Chelsea Technologies Group

FastBallast System Handbook

Includes: Operating instructions for the FastBallast system
Installation and operating instructions for FaBtest software (version 3.x)

EDNS: 2355-001-HB

Authored by:	Kevin Oxborough
Checked by:	Toby Boatman
Approved by:	Kevin Oxborough

Issue G

Changes from the last release

This version of the handbook references the production version of FastBallast and FaBtest version 3.x.

Contents

Introductory section

1.1 Scope

This handbook references the production version of the FastBallast Compliance Monitor, as shown right. It is important that the included Panasonic Toughpad is stored with the screen facing into the lid (as shown) when the lid of the FastBallast unit is closed. This provides maximum protection for the Toughpad screen.

The production version of FastBallast can only be used with FaBtest V3.x.

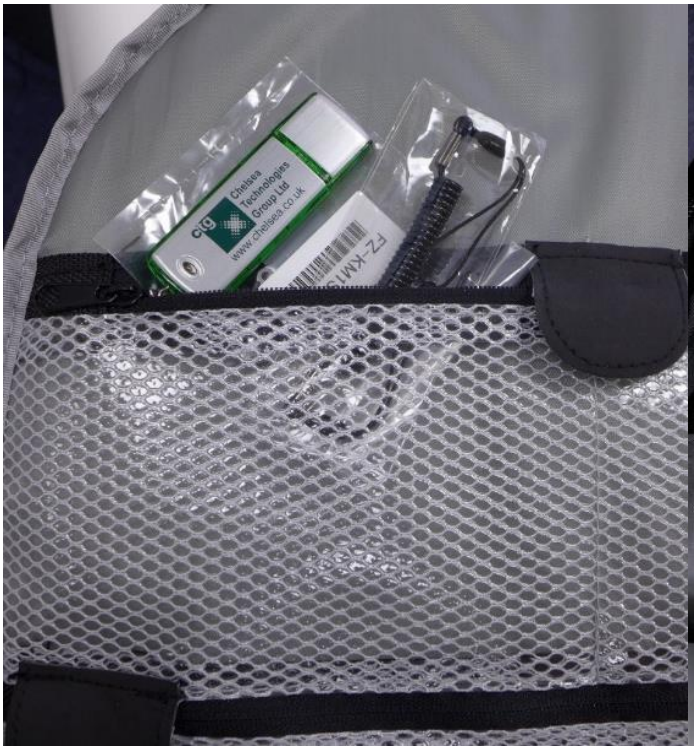
1.2 Parts list check

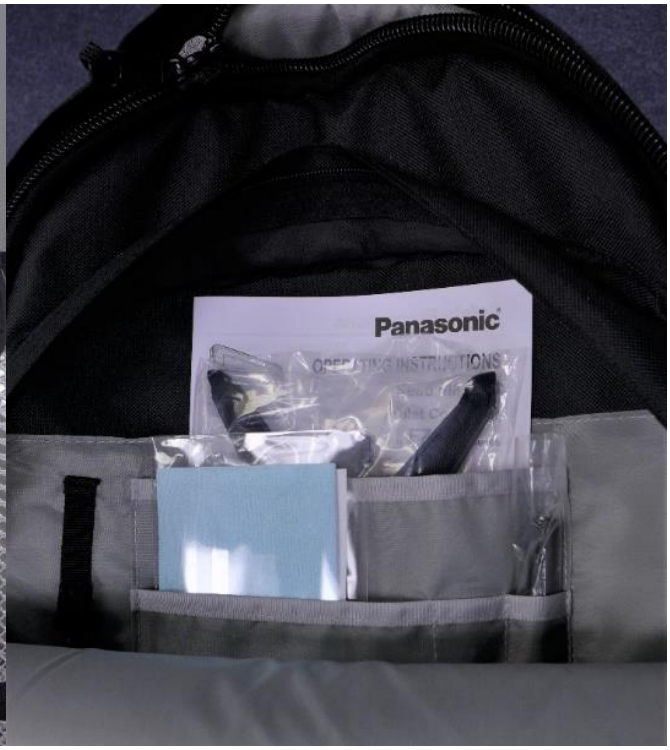
The FastBallast system delivery pack should include the following:

- Backpack (113920)
- The main compartment of the backpack should contain:
  - FastBallast unit (2355-130-PL-A) containing:
    - FastBallast stir unit (2355-138-PL-A)
    - Panasonic ToughPad with FaBtest installed (2355-149-PL-A)
  - FastBallast charger with 15 V at 1.2 A output (2355-137-PL-A)
  - FastBallast to USB cable (2355-085-PL-A)
  - 40 x 20 mL sample cups (119125)
  - 2 x 350 mL empty bottles for tap water (120021)
  - 6 x 3 mL Pasteur pipettes (925791264)
  - 12 x 1 mL Pasteur pipettes (925791271)
  - 2 x 30 mL syringes (120020)
- The inner front panel of the backpack should contain:
  - USB key containing electronic documentation and FaBtest installation (2355-151-PL-A)
  - Small parts for the Panasonic ToughPad (2355-149-PL-A)
  - 6 x spare screws for the stir unit (113951)
- The outer front panel of the backpack should contain the following Panasonic ToughPad parts:
  - Operating instructions (2355-149-PL-A)
  - Soft cloth (2355-149-PL-A)
  - Grab handle (2355-149-PL-A)
  - Stylus (2355-149-PL-A)
- The following parts should be included within the shipping case, but outside the backpack:
  - FastBallast Ethernet cable (2355-086-PL-A)
  - FastBallast charger UK power mains lead (111002)
  - FastBallast charger EU power mains lead (111003)
  - FastBallast charger US power mains lead (111004)
  - Panasonic ToughPad UK power mains lead (2355-149-PL-A)
  - Panasonic ToughPad EU power mains lead (113948)
  - Panasonic ToughPad US power mains lead (113949)
  - Quick start guide (2355-001-HB)

Inner compartment of the backpack (Pasteur pipettes and 30 mL syringes not shown)

Inner and outer pockets of the backpack





1.3 Targeted test requirements

It has long been accepted that ballast water functions as a vector for the transfer of harmful species. In an attempt to prevent such transfer, the U.S. Coast Guard Ballast Water Discharge Standards (USCG BWDS) are currently being implemented and will be applied to all ships visiting U.S. waters by December 1st, 2016. These standards (summarized in Table 1) are largely consistent with the 'International Convention for the Control and Management of Ships' Ballast Water and Sediments' (IMO, 2004). This convention has now been ratified and will be enforced on September 8th 2017.

