FIReCom and stored locally.

Their local location is platform specific, see the below table for their default location.

System	Application Folder
Windows XP	C:\Documents and Settings\%USERNAME%\My Documents\Satlantic\FIReCom X.X
Windows Vista	C:\Users\%USERNAME%\My Documents\Satlantic\FIReCom X.X
Windows 7	C:\Users\%USERNAME%\My Documents\Satlantic\FIReCom X.X
Windows 8	C:\Users\%USERNAME%\My Documents\Satlantic\FIReCom X.X

It is important to manage instrument package files so that they can be used for file post processing. These files define instrument configuration at the time of the data collection. Sequence files(fireSequence.xml) and Session Files(FIRe_SESSION.ser) are only used by FIReCom for display and are not critical to maintain.

FIReCom also stores its application log in this directory, see section FIReCom Message Logging for more information on these log files.

Data Processing

In Situ FIRe collects data profiles per the protocol introduced in Z. S. Kolber et. al.: *Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocol* , Biochimica et Biophysica Acta 1367 (1998) 88-106. FIReCom derives physiological parameters defined in that publication from such profiles.

Before the parameters are estimated, FIReCom normalizes the fluorescent signal with the reference excitation signal. Each fluorescence datum is divided by the associated reference excitation datum and multiplied by the maximum reference excitation for the flash. In this way both short term intra-flash and long term variations in excitaiton intensity are accounted for.

FIReCom implements an estimation algorithm to find parameter values that best match a given profile (in the least square sense). The estimation is an iterative search, with subsequent steps closing in on increasingly better parameter estimates. However, depending on the quality of the measured data and some intentional randomness, a search may run into a dead end. When a search has not converged after the *Maximum Iterations per Estimation* , a new estimation is started using slightly different initial conditions.

Since the parameter estimation algorithm is non-deterministic, there is no guarantee that a good parameter set will be found. However, tests with many types of measured and artificial data have shown that attempting at least 40, but preferably 50 or up to 100 independent estimations (*Number of Estimations* in the Advanced Processing Settings section), will find parameters close to the true parameters.

Besides using the *Maximum Iterations per Estimation* , the operator can shorten or lengthen the searches by increasing or decreasing the *Estimation Tolerance* . The tolerance is a measure of the expected precision of the search result. Reasonable values are in the 0.0001 to 0.2 range. Here, a large value is acceptable when profile data are noisy and precise results cannot be achieved anyways. Otherwise, a value below 0.01 is preferred. Values below 0.001 have only marginal benefit while significantly increasing the computation time.

The *Estimation Scaling* provides a way to control the data range searched by the Estimations. While the first estimate begins as wide as possible, subsequent estimates begin around the latest best estimate, and reduce the searched range by the scaling value. Reasonable values are between 1.0 (no reduction) to 0.1 (severe reduction), with the default value of 0.5.

The scaling value should only be changed if the estimated model parameters are of poor quality. In such a situation, the operator may attempt to widen the scaling value to a value closer to 1.0, while at the same time significantly increasing both the *Number of Estimations* and the *Maximum Iterations per Estimation* . Those settings force the search to be cast wider, at the expense of a substantial increase in computation time.

If, on the other hand, a very slow computer is used to perform the estimates, and the data are known to have very little noise, the operator may attempt to reduce the search scaling to 0.1, potentially reducing the processing time while compromising the data quality.

Configure Data Processing

When *In Situ* FIRe starts acquiring data in real time mode, it transmits raw data frames to FIReCom. FIReCom has the ability to process these transmitted raw data frames and create and display processed data frames. The following settings control which parts of the raw profiles are processed and what values are produced.

Post Processing Options

The *Post Processing Options* allow you to specify which parts of the raw profiles to use to produce processed values. Detailed descriptions of processed frames are specified in the Data Format Reference section of this manual.

Advanced Post Processing Settings

The *Advanced Post Processing Settings* allow you to specify processing algorithm parameters and LED calibration values.

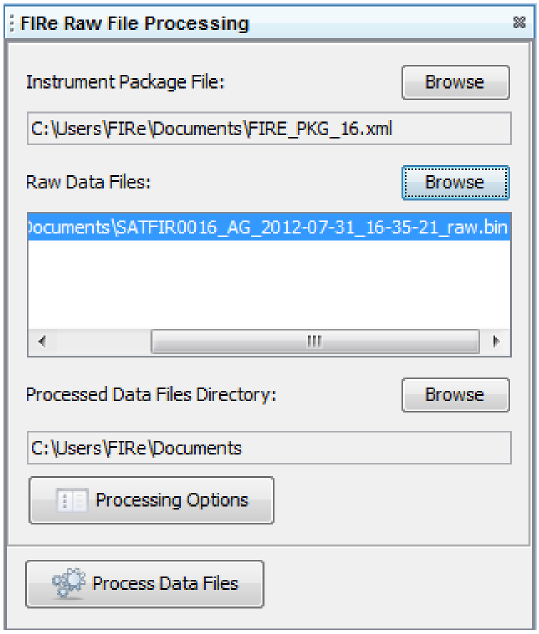
On Board Processing

In Situ FIRe may also be outputting STF processed frames that can be produced in real time on board the instrument. This feature is controlled via the **Operation Mode** option **Process Single Turnover Flash Data** described in the FIRe Settings section.

Processing Raw Data

Raw data files can be retrieved from *In Situ* FIRe as described in Transferring Files . Raw data files can also be logged directly to a computer when acquiring data in real time as described in Logging Real Time Data .

To process and/or replay (plot) raw files saved to the local computer, first ensure that *In Situ* FIRe is **not** connected. Select *Data -> Process Raw Data* from the main menu to open the *FIRe Raw File Processing* panel in the FIReCom dashboard.



Enter the following information in the *FIRe Raw File Processing* dashboard panel:

- **Instrument Package File:** Press the *Browse* button to select the instrument package XML file for the *In Situ* FIRe that generated the raw data to be processed. This XML file can be found on the CD-ROM provided with your *In Situ* FIRe. If you are managing data from more than one *In Situ* FIRe, ensure that you select the *Instrument Package File* that corresponds with the raw data files specified below.

- **Raw Data Files:** Press the *Browse* button to search for raw data files that have been transferred from *In Situ* FIRe to the local computer. Files selected for processing are then listed in the area below. If you are managing data from more than one *In Situ* FIRe, ensure that you select only raw data files that correspond to the *Instrument Package File* specified above.

- **Processed Data Files Directory** : Press the *Browse* button to select the directory on the local computer to which processed files should be saved.

Press the *Processing Options* button to show the Post-Processing Options dialog. Select one or more of the *Turnover Flash* options in the Post-Processing Options dialog to enable creation of processed data files. If none of these is selected, normalized induction and relaxation profiles will be plotted in the graphical display, but no processed data files will be generated.

Press the *Process Data Files* button to process the selected raw files. This button is alternately labeled *Playback Data Files* when no STF/MTF options are selected in the Post-Processing Options dialog.