Division of the USCG BWDS		Limit (SI units)	Limit (BWDS units)
1	Living cells greater than or equal to 50 µm in the minimum dimension	< 10 cells m^{-3}	< 10 cells mL^{-1}
2	Living cells greater than or equal to 10 µm and less than 50 µm in the minimum dimension	< 1 x 10^7 cells m^{-3}	< 10 cells mL^{-1}
3i	Toxicogenic *Vibrio cholerae* (O1 and O139)	< 1 x 10^4 cfu m^{-3}	< 1 cfu 100 mL^{-1}
3ii	*Escherichia coli*	< 2.5 x 10^6 cfu m^{-3}	< 250 cfu 100 mL^{-1}
3iii	Enterococci	< 1 x 10^6 cfu m^{-3}	< 100 cfu 100 mL^{-1}

Table 1.1: Summary of the U.S. Coast Guard Ballast Water Discharge Standards (2012).

Although zooplankton and phytoplankton are both represented within Division 1 and Division 2, it is generally the case that zooplankton dominate the former while phytoplankton dominate the latter. FastBallast specifically targets Division 2 of the USCG BWDS by interrogating chlorophyll fluorescence emission from photosynthetically active phytoplankton. Throughout this document, the PASS/FAIL threshold for a FastBallast test should be taken to mean 10 phytoplankton cells between 10 µm and 50 µm in the smallest dimension.

1.4 The FastBallast system

1.4.1 Summary of advantages

- Provides rapid, on board compliance testing
- Detection limit of < 1 cell/mL
- Does not depend on an assumed level of fluorescence per cell
- Sampling issues associated with analyzing small static volumes at close to the D2 threshold are overcome by using a larger stirred volume of 20 mL
- Very low level of false negatives and negligible possibility of false positives
- Wide dynamic range provides a high tolerance of background fluorescence (from dead cells, CDOM and other sources)
- High level of turbidity rejection
- Minimal consumables and no sample preparation required
- Long service intervals (two years)

1.4.2 In more detail

FastBallast is an ultra-sensitive active chlorophyll fluorometer system designed to allow for rapid assessment of the density of living phytoplankton cells within ballast water discharge. It is a highly portable device, providing 8 h operation on a single battery charge. Its uncompromising design provides the resolving power and dynamic range to detect fluorescence from living phytoplankton cells at well below the density allowed within Division 2 of the USCG BWDS standards, even in the presence of a high level of fluorescence from other sources.

The FastBallast system incorporates four LED arrays to excite chlorophyll and other light harvesting pigments within phytoplankton cells. The emission wavebands for these LEDs are centred at 455, 470, 530 and 624 nm (royal blue, blue, green and orange-red). This combination not only allows for optimisation of LED spectral output to the current regulations but also provides the capacity to adapt to possible future changes: for example, a decrease in the 10 µm lower limit of Division 2 to include the cyanobacteria.

The fluorescence emission stimulated by light from the LEDs is detected within a waveband centred at 682 nm (deep red). This waveband is very closely matched to the variable fluorescence emission from functional photosystem II (PSII) complexes within phytoplankton, which provides the best indication of cell vitality.

FastBallast employs a variant of the so-called Single Turnover (ST) method, which involves the application of 400 microsecond pulses from the excitation LEDs to saturate photosynthesis. Most alternative systems use the Multiple Turnover (MT) method which applies pulses of several hundred milliseconds. The ST method is more difficult (and more expensive) to implement than MT-based methods. However, the thousand-fold decrease in pulse duration provided by ST allows for a dramatic increase in sampling rate. As a consequence, the signal to noise (S:N) ratio of ST-based systems is orders of magnitude higher than MT-based systems. With FastBallast, this S:N ratio advantage is pushed further through optimisation of:

- The optical configuration
- The LED drive system
- The measurement protocol
- The algorithm used to analyse primary data

The overall result is a level of sensitivity that allows for the detection of individual phytoplankton cells, even at the low end of the 10 – 50 µm size range.

The sample chamber within FastBallast holds 20 mL of sample. The interrogated volume, from which the fluorescence emission is recorded at any point during a test, is 0.5 mL (2.5% of the total sample volume). Because the ST pulses applied by FastBallast are so short, it is possible to take reliable measurements as the incorporated stir unit continuously exchanges the interrogated volume. The ST measurement pulses are applied at 40 Hz throughout a test, with 40 consecutive pulses being averaged to generate a data point (an acquisition) every second. The stir speed is slow enough to provide a low level of exchange during each acquisition but fast enough to ensure that the entire 20 mL volume is interrogated over the duration of the test. This setup:

- Eliminates potential sampling errors at cell densities close to the PASS/FAIL threshold
- Allows for a cell size-independent estimation of cell density using a proprietary data analysis algorithm

A more detailed discussion of active chlorophyll fluorometry and the analysis methods embedded within FastBallast is provided within Background information.

2 Operation of the unit

2.1 First steps

If the battery within the FastBallast unit is completely discharged, it should be charged for at least 30 minutes before use.

A

Open the lid.

B

Pull out the stir unit.

C

Slide the stir unit collar from the storage position to the test position (all the way through the J grooves on the side of the unit).

D

Fill a measuring cup up to the bottom of the rim grooves with sample (20 mL).

E

Pour into the sample chamber.

F

Install the stir unit within the sample chamber and plug it into the **Stirrer** socket.

G

Remove the Panasonic ToughPad from the lid, turn it on and log in.

The ToughPad can be kept within the lid or hand-held while a test is running.