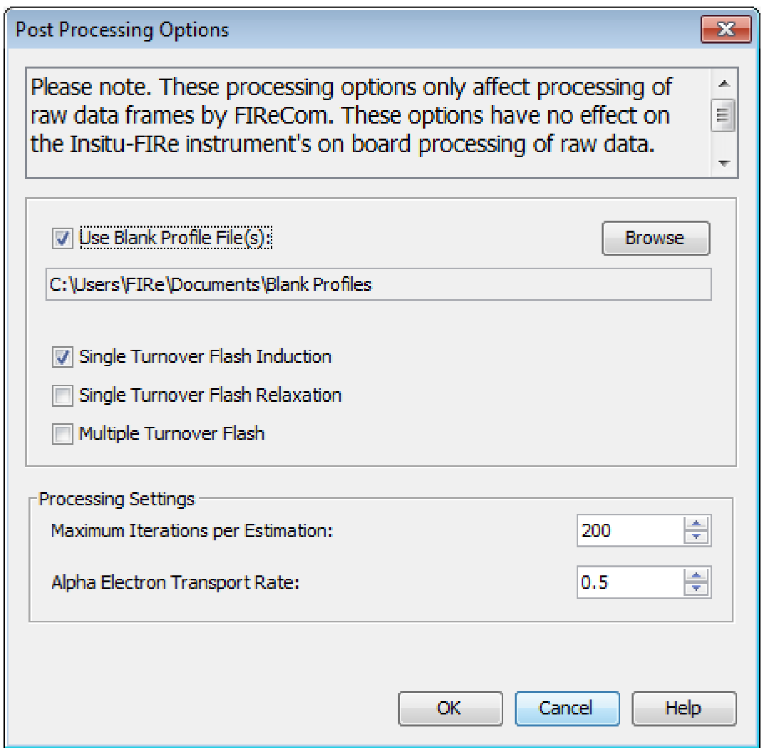
Post-Processing Options

When acquiring real-time data from *In Situ* FIRe, or when processing retrieved or locally logged raw data files, FIReCom processes and displays sensor data according to options specified in the *Post-Processing Options* dialog.

There are two ways to show the *Post-Processing Options* dialog:

1. Select *Data -> Post-Processing Options* from the main menu.

2. Press the *Processing Options* on the *FIRe Raw File Processing* dashboard panel as described in Processing Raw Data.



Note that all settings on this dialog apply only to processing performed by FIReCom software. These settings have no effect on the processing performed on-board by *In Situ* FIRe.

- **Use Blank Profile File(s):** Select this option to apply one or more *blank profile* files when processing raw data. Press the *Browse* button to identify a folder on the local computer that holds the blank profile file(s) to be applied. When enabled determination of which Blank Profile(s) are used for processing is based on the gain and the time-stamp of each profile that is processed. A more detailed description of what a blank profile is and why a blank profile might be required is described in the Deployment Procedures section.

- **Single Turnover Flash Induction:** Select this option to enable processing of the single turnover flash induction phase.

- **Single Turnover Flash Relaxation:** Select this option to enable processing of the single turnover flash relaxation phase.
- **Multiple Turnover Flash:** Select this option to enable processing of the multiple turnover flash. Note that only the relaxation phase of the multiple turnover flash is processed.

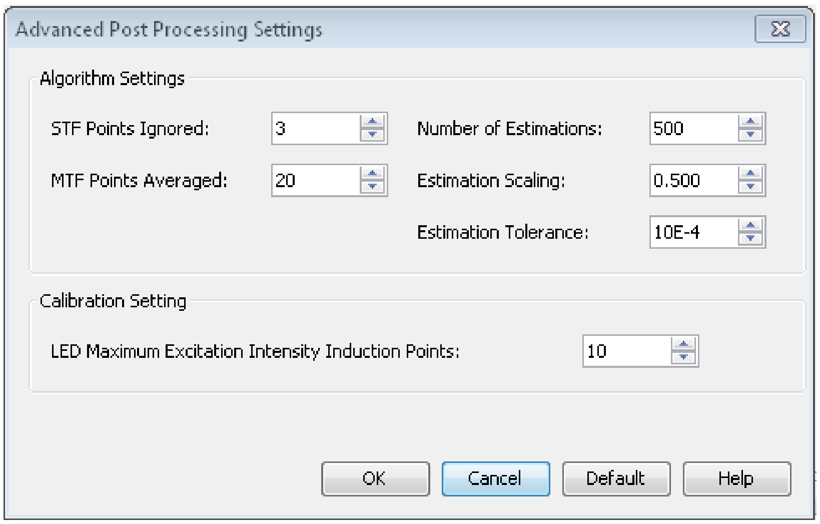
The following *Processing Settings* are for expert users only. Modification of these options can significantly change the output of the processing routines and should not be changed without understanding their effects.

Maximum Iterations per Estimation: This cutoff value controls the duration of data processing, at the expense of data precision.
Alpha Electron Transport Rate: This parameter governs the maximum quantum yield for whole chain electron transport at low light intensities, as a fraction of absorbed quanta available for photosystem II (nominally 0.5).

Advanced Processing Settings

When acquiring real-time data from *In Situ* FIRe, or when processing retrieved or locally logged raw data files, FIReCom processes and displays sensor data according to options specified in the Post-Processing Options dialog. The default settings for averaging and parameter estimation are recommended for all processing activity.

Expert users may, however, wish to experiment with alternate setting for averaging and parameter estimation. To adjust advanced settings for offline processing ("post processing"), select *Data -> Advanced Post Processing Settings* from the main menu to show the *Advanced Processing Settings* dialog.



Note that all settings in the *Advanced Processing Settings* dialog apply only to processing performed by FIReCom software. These settings do not apply to on-board processing performed by *In Situ* FIRe.

- **STF Points Ignored** : The initial three measurement points of the ST induction phase are normally of low quality and not suitable for model parameter estimation. This setting determines how many of the initial ST induction points should be discarded before processing. Do not change this setting from the default (3) unless there is a confirmed deterioration of optical performance affecting one or more subsequent induction points.

- **MTF Points Averaged** : This parameter controls how many of the last points of the MT induction profile are averaged in order to estimate the maximum fluorescence. The default value of twenty (20) is recommended for most situations. If data at the end of the induction are relatively noisy, increasing this parameter may yield slightly better results, but increasing it too much can skew results to the steeper start of the induction curve.

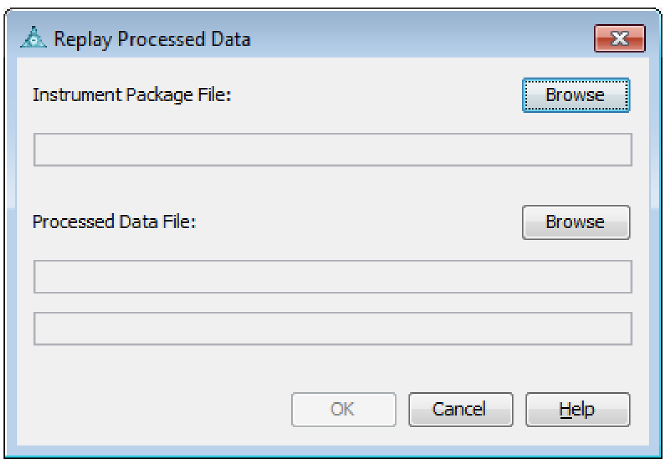
- **Number of Estimations** : This parameter determines how many independent estimations are attempted. A small value reduces computation time at the expense of data accuracy. The value should always be above 40. Values above 250 are of marginal benefit. Lower vales reduce computation time, especially on low-performance computer hardware, at the expense of data accuracy.

- **Estimation Scaling** : This processing setting determines how far subsequent estimations search for model parameters. A value of 1.0 searches for each subsequent estimation over the full region, whereas a smaller value focuses on the interval centered around the current best result. Setting the value below 0.1 is inadvisable, as the search may be too constrained, and the best result may be missed.
- **Estimation Tolerance** : This processing setting defines when an estimation has converged. The search within an estimation is terminated when subsequent search steps are advancing the quality of the result by less than the specified tolerance.
- **LED Maximum Excitation Intensity Induction Points** : The number of trailing LED excitation measurements from the induction to use in the calculation of the maximum LED light intensity. Minimum 1, default 10, maximum 20.

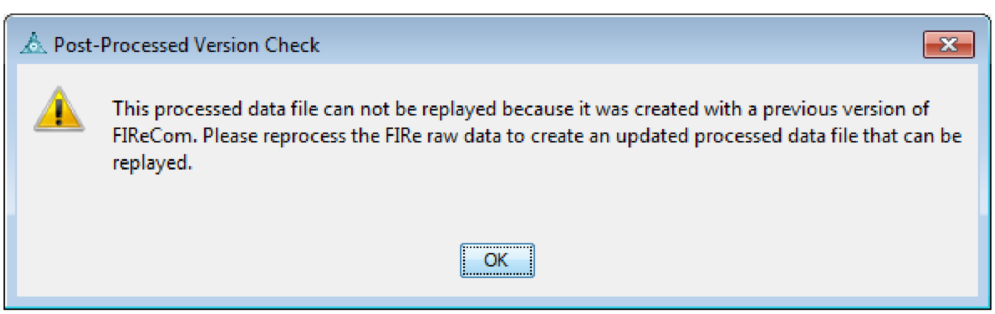
Replay Processed Data

FIReCom can graph or "replay" processed data files produced as described in Processing Raw Data . To replay processed data;

1. Ensure that *In Situ* FIRe is **not** connected.
2. Select *Data -> Replay Processed Data* from the main menu to show the *Replay Processed Data* dialog.
3. Press the first *Browse* button to select the *Instrument Package File* for the *In Situ* FIRe that produced the processed file to be replayed.
4. Press the second *Browse* button to select the processed data file to be replayed. The corresponding profile data file must be located in the same local folder.
5. Press *OK* to replay the processed data file and corresponding profile data file.



Processed data files that were produced by older versions of FIReCom software may not be compatible with the replay feature. If the selected processed data file is incompatible, the original raw data must be reprocessed to create a compatible processed data file.



Troubleshooting

If FIReCom is unable to establish a connection to *In Situ* FIRe, check the physical connectors using a multimeter with DC voltage measurement, resistance measurement, and continuity check capability.

WARNING! While checking voltages, extreme care should be used so as not to short the probe leads. A shorted power supply or battery can output many amperes of current, potentially harming people, starting fires, or damaging equipment.

Check Connections

The cable connections of the system should be checked for continuity and correctness. Make sure that all Subconn ® connectors are free of dirt and lightly lubricated before mating. Do not use petroleum-based lubricants. Satlantic recommends using a light coating of DC 111 silicone grease (made by Dow Corning ®) on the male pins prior to connection. Also, ensure that the connections are complete and, if applicable, the locking sleeves are secure.

- Check that the power cable is properly connected to the power supply and to *In Situ* FIRe.
- Check that the *In Situ* FIRe interconnect cable is in place and properly connected.
- Check that the RS 232 cable is connected to the correct communications port on the host computer or data logger.

Check the Supply Voltage

To check voltages, a multimeter with DC voltage measurement is required.

Procedure:

1. Set the multimeter to measure a DC voltage.
2. If using a battery as the power source, measure the voltage directly at the battery terminals with the multimeter. A new or fully charged 12 V battery usually measures in the 13 - 15 V range. If the voltage is low (under 11 V), recharge or replace the battery. If using a DC power supply, set the output voltage in the range from 10 - 18 V and then check the voltage with the multimeter.
3. Connect the power supply cable to the power source.
4. **Being extremely careful not to short the probe leads** , measure the voltage between the pins on the supply cable. It should read approximately the same as the measurement taken in step 2. If the voltages are not the same, recheck the power supply cable connections. If the voltages are still not the same, the cable is likely broken and will need repair. A wire break can be confirmed with a *continuity check* .

5. If the voltage is within tolerance, connect the power supply cable to the FIRe.

6. Again, measure the voltage at the power supply terminals. The voltage should remain approximately the same as before, although there may be a small voltage drop when using a battery (battery voltage drops under load). If there is a significant voltage drop, disconnect the power immediately and check for shorts in the cable.

Check Cable Continuity

Often, system problems can be traced to cable breaks or shorts. Usually, these cable failures are a result of improper handling or storage. Cable continuity can be checked as outlined below. **Make sure all cables are completely disconnected before performing this test.**

Procedure:

1. Set the multimeter to measure continuity. The resistance measurement setting can also be used.

2. Check for continuity by measuring from pin 1 on one end of the cable to pin 1 on the other end. The meter should confirm that the connection is continuous by either giving an audible signal or measuring a low resistance. If there is not continuity, there is a break in the cable, which will require repair.

3. Repeat step 2 for all pins in the cable.

4. Check for shorts from pin 1 to all other pins by keeping one probe lead on pin 1 and touching the other probe lead to each of the other pins in the same connector in turn. Repeat this for all pins on the cable to make sure that all the pins are isolated from each other. The meter should read this as open or measure a very high resistance. If any of the pins are not isolated, there is a short in the cable, which will require repair.

Check Powerdown/Powerup

The FIRe has internal capacitors that protect the computer and file system when power is removed from the system. After power down, it is necessary to wait about 20 seconds to allow the system to shut down properly before applying power again. When the instrument is restarted, the boot up time is about 1 minute. After the 1 minute, if the FIRe is not connect via a USB cable, periodic flashing of the LEDs should be visible.

Invalid Frames in Data

In any data logging application, there is a possibility that frames of data can be corrupted during transmission. For each corrupt frame, a message indicating *Lost Bytes* is displayed in the Application tab of the output panel. In addition to this, the FIReCom Error Event panel should be displayed. FIReCom will display this frame corruption warning, hen ignore these corrupt data points and continue.