H

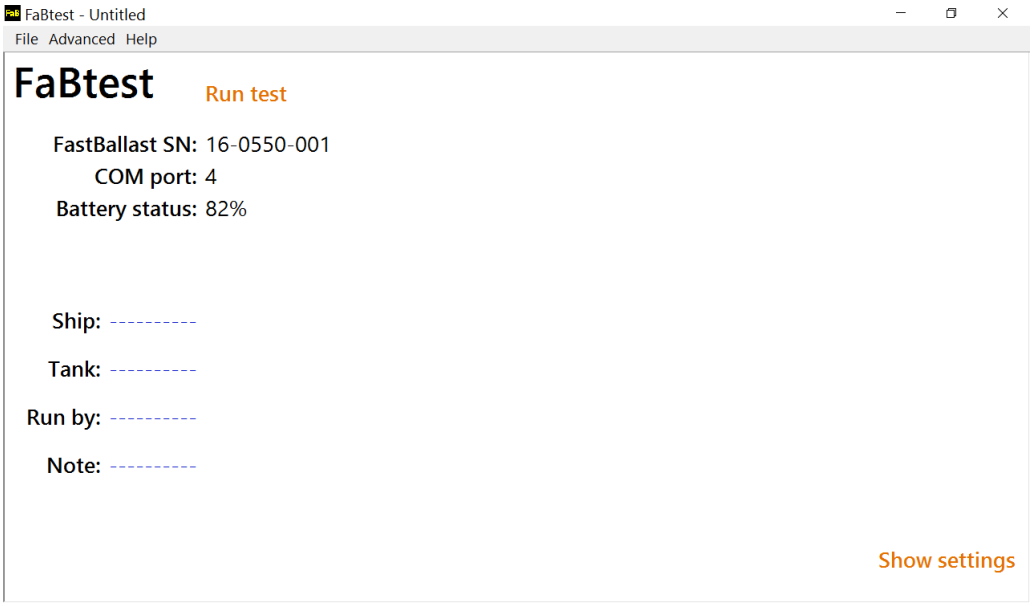
Turn the FastBallast on by pressing the power button on the top panel. Wait 10 s before starting FaBtest. The FastBallast should attach within half a minute. The Bluetooth connection is normally effective to at least 10 m with a clear line of sight.

I

If the FastBallast doesn't attach to FaBtest, make sure that the power button is showing a blue light.

If the blue light isn't showing, the FastBallast unit probably needs charging.

If the blue light is showing, follow the instructions provided within Bluetooth problems.



2.2 The test

Depending on the sample, a test will run for under two minutes (level 1) or just over eight minutes (level 2).

FaBtest will automatically turn the FastBallast off once a test has completed or if a test is halted by the user.

- A level 1 test reports a PASS or a FAIL and an accompanying numeric
  - A PASS will always have a numeric of no more than 0.04
  - A FAIL will always have a numeric of at least 40
- If the numeric is greater than 0.04 and less than 40 at the end of a level 1 test, FaBtest will jump straight into a level 2 test
  - The boundary between a level 2 PASS or FAIL is a numeric of 1.0

The first test shown here is a blank test conducted using tap water (Tap water test). This is a good test to apply between ballast water samples as it ensures that there is no carry over of cells between tests.

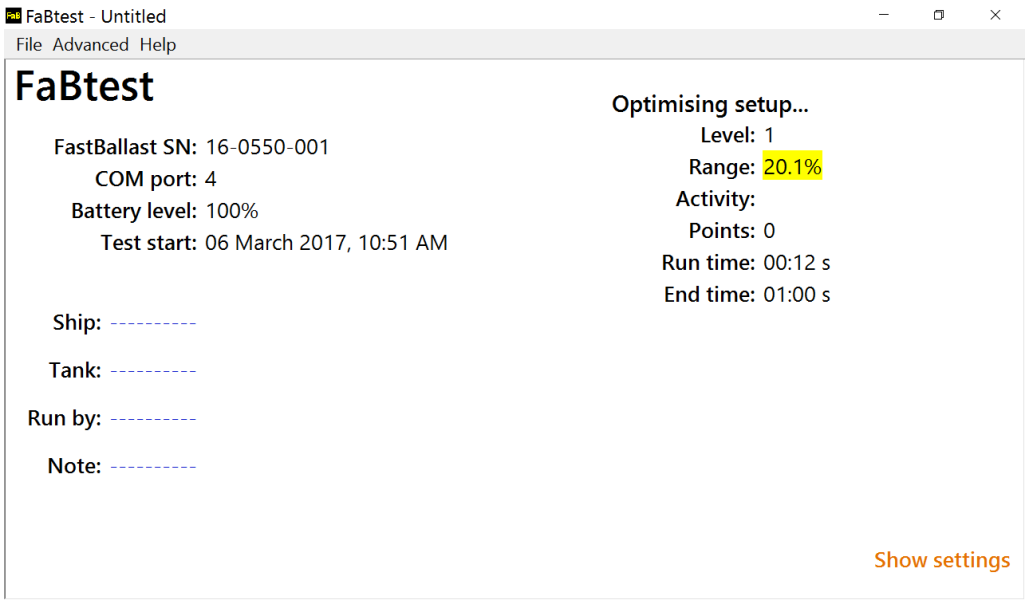
The second test is with a suspension of living phytoplankton cells (Live cells test).

Tap water test – A

Initially, FaBtest will optimise the setup to generate a **Range:** value of 30 – 100%.

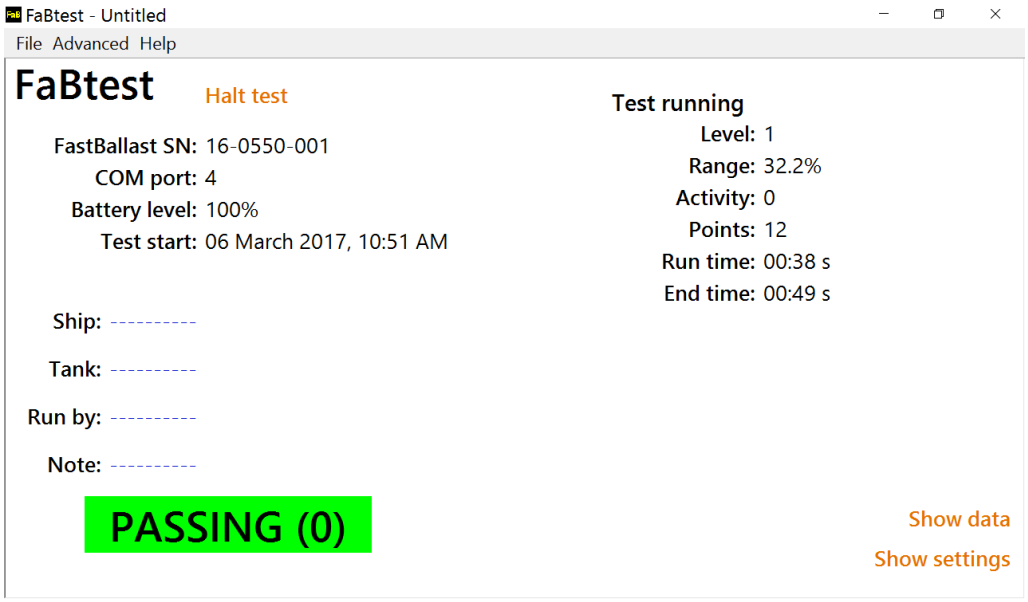
In the screenshot shown right, the range value has a yellow background because it is below 30%.

A range value of more than 100% is shown with a red background.



Tap water test – B

Following optimisation, FaBtest reports the current PASSING or FAILING status with a numeric. The run time and time until the end of the test are also shown.

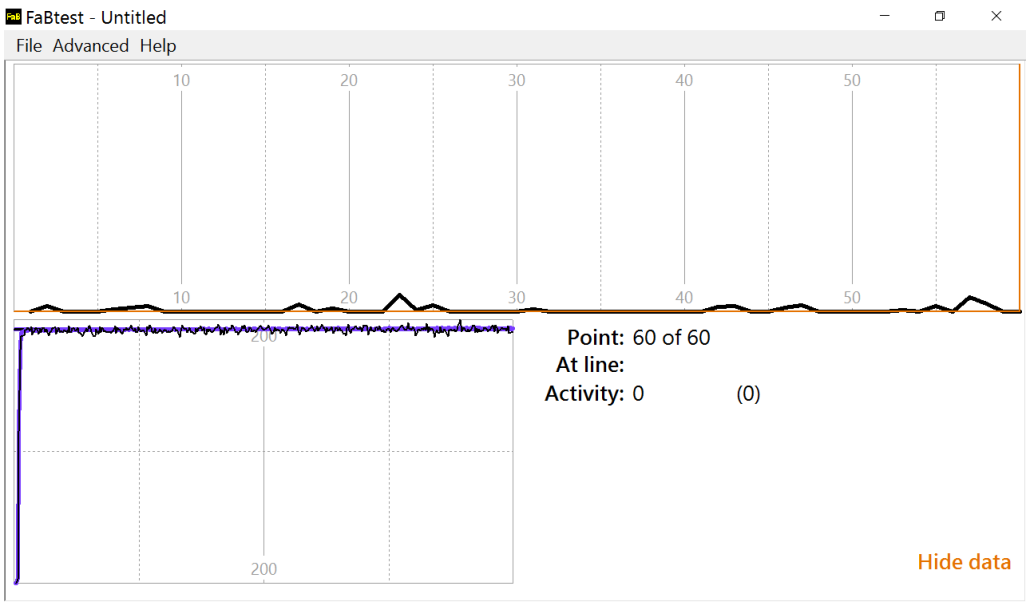


Tap water test – C

Pressing the Show data button switches to the data window.

Tap water test – D

Pressing the Hide data button switches back to the main window.

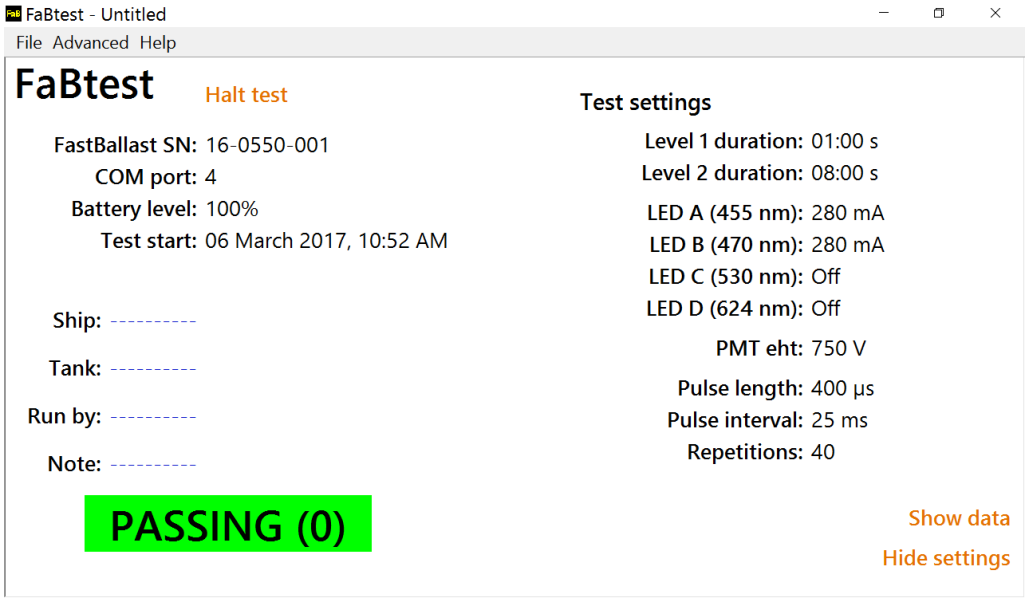


Tap water test – E

Pressing the Show settings button switches to the settings window.

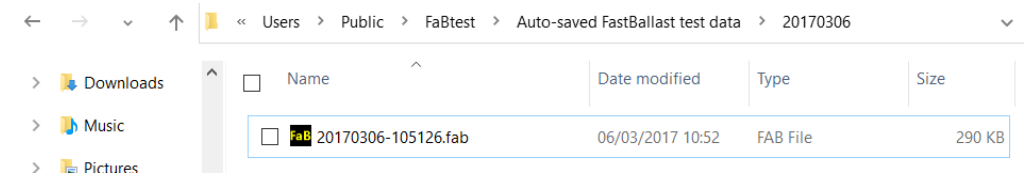
Tap water test – F

Pressing the Hide settings button switches back to the main window.