No Connection to *In Situ* FIRe

If you are unsure if FIReCom is connecting to *In Situ* FIRe, try to manually connect using a terminal emulation program such as HyperTerm. *In Situ* FIRe uses the following serial connection settings:

Data Bits 8
Parity None
Stop Bits 1
Flow ControlNone

The baud rate is set to 115200 in the default FIRe configuration. The baud rate may be changed for custom applications. Finally, both a carriage return *and* linefeed are required for communication. See the External Interfaces section for more details.

If a connection cannot be established, please Contact Satlantic for support.

FIReCom Event Logs

All activity that occurs with the FIReCom application is logged to disk in the event log. The event log is rotated daily, the current log can be found at:

```
<INSTALL_DIR>\eventlogs\FIReCom.log
```

Although the contents of the log are somewhat cryptic and mainly for use by Satlantic personnel to diagnose problems within the application, it will often provide clues to errors that exist within the application. Each line in the log represents an event that occurs within the application. Below is a short explanation of the format of the log file event:

Event Logs have the following format:

```
<Date/Time>, <Module>:<Event Code> ~ <Message>,<optional stack trace>
```

Date/Time - Displays the date and time when the message was logged.

Module - Displays the module that initiated the log message (either Internal, Core or GUI)

Event Code - internal code (i.e. ISC-1100-ERR) describing the module, internal error number and event severity. Severity is either INF(info), WRN(warning) or ERR(error).

Message - A text based representation of the event

Stack Trace - Optionally occurs when unexpected errors happen to show the state of the application when the error occurred.

When a problem occurs that cannot be easily explained, it may be necessary to send files to Satlantic to determine the nature of the problem. When submitting an issue, please have the following information on hand:

- The version of FIReCom you are using (select *Help -> About* on the main menu)

- The version of firmware and serial number of the *IN Situ* FIRe you are using (see FIReCom Dashboard)
- The raw data file that was logged on *In Situ* FIRe
- The FIReCom event log files

Change in Excitation Intensity

In Situ FIRe has eight excitation LEDs. The unlikely event of a failure in one of these LEDs can be identified by a change in reference counts in the Sequence Plot. Any previous data from blanks or samples could be used to obtain historical reference levels for the FIRe, provided they were low-turbidity samples. If drops in excitation are observed, please Contact Satlantic.

Incorrect Time

If the *In Situ* FIRe internal clock loses its time setting every time it is restarted, then it is likely that the clock battery is drained. Please Contact Satlantic to arrange a battery replacement.

Maintenance

Preventative Maintenance

In Situ FIRe requires little maintenance. To prolong the life of the instrument:

- Protect *In Situ* FIRe from impacts
- Rinse *In Situ* FIRe in fresh water after each use
- Clean the optional flow cell with a pipe cleaner and mild detergent and de-ionized water rinse
- Always replace dummy connectors when *In Situ* FIRe is stored
- Carefully clean the optical windows (see below)

Cleaning the Optical Windows

In order to limit the possibility of damaging the optical windows, we suggest first rinsing the optics with clean water to remove particulate matter and dissolve any salt residue. Then gently wipe the windows clean with a cotton swab applicator and methanol or isopropanol, being careful to avoid leaving any cotton fibers behind. De-ionized water may be used, however it may leave spots that can affect transmission. Kimwipes or other lens tissue and methanol or isopropanol may also be used. In this case, care must be taken to avoid getting finger grease on the windows. Wearing disposable gloves is suggested to avoid grease transfer.

Do not soak the cotton swab or lens tissue with the cleaning agent. It will leave pools of liquid that will not dry properly. Just dampen the applicator with the agent. The applicator should be moved over the window in one direction and then discarded, or rotated to a different position so that contaminants are not rubbed across the surface.

The optics should be rinsed with water before they are stored, or if they will not be used for a period of time.

Safety and Hazards

Personal Safety

WARNING!

If you suspect that *In Situ* FIRe has flooded, use **EXTREME CAUTION** around the instrument. *In Situ* FIRe can operate at depths of up to 200 meters. If the instrument leaked at depth it might remain **highly pressurized** when recovered and could cause the lamp housing or the spectrometer housing to be launched from the coupler with **extreme force** if the restraining screws are removed.

An indication for flooding is that the instrument stops operating or that there is a short-circuit condition in the instrument. The humidity readings increase when water enters the instrument. If you suspect a flood, check the instrument for signs of pressurization. In a pressurized instrument, the gap between the housings and coupler may be extended. Also an instrument flooded with salt water may short all of the connector pins together. An electric continuity test between random pins on any of the bulkheads may confirm this suspicion. **Place the instrument in a safe location and contact Satlantic for further instructions.**

If the instrument cannot be safely stored for return, the following steps can be taken:

CONTINUE AT YOUR ON RISK!

The safest method to depressurize the FIRe sensor is to **slowly** back off the bulkhead connector. The connector only has to be loosened so that the face seal o-ring is no longer sealed against the housing. Pressurized water can then escape along the threaded portion of the bulkhead. When all of the pressure has been released the instrument can be stored and safely shipped to Satlantic for repairs.

- The FIRe has an internal high-voltage supply. **Do not operate with the housing removed!**
- When operating on the bench top, the FIRe housing should be connected to earth ground to minimize the risk of **electric shock**.

Instruments

- Do not leave instruments in direct sunlight. Direct sunlight can easily increase the internal temperature of the instrument beyond its maximum rating.

Connections

- Handle electrical terminations carefully, as they are not designed to withstand strain. Disconnect the cables from the components by pulling on the connector heads and not the cables. Do not twist the connector while pulling, as this will damage the connector pins.

- Do not use petroleum-based lubricants on Subconn® connectors. Connectors should be free of dirt and lightly lubricated before mating. Satlantic recommends using DC-111 silicone grease (made by Dow-Corning®) on the male pins prior to connection.

Troubleshooting

- While checking voltages with a multimeter, use extreme care to avoid shorting the probe leads. A shorted power supply or battery can output many amperes of current, potentially harming the user, starting fires, or damaging equipment.

Recovery

- Remember never to grab the electrical portion of the instrument cable during recovery. This can cause damage to the bulkhead connector and the underwater splice.

- Be sure to rinse seawater from the instrument with fresh water prior to storage. Corrosion resulting from failure to do so is not covered under warranty.

Warranty

Warranty Period

All Satlantic equipment is covered under a one-year parts and labor warranty from date of purchase.

Restrictions

Warranty does not apply to products that are deemed by Satlantic to be damaged by misuse, abuse, accident, or modifications by the customer. The warranty is considered void if any optical or mechanical housing is opened. In addition, the warranty is void if the warranty seal is removed, broken or otherwise damaged.

Provisions

During the one year from date of purchase warranty period, Satlantic will replace or repair, as deemed necessary, components that are defective, except as noted above, without charge to the customer. This warranty does not include shipping charges to and from Satlantic.

Returns

To return products to Satlantic, whether under warranty or not, contact the Satlantic Customer Support Department and request a Returned Material Authorization (RMA) number and provide shipping details. All claims under warranty must be made promptly after occurrence of circumstances giving rise thereto and must be received by Satlantic within the applicable warranty period. Such claims should state clearly the product serial number, date of purchase (and proof thereof) and a full description of the circumstances giving rise to the claim. All replacement parts and/or products covered under the warranty period become the property of Satlantic LP

Liability

IF SATLANTIC EQUIPMENT SHOULD BE DEFECTIVE OR FAIL TO BE IN GOOD WORKING ORDER THE CUSTOMER'S SOLE REMEDY SHALL BE REPAIR OR REPLACEMENT AS STATED ABOVE. IN NO EVENT WILL SATLANTIC LP BE LIABLE FOR ANY DAMAGES, INCLUDING LOSS OF PROFITS, LOSS OF SAVINGS OR OTHER INCIDENTAL OR CONSEQUENTIAL DAMAGES ARISING FROM THE USE OR INABILITY TO USE THE EQUIPMENT OR COMPONENTS THEREOF.

Contact Satlantic

If you have any questions, comments or concerns about your *In Situ* FIRe, please contact us.

Satlantic
3481 North Marginal Road
Halifax, Nova Scotia
B3K 5X8 Canada
PHONE: (902) 492-4780
FAX: (902) 492-4781
Email: Technical Support: support@satlantic.com
General Inquiries: info@satlantic.com
Web: http://www.satlantic.com

Business Hours

Satlantic is normally open for business between the hours of 9:00 AM and 5:00 PM Atlantic Time. Atlantic Time is one hour ahead of the Eastern Time. Daylight saving time is in effect from 2:00 AM on the second Sunday in March through 2:00 AM on the first Sunday in November. Atlantic Standard Time (AST) is UTC-4. Atlantic Daylight Saving Time (ADT) is UTC-3.

Satlantic is not open for business during Canadian statutory holidays, which are as follows:

- **New Year's Day** January 1st
- **Good Friday** The Friday before Easter Sunday (Easter Sunday is the first Sunday after the full moon on or following March 21 st , or one week later if the full moon falls on Sunday)
- **Victoria Day** The first Monday before May 25 th
- **Canada Day** July 1 st
- **Civic Holiday** The first Monday in August
- **Labor Day** The first Monday in September
- **Thanksgiving Day** The second Monday in October
- **Remembrance Day** November 11 th
- **Christmas Day** December 25 th
- **Boxing Day** December 26 th

Data Format Reference

In Situ FIRe is capable of emitting and or logging several different telemetry data frame formats, depending on the operation mode of the FIRe. These telemetry data frame formats are described in the following sections.

Raw Data Format

The telemetry raw data frame format for the FIRe complies with the *Satlantic Data Format Standard* for the Variable Binary Telemetry Frame. For every sample taken, the instrument will compose one frame of *raw* data. If configured to do so, it will internally log the frame and transmit the frame via the serial telemetry interface. The FIRe raw telemetry frame is described in detail in the table below. All fields occurring in the frame are listed, and their meaning is explained. The fields' format and sizes are also listed. The ASCI String (AS), the Binary Unsigned Integer (BU), and the Binary Floating Point (BD) data types are used in the raw frame.

Table 1: FIRe Raw Data Frame Format

Field Name	Format	Description
Frame Header	AS 10	The frame header or synchronization string starts with *SAT* for a Satlantic instrument, followed by three characters identifying the frame type. For the In-Situ FIRe this is *FIR* . The last four characters are the instrument serial number. Examples are SATFIR0001, SATFIR0301, SATFIR1005
Time	BU 7	The date and time of the frame. This field follows Satlantic's 7-byte time stamp format.
Flash Type	AS 1	Indicates the flash type used. Value 'S' for a Single Turnover Flash and 'M' for a Multiple Turnover Flash.
Sequence Profile Total Number	BU 2	The total number of profiles in the current flash sequence.
Sequence Ordinal Number	BU 2	The ordinal position of the profile represented by this frame in the current flash sequence.
Induction Phase number	BU 2	Number of data points in the induction phase of the profile.
Relaxation Phase number	BU 2	Number of data points in the relaxation phase of the profile.
Excitation Induction Phase number	BU 2	Number of data points in the induction phase of the reference excitation profile.
Excitation Relaxation Phase number	BU 2	Number of data points in the relaxation phase of the reference excitation profile.

Gain Setting	BU 1	The gain setting of the instrument when the profile was collected. This indicates the relative gain with a range of 1-10.
Iteration Delay	BU 2	The delay (ms) between the flash iterations during the collection of one profile.
Number iterations	BU 2	The number of flash iterations to use during the collection of one profile.
Induction Phase duration	BU 4	The length(µs) of the induction phase.
Relaxation Phase duration	BU 2	The duration (µs) of the relaxation phase.
RPI	BU 2	The initial interval (µs) between the relaxation phase pulses.
Induction Phase	BU 2	Raw counts from the induction phase of the fluorescence profile
...	...	...
Relaxation Phase	BU 2	Raw counts from the relaxation phase of the fluorescence profile
...	...	...
Reference Excitation Induction Phase	BU 2	Raw counts from the induction phase of the fluorescence reference excitation profile
...	...	...
Reference Excitation Relaxation Phase	BU 2	Raw counts from the relaxation phase of the fluorescence reference excitation profile
...	...	...
PAR	BD 8	Measured PAR value read from analog-to-digital converter
Battery Voltage	BU 2	Internal measurement of voltage read from analog-to-digital converter
Pressure	BU 4	Measured value from digital pressure sensor
Auxiliary Status Flag	BU 2	A flag indicating auxiliary sensor status. The PAR sensor is enabled if bit 0 is set and the pressure sensor is set if bit 1 is set.
Profile Error Flag	BU 1	Error indicator for the collected profile data. A 0 value indicates no error. A 1 indicates an error occurred during the collection of the profile.
PAR Error Flag	BU 1	Error indicator for the PAR data value. A 0 value indicates no error. 1 indicates an error occurred when reading the value.

Voltage Error Flag	BU 1	Error indicator for the voltage data value. A 0 value indicates no error. 1 indicates an error occurred when reading the value.
Pressure Error Flag	BU 1	Error indicator for the pressure data value. A 0 value indicates no error. 1 indicates an error occurred when reading the value.
LED Intensity Calibration Coefficient	BF	Calibration coefficient used during the calculation of the maximum LED light intensity.
Frame Counter	BU 1	Indicates the frame number. Resets after 255.
Check Sum	BU 4	CRC32 checksum for the frame excluding the checksum and the terminator.
Terminator	BU 4	0xFF00FF00

Processed Data Format

If configured to do so, the FIRe will process the raw data, transmit a processed frame, and internally log the processed frame. The telemetry processed data frame format follows the Satlantic Data Format Standard for the Variable Length ASCII Telemetry Frame, with a ',' used as a field delimiter for every field. The processed telemetry frame is described in detail in the table below.