Tap water test – G

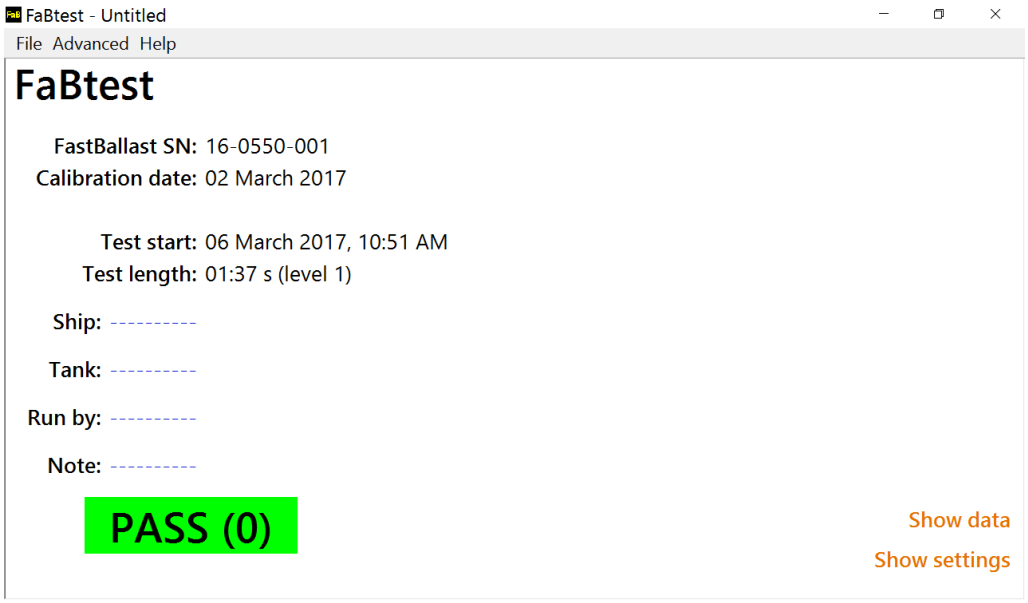
The test data are automatically archived within a timestamped file (14th December 2016 at 15:05:52 in this instance). The sub-folder name is the test date. A new sub folder is automatically generated each day that a test is run.



Tap water test – H

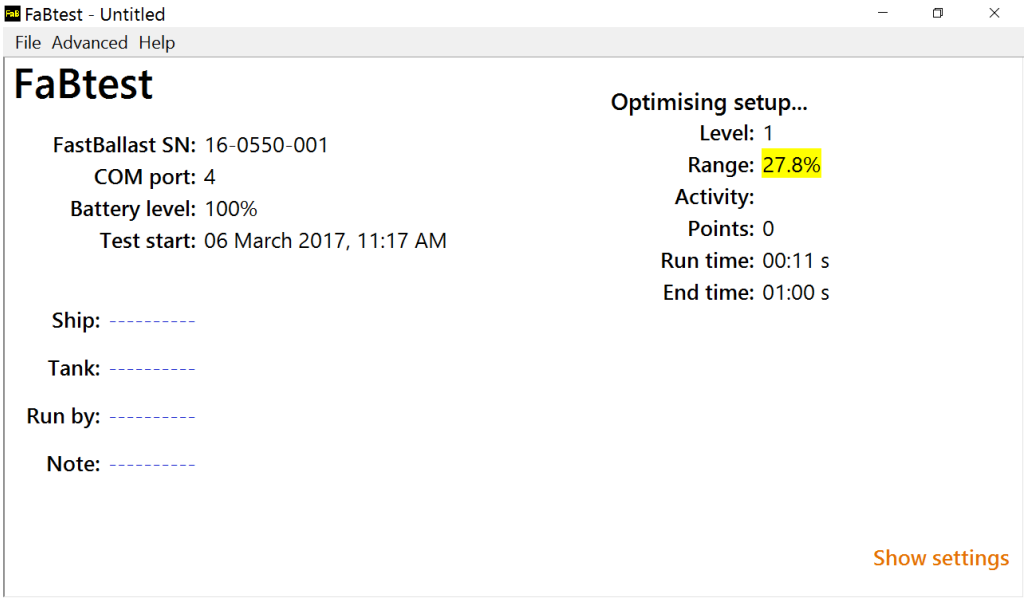
This timestamped file includes all primary data from the test.

In this instance, the test result clearly indicates that the sample chamber and stir unit are not contaminated with living phytoplankton cells.



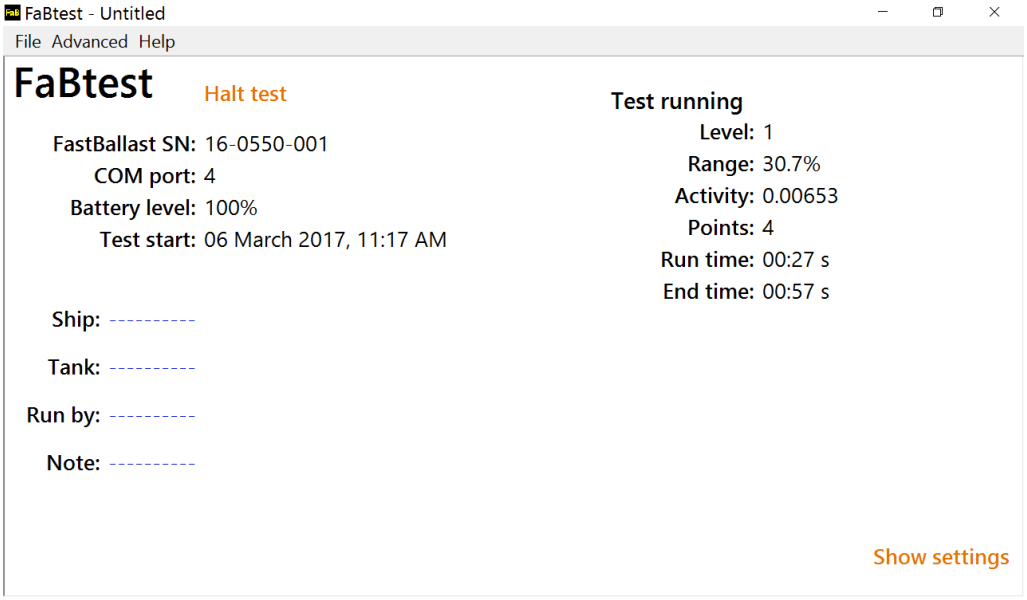
Live cells test – A

Initially, FaBtest will optimise the setup.