Table 2: FIRe Processed STF Data Frame Format

Field Name	Description
Frame Header	The frame header or synchronization string starts with *SAT* for a Satlantic instrument, followed by three characters identifying the frame type. For the FIRe this is *FIS* indicating data processed from a Single Turnover Flash. The last four characters are the instrument serial number. Examples are: SATFIS0003, SATFIS2222
Date	Data collection date using the format YYYY-MM-DD
Time	Data collection time using the format hh:mm:ss.sss
Pressure	Pressure sensor value (pascals)
F_o	Calculated initial fluorescence (relative)
F_m	Calculated maximum fluorescence (relative)
F_v	Calculated variable fluorescence (F_m -F_o) (relative)
F_v /F_m	Calculated maximum quantum yield of photochemistry in PSII (F_m -F_o)/F_m

p	*Connectivity factor* (dimensionless). Defines the energy transfer between PSII units.
Relative σ	Relative functional absorption cross-section- sigma (relative)
Absolute σ	Absolute functional absorption cross-section- sigma ($Å^2$)
Maximum LED intensity	Calculated maximum LED light intensity (units of PAR)
ETR	Calculated Electron Transport Rate
α 1	Reoxidation distribution state 1 **(not calculated on FIRe)**
α 2	Reoxidation distribution state 2 **(not calculated on FIRe)**
α 3	Reoxidation distribution state 3 **(not calculated on FIRe)**
τ 1	Reoxidation time constant 1 **(not calculated on FIRe)**
τ 2	Reoxidation time constant 2 **(not calculated on FIRe)**
τ 3	Reoxidation time constant 3 **(not calculated on FIRe)**
Normalized RMSe	Normalized root mean square error
Gain Setting	The gain setting of the instrument when the profile was collected. This indicates the relative gain with a range of 1-10.
PAR	Calibrated measured PAR value
Internal Voltage	Calibrated measured voltage value (V)
Processing Results Error Flag	Error indicator for the processed data. A 0 value indicates no error. A 1 indicates that an error was flagged for the data in the raw data or that an error occurred during processing.
PAR Error Flag	Error indicator for the calibrated PAR value. A 0 value indicates no error. A 1 indicates that an error was flagged for the raw PAR value.
Voltage Error Flag	Error indicator for the calibrated voltage value. A 0 value indicates no error. A 1 indicates that an error was flagged for the raw voltage value.
Pressure Error Flag	Error indicator for the pressure value. A 0 value indicates no error. A 1 indicates that an error was flagged for the pressure value in the raw frame.
Check Sum	CRC32 checksum for the frame excluding the checksum and the terminator fields.

Terminator	CR/LF (Carriage return, Linefeed)

Post-Processed Data Format

The FIReCom program can be used to process FIRe raw data log files. The telemetry processed data frame format follows the Satlantic Data Format Standard for the Variable Length ASCII Telemetry Frame, with a ',' used as a field delimiter for every field. The processed telemetry frame is described in detail in the table below.

Table 3: FIReCom Post Processed STF or MTF Data Frame Format

Field Name	Description
Frame Header	The frame header or synchronization string starts with *SAT* for a Satlantic instrument, followed by three characters identifying the frame type. For FIReCom this is either *FPS* indicating data processed from a Single Turnover Flash or *FPM* indicating data processed from a Multiple Turnover Flash. The last four characters are the instrument serial number. Examples are SATFPS0003, SATFPM2222
Date	Data collection date using the format YYYY-MM-DD
Time	Data collection time using the format hh:mm:ss.sss
Pressure	Pressure sensor value (pascals)
F_o	Calculated initial fluorescence (relative)
F_m	Calculated maximum fluorescence (relative)
F_v	Calculated variable fluorescence (F_m -F_o) (relative)
F_v /F_m	Calculated maximum quantum yield of photochemistry in PSII (F_m -F_o)/F_m
p	*Connectivity factor* (dimensionless). Defines the energy transfer between PSII units.
Relative σ	Relative functional absorption cross-section--sigma (relative)
Absolute σ	Absolute functional absorption cross-section--sigma ($Å^2$)
Maximum LED intensity	Calculated maximum LED light intensity (units of PAR)
ETR	Calculated Electron Transport Rate
α 1	Reoxidation distribution state 1

α 2	Reoxidation distribution state 2
α 3	Reoxidation distribution state 3
τ 1	Reoxidation time constant 1
τ 2	Reoxidation time constant 2
τ 3	Reoxidation time constant 3
Normalized RMSe	Normalized root mean square error
Gain Setting	The gain setting of the instrument when the profile was collected. This indicates the relative gain with a range of 1-10.
PAR	Calibrated measured PAR value
Internal Voltage	Calibrated measured voltage value (V)
Processing Results Error Flag	Error indicator for the processed data. A 0 value indicates no error. A 1 indicates that an error was flagged for the data in the raw data or that an error occurred during processing.
PAR Error Flag	Error indicator for the calibrated PAR value. A 0 value indicates no error. A 1 indicates that an error was flagged for the raw PAR value.
Voltage Error Flag	Error indicator for the calibrated voltage value. A 0 value indicates no error. A 1 indicates that an error was flagged for the raw voltage value.
Pressure Error Flag	Error indicator for the pressure value. A 0 value indicates no error. A 1 indicates that an error was flagged for the pressure value in the raw frame.
Check Sum	CRC32 checksum for the frame excluding the checksum and the terminator fields.
Terminator	CR/LF (Carriage return, Linefeed)

Post-Processed Profile Data Format

During post processing the FIReCom program also produces a more detailed output data product with contains detailed intermediate processing products for each profile. The file is output in the same directory and has the same name as the post processed data product with an appended "_profile" postfix in the filename, It contains a data product for each frame. Each data product contains the following data.

- An list of ancillary data from the data original data frame used to produce the profile data product, described in Table 4

- A table of profile data, described in Table 5

Table 4: FIReCom Profile Header *Data Format* for *Post Processed STF or MTF*

Field Name	Description
Frame Header	The frame header or synchronization string starts with *SAT* for a Satlantic instrument, followed by three characters identifying the frame type, SATFPH0001 (FPH="FIRe profile header")
Date	Data collection date using the format YYYY-MM-DD
Time	Data collection time using the format hh:mm:ss.sss
Pressure	Pressure sensor value (pascals)
Maximum LED intensity	Calculated maximum LED light intensity (units of PAR)
PAR	Calibrated measured PAR value
Internal Voltage	Calibrated measured voltage value (V)
Induction Phase number	Number of data points in the induction phase of the profile.
Relaxation Phase number	Number of data points in the relaxation phase of the profile.
Gain Setting	The gain setting of the instrument when the profile was collected. This indicates the relative gain with a range of 1-10.
Iteration Delay	The delay (ms) between the flash iterations during the collection of one profile.
Number iterations	The number of flash iterations to use during the collection of one profile.
Induction Phase duration	The length(µs) of the induction phase.
Relaxation Phase duration	The duration (µs) of the relaxation phase.
RPI	The initial interval (µs) between the relaxation phase pulses.
Terminator	CR/LF (Carriage return, Linefeed)