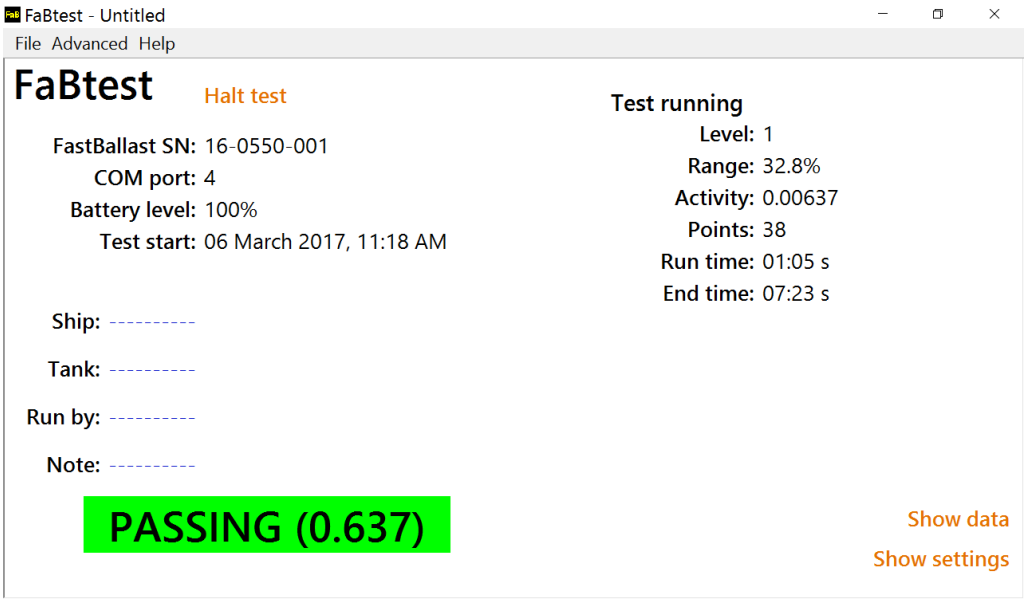
Live cells test – B

For the first few points after optimisation, FaBtest will report an end time that is consistent with a level 1 test (one minute or less by default).



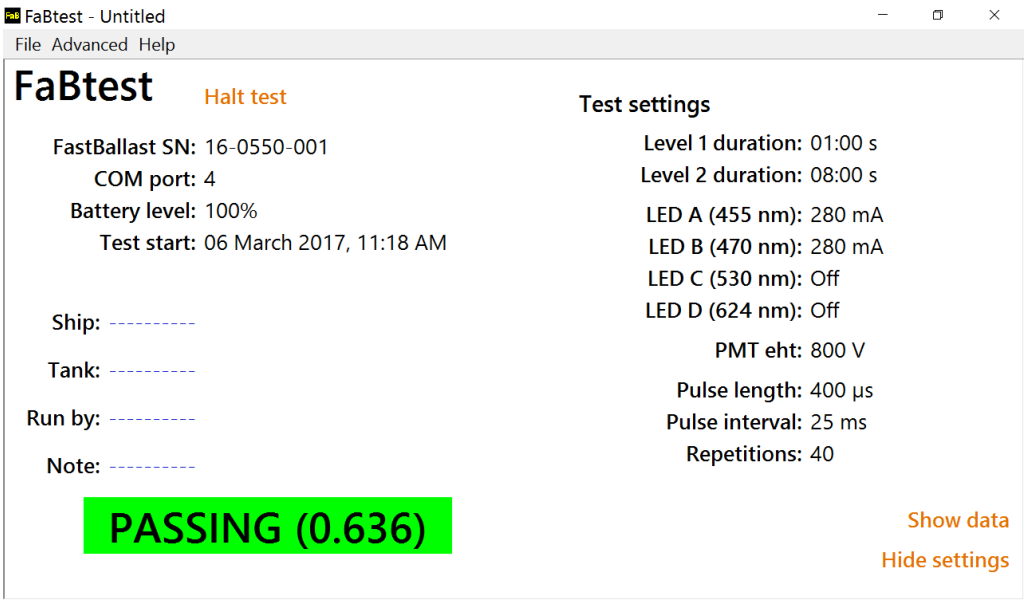
Live cells test – C

Once the number of points acquired passes 10, the end time may increase to reflect a level 2 test time. However, the switch to level 2 won't happen until at least 120 points have been acquired. In this case, the level 1 test analysis is reporting a PASSING result with a numeric of 0.637.



Live cells test – D

Pressing the Show settings button switches to the Test settings window. This only changes the right hand column.



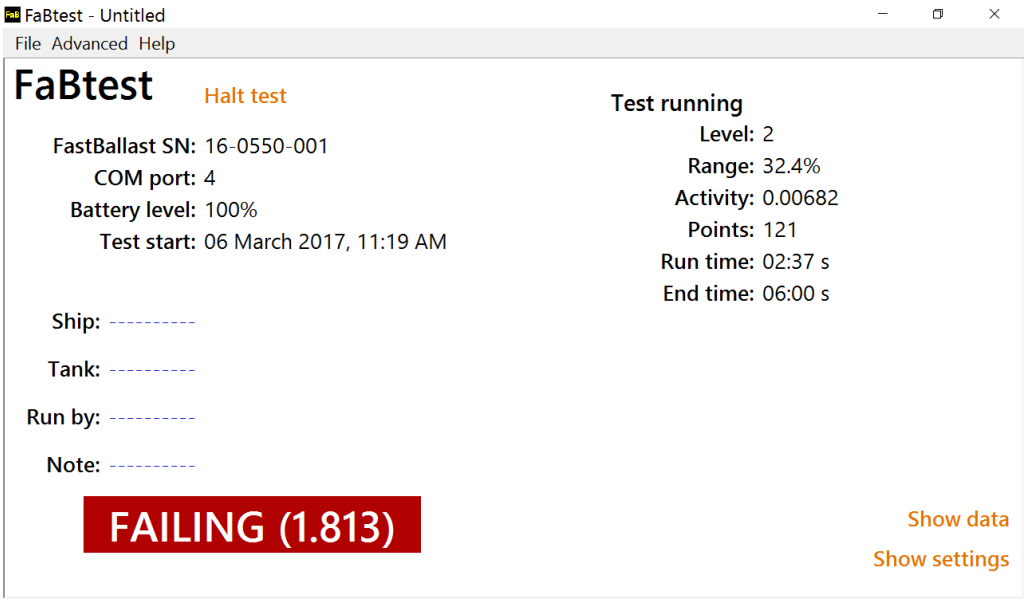
Live cells test – E

Pressing the Hide settings button switches back to the main window.