Table 5: FIReCom Profile *Data Format* for *Post Processed STF or MTF*

Field Name	Description
Frame Header	The frame header or synchronization string starts with *SAT* for a Satlantic instrument, followed by three characters identifying the frame type, SATFPD0001 (FPD="FIRe profile data")
Time (offset)	The time offset in (µs) from the start of the profile
Fluorescence counts	Dark corrected counts from the fluorescence profile

Reference counts	Dark corrected counts from the reference profile
Fluorescence Yield	The total fluorescence Yield for this time offset
Terminator	CR/LF (Carriage return, Linefeed)

Data Log File Naming

In Situ FIRe is capable of internally logging its telemetry in local data files. The format for the names of the FIRe telemetry files, specified as *prefix_gain_timestamp_suffix.extension* , is described in the table below. Examples of file names are:

- SATFIR0001_01_2009-06-19_13-45-11_raw.bin
- SATFIR0001_01_2009-06-22_13-52-36_blk.bin
- SATFIS0001_01_2009-06-19_16-07-30_pro.csv

Table 5: Data Log File Naming Format

Section	Description	Example
prefix	The frame header (see description in the preceding tables) is the default file name prefix.	• SATFIR001(raw frame) • SATFIS001(processed frame)
gain	Indicates the relative gain that was used when the telemetry was collected.	• *03* • *10* • *AG* (autogain)
timestamp	Denotes the date and time of the telemetry file creation. The format is *yyyy-MM-dd_HH-mm-ss* .	• *2009-06-19_13-45-11*
suffix	Denotes the data product type.	• *raw* (raw telemetry) • *blk* (blank profile) • *pro* (processed telemetry)
extension	Denotes the file data format.	• *.bin* (binary) • *.csv* (comma separated ASCII values)

Command Reference

In Situ FIRe can be configured and controlled via the serial command interface detailed below. Once a $bk break command has been issured to put *In Situ* FIRe in commanded mode, it will resond to commands for configuration, status, data operations, and primary control.

Configuration Commands

The *$conf* directive provides the following configuration commands to set operating parameters of *In Situ* FIRe.

Command	Parameter	Description
$conf autogain	on | off	Turns the autogain function on or off.
$conf baudrate	1200 | 2400 | 4800 | 9600 | 19200 | 38400 | 57600 | 115200 | 230400	Sets the serial port baud rate.
$conf blank	on | off	Controls whether a blank subtraction is used during processing of the STF induction.
$conf calibpresssensor	Floating point number representing atmospheric pressure in kPa.	Resets the calibration of the sensor pressure.
$conf configuration	None	Displays the current configuration of the FIRe instrument.
$conf datafileheader	String	ASCII comment inserted at the beginning of all telemetry data files.
$conf datafileprefix	String containing only the following characters: 'A-Z', 'a-z', '0-9', '-', '_', and '.'	String used as the prefix in the file name of all telemetry data files.
$conf etralpha	Floating point number with range [0.0, 20.0]	Sets the # used in the electron transport rate calculation.
$conf gain	Integer with range [1, 10]	Sets the relative gain of the FIRe detection circuit. Used when the automatic gain function is turned off.
$conf iterations	Integer with range [1, 1000]	Sets the number of repeated profiles taken for a single profile. Used only when in fixed iteration mode.
$conf itergain <gainLevel>	Integer with range [1, 1000]	Sets the number of iterations for the specified gain level. Used only when in variable iteration mode. *gainLevel* is an integer with range [1, 10]
$conf itergainlow	Integer with range [1, 1000]	Sets the number of iterations for gain levels 1 to 9. Used only when in variable iteration mode.
$conf itergainmax	Integer with range [1, 1000]	Sets the number of iterations for gain level 10. Used only when in variable

		iteration mode.
$conf itermode	fixed | variable	Sets the iteration mode to either fixed or variable.
$conf ledintcalibcoef	Floating point 0.0 or greater	Sets the LED intensity calibration coefficient used in the calculation of the maximum LED excitation intensity
$conf ledintnoindpts	Integer with range [1, 20]	Sets the number of induction points used in the calculation of the maximum LED excitation intensity
$conf mtflen	Integer with range [1000, 1000000]	Sets the duration in microseconds of the Multiple Turnover Flash induction phase.
$conf mtri	Integer with range [5, 200]	Sets the initial interval in microseconds between the pulses of the Multiple Turnover Flash relaxation phase.
$conf mtrp	Integer with range [500, 50000]	Sets the duration in microseconds of the Multiple Turnover Flash relaxation phase.
$conf para0	Floating point number	Sets the PAR sensor calibration coefficient *a 0 .*
$conf para1	Floating point number	Sets the PAR sensor calibration coefficient *a 1 .*
$conf parenabled	on | off	Controls whether the PAR sensor is enabled or not.
$conf parim	Floating point number	Sets the PAR sensor calibration immersion coefficient.
$conf pressureenabled	on | off	Controls whether the pressure sensor is enabled or not.
$conf processstf	on | off	Controls whether the STF induction is processed or not when collecting profiles.
$conf profiledelay	Integer with range [0, 1000]	Sets the delay in milliseconds between the individual iterations of a fluorescence profile collection.
$conf serialport	rs232 | rs422	Sets the serial communication channel to be used by the FIRe instrument the next time it is powered on.
$conf stflen	Integer with range [1, 1000]	Sets the duration in microseconds of the Single Turnover Flash induction phase.
$conf storeprocessed	on | off	Controls whether or not processed data is stored on board the FIRe instrument. Has no effect if processing is not enabled.

$conf storeraw	on | off	Controls whether or not raw data is stored on board the FIRe instrument.
$conf stri	Integer with range [5, 200]	Sets the initial interval in microseconds between the pulses of the Single Turnover Flash relaxation phase.
$conf strp	Integer with range [500, 50000]	Sets the duration in microseconds of the Single Turnover Flash relaxation phase.
$conf synctime	Integer greater than or equal to 0	Sets the FIRe instrument clock. The input value represents the number of seconds since 00:00:00 1 Jan 1970 UTC.
$conf transmitprocessed	on | off	Controls whether or not processed data is transmitted from the FIRe instrument during data acquisition. Has no effect if processing is not enabled.
$conf transmitraw	on | off	Controls whether or not raw data is transmitted from the FIRe instrument during data acquisition.

Get Commands

The *$get* command returns the current setting of the named parameter passed as the first argument.

Command	Return Value	Description
$get autogain	on | off	Retrieves whether the autogain function is turned on or off.
$get baudrate	1200 | 2400 | 4800 | 9600 | 19200 | 38400 | 57600 | 115200 | 230400	Retrieves the serial port baud-rate.
$get blank	on | off	Retrieves whether or not blank subtraction is used during STF induction processing.
$get datafileheader	String	Retrieves the ASCII comment inserted at the beginning of all telemetry data files.
$get datafileprefix	String containing only the following characters: 'A-Z', 'a-z', '0-9', '-', '_', and '.'	Retrieves the string used as the prefix in the file name of all telemetry data files.
$get diskspace	Integer Integer	Retrieves the unallocated space, in bytes, and the total size, in bytes, of the data directory partition
$get etralpha	Floating point number with range [0.0, 20.0]	Retrieves the # used in the electron transport rate calculation.

$get gain	Integer with range [1, 10]	Retrieves the relative gain of the FIRe detection circuit used when the automatic gain function is turned off.
$get iterations	Integer with range [1, 1000]	Retrieves the number of repeated profiles taken for a single profile when in fixed iteration mode.
$get itergain <gainLevel>	Integer with range [1, 1000]	Retrieves the number of iterations for the specified gain level when in variable iteration mode. *gainLevel* is an integer with range [1, 10]
$get itermode	fixed | variable	Retrieves the iteration mode.
$get ledintcalibcoef	Floating point greater than 0.0	Retrieves the LED intensity calibration coefficient used in the calculation of the maximum LED excitation intensity
$get ledintnoindpts	Integer with range [1, 20]	Retrieves the number of induction points used in the calculation of the maximum LED excitation intensity
$get mtflen	Integer with range [1000, 1000000]	Retrieves the duration in microseconds of the Multiple Turnover Flash induction phase.
$get mtri	Integer with range [5, 200]	Retrieves the initial interval in microseconds between the pulses of the Multiple Turnover Flash relaxation phase.
$get mtrp	Integer with range [500, 50000]	Retrieves the duration in microseconds of the Multiple Turnover Flash relaxation phase.
$get packagecrc	8 digit hexadecimal integer	Retrieves the CRC-32 check sum of the instrument package configuration XML file.
$get para0	Floating point number	Retrieves the PAR sensor calibration coefficient *a 0 .*
$get para1	Floating point number	Retrieves the PAR sensor calibration coefficient *a 1 .*
$get parenabled	on | off	Retrieves whether the PAR sensor is enabled or not.
$get paraim	Floating point number	Retrieves the PAR sensor calibration immersion coefficient.
$get pressureenabled	on | off	Retrieves whether the pressure sensor is enabled or not.
$get processstf	on | off	Retrieves whether or not the STF induction is processed when collecting profiles.

$get profiledelay	Integer with range [0, 1000]	Retrieves the delay in milliseconds between the individual iterations of a fluorescence profile collection.
$get sequencecrc	8 digit hexadecimal integer	Retrieves the CRC-32 check sum of the profile sequence configuration XML file.
$get serialnumber	Integer between 1 and 9999	Retrieves the FIRe instrument serial number.
$get serialport	rs232 | rs485	Retrieves the serial communication channel to be used by the FIRe instrument the next time it is powered on.
$get startupstatus	[STATUS] where STATUS is either GOOD or any combination of MEDIACARD, INSTRCONFIG, SEQUENCE	Retrieves the startup status of the FIRe instrument. Indicates whether the media card is missing, whether the instrument config XML file is missing, and whether the sequence file is missing/corrupt.
$get stflen	Integer with range [1, 1000]	Retrieves the duration in microseconds of the Single Turnover Flash induction phase.
$get storeprocessed	on | off	Retrieves whether or not processed data is stored on board the FIRe instrument. Has no effect if processing is not enabled.
$get storeraw	on | off	Retrieves whether or not raw data is stored on board the FIRe instrument.
$get stri	Integer with range [5, 200]	Retrieves the initial interval in microseconds between the pulses of the Single Turnover Flash relaxation phase.
$get strp	Integer with range [500, 50000]	Retrieves the duration in microseconds of the Single Turnover Flash relaxation phase.
$get time	Integer greater than or equal to 0	Retrieves the FIRe instrument clock time representing the number of seconds since 00:00:00 1 Jan 1970 UTC.
$get transmitprocessed	on | off	Retrieves whether or not processed data is transmitted from the FIRe instrument during data acquisition. Has no effect if processing is not enabled.

$get transmitraw	on | off	Retrieves whether or not raw data is transmitted from the FIRe instrument during data acquisition.
$get version	String	Retrieves the FIReOS software version.

Data Commands

The *$data* directive provides commands for collecting, listing, downloading, uploading, and deleting data, blanks, and metadata.

Command	Parameter	Description
$data blank	none	Requests the FIRe instrument to collect a blank profile.
$data delete	filename	Requests the FIRe instrument to delete the specified file from the telemetry data directory.
$data deleteblank	filename	Requests the FIRe instrument to delete the specified blank profile file.
$data deletefiles	none	Requests the FIRe instrument to delete all telemetry data files. Note this does not delete any blank profile files.
$data deletelog	filename	Requests the FIRe instrument to delete the specified log file.
$data deleteprocessed	filename	Requests the FIRe instrument to delete the specified processed telemetry file.
$data deleteraw	filename	Requests the FIRe instrument to delete the specified raw telemetry file.
$data deletesequence	none	Requests the FIRe instrument to delete the profile sequence configuration file.
$data downloadblank	filename	Requests the FIRe instrument to download the specified blank file.
$data downloadlog	filename	Requests the FIRe instrument to download the specified log file.
$data downloadprocessed	filename	Requests the FIRe instrument to download the specified processed telemetry file.
$data downloadraw	filename	Requests the FIRe instrument to download the specified raw

		telemetry file.
$data downloadsequence	none	Requests the FIRe instrument to download the profile sequence configuration file.
$data downloadxml	none	Requests the FIRe instrument to download the instrument package XML configuration file.
$data listblank	none	Retrieves a listing of the blank profile files on the FIRe instrument.
$data listfiles	none	Retrieves a listing of all files in the telemetry data directory on the FIRe instrument.
$data listlog	none	Retrieves a listing of the log files on the FIRe instrument.
$data listprocessed	none	Retrieves a listing of the processed telemetry files on the FIRe instrument.
$data listraw	none	Retrieves a listing of the raw telemetry files on the FIRe instrument.
$data profile	none	Requests the FIRe instrument to collect a single fluorescence profile.
$data run	none	Requests the FIRe instrument to start collecting data continuously.
$data transmit	none	Requests the FIRe instrument to download all telemetry data files. This includes all raw, processed, and blank profile files.
$data uploadpatch	filename crc32checksum	Requests the FIRe instrument to upload the specified patch file. If the CRC32 checksum of the uploaded file does not match the specified one, an error response is returned.
$data uploadsequence	none	Requests the FIRe instrument to upload a profile sequence configuration file.
$data uploadxml	filename	Requests the FIRe instrument to upload an instrument package XML configuration file with the specified file name.

Action Commands

The following action commands control the mode and operating state of *In Situ* FIRe.

Command	Description
$bk	Requests the FIRe instrument to go to *commanded* mode.
$qt	Requests an exit of the FIReOS software running on the FIRe instrument.
$rb	Requests a reboot of the operating system on the FIRe instrument.
$sd	Requests a shut down of the operating system on the FIRe instrument.

Index