Live cells test – F

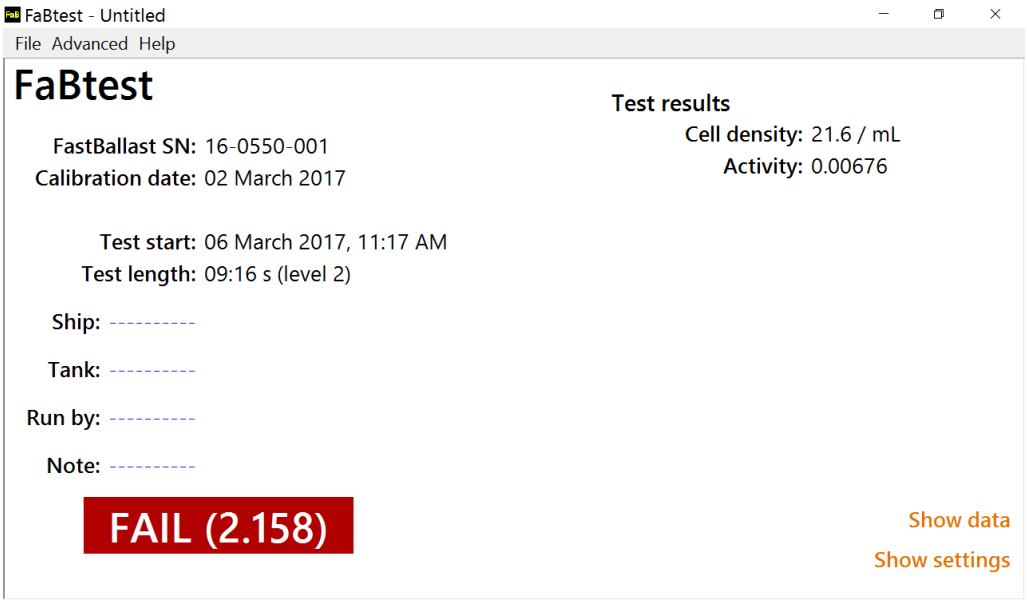
Once the points count has reached 120, FaBtest will report at level 2. In this case, FaBtest has gone from a PASSING result at level 1 to a FAILING result at level 2.

The difference between a level 1 and level 2 test is explained in detail within Interpretation of test data.



Live cells test – G

At completion of a level 2 test, FaBtest reports a PASS or FAIL plus an estimate of cell density. In this case, the result is a FAIL with an estimate of 21.6 cells / mL (2.16 times the PASS/FAIL threshold for division 2 of the IMO / USCG standards).



2.3 After the test

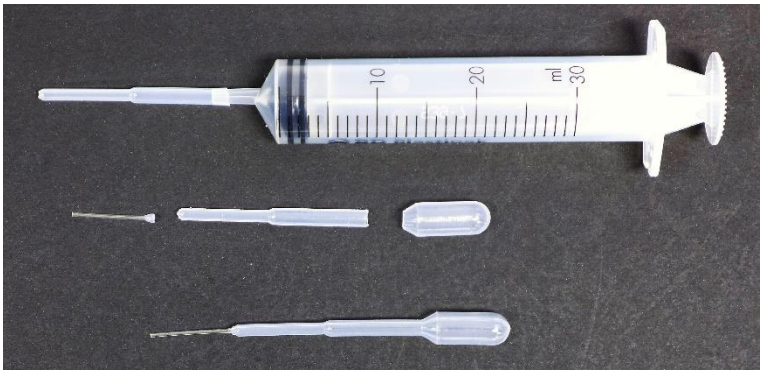
FaBtest will automatically turn the FastBallast unit off once a test has completed, to save battery life.

To prepare for the next test:

A

Remove the stir unit and empty the sample chamber using a 30 mL syringe with cropped 1 mL Pasteur pipette installed, as shown right.

As an alternative to the syringe, a piece of kitchen towel can be used to soak up the sample.



B

Wash the sample chamber out with tap water at least twice, using the 30 mL syringe or kitchen towel to empty the chamber each time.

C

Dry the chamber out with a piece of kitchen towel.

D

Wash the stir unit with tap water and dry with a piece of kitchen towel.

3 Routine checks

3.1.1 Battery check

The FastBallast unit includes a fuel gauge, which provides an estimate of battery charge remaining. The current battery status is reported by FaBtest…

FaBtest Run test

FastBallast SN: 16-0550-001
COM port: 4
Battery status: 77%

A 100% charge will provide at least 8 h operation.

A full charge requires approximately 6 h, whether or not the unit is switched on.

3.1.2 Consumables

The 20 mL sample cups provide the correct volume for a sample. These can be washed and re-used many times.

Kitchen towel is ideal for soaking up the sample once a test has completed. However, the provided syringe can also be used to empty the sample chamber (see step-by-step instructions).

Tap water is ideal for flushing out the sample chamber between tests and running as a blank test. Suitable bottles are provided within the storage bag, for situations where tap water is not going to be available on-site. If these bottles are used, the tap water should be refreshed each day.

4 FaBtest

FaBtest is a Windows desktop application for controlling FastBallast. A copy of FaBtest is installed on the Panasonic Toughpad supplied as part of the FastBallast system. Installation files for FaBtest are also included on the USB key supplied as part of the FastBallast system. FaBtest can be installed on most Windows computers running 32-bit or 64-bit versions of Windows 10.

System requirements for FaBtest are modest: it is extremely unlikely that any hardware capable of running Windows 10 will not also run FaBtest. Attaching FastBallast to the hardware running FaBtest requires a free USB 2.0 port or Bluetooth capability. When run, FaBtest will search all available COM ports and attach to the first FastBallast found. The required COM port can be provided by the Bluetooth device incorporated within FastBallast or physical connection through the USB port.

Most users will only ever use a very limited range of the functionality provided by FaBtest. Running a test will generally involve starting FaBtest, waiting a few seconds for the FastBallast to attach and then pressing the Run test button. FaBtest will optimise settings, run the most appropriate test (level 1 or level 2), report the result on screen and archive the test data within a timestamped file.

In addition to the highly automated test procedures, FaBtest provides low level control of FastBallast, including instrument calibration. It also incorporates a number of functions for visualisation, processing, archiving and export of FaBtest data.

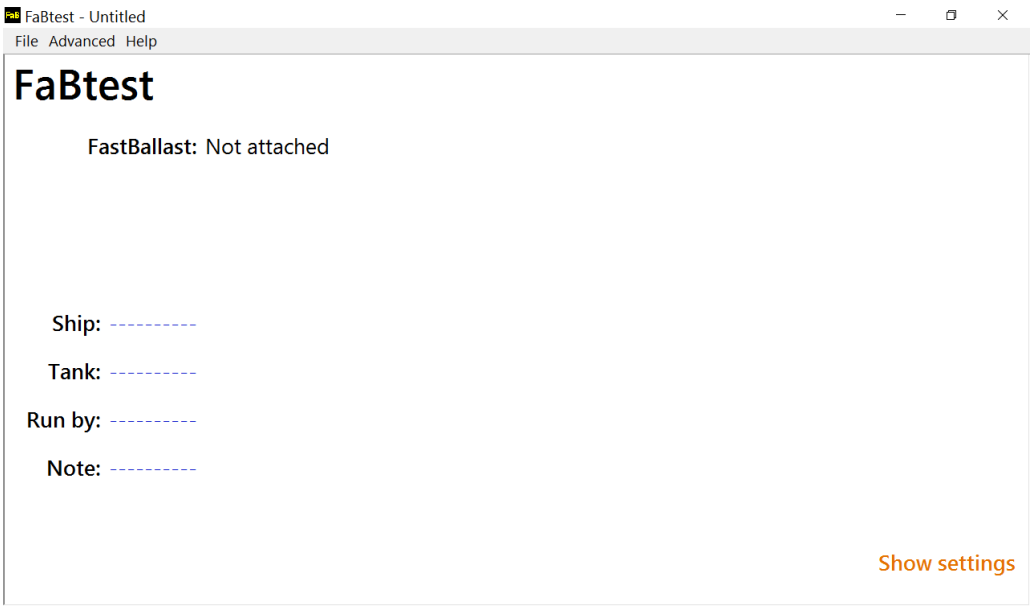
4.1 The home screen, settings screen and data screen

This section provides a basic introduction to the three screen views available in FaBtest. A more detailed description of the terminology used is provided within other sections of this handbook.

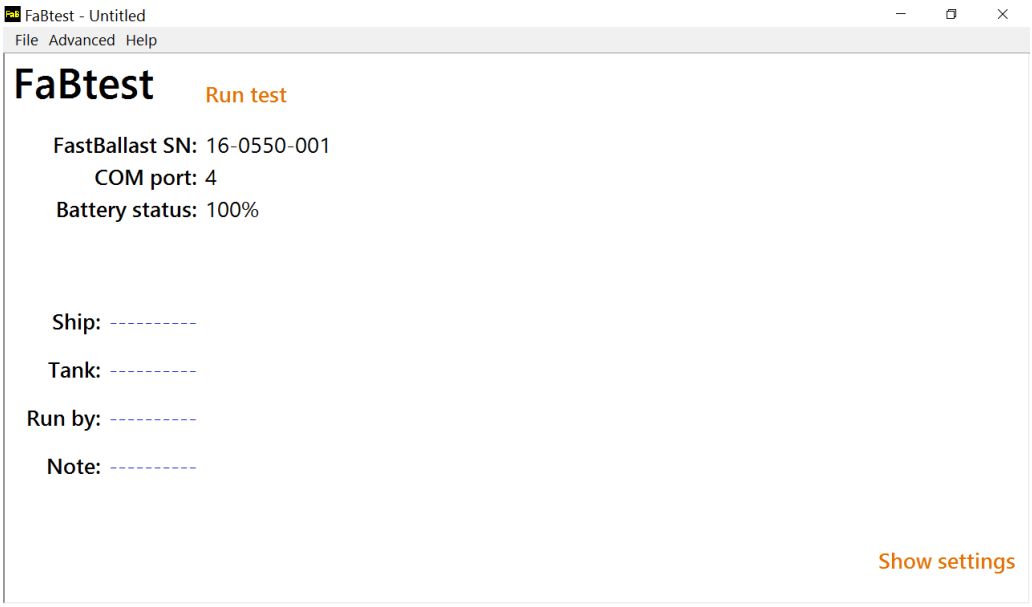
4.1.1 Home screen

The initial FaBtest home screen looks like this. The **Ship:**, **Tank:**, **Run by:** and **Note:** fields can be edited at any time by pressing the dashes after each label.

Text entered within these fields is saved with the file.

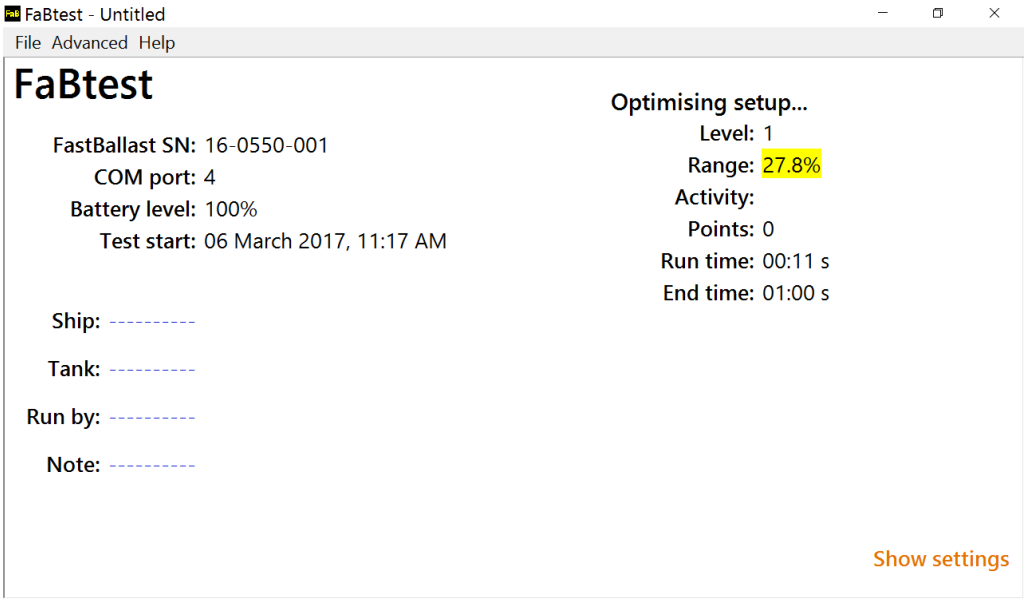


If there is a FastBallast unit available (through Bluetooth or USB cable), FaBtest will automatically connect to it. The first time FaBtest connects to a FastBallast, connection may take as much as 30 s. Subsequent connections to the same unit will generally be much quicker.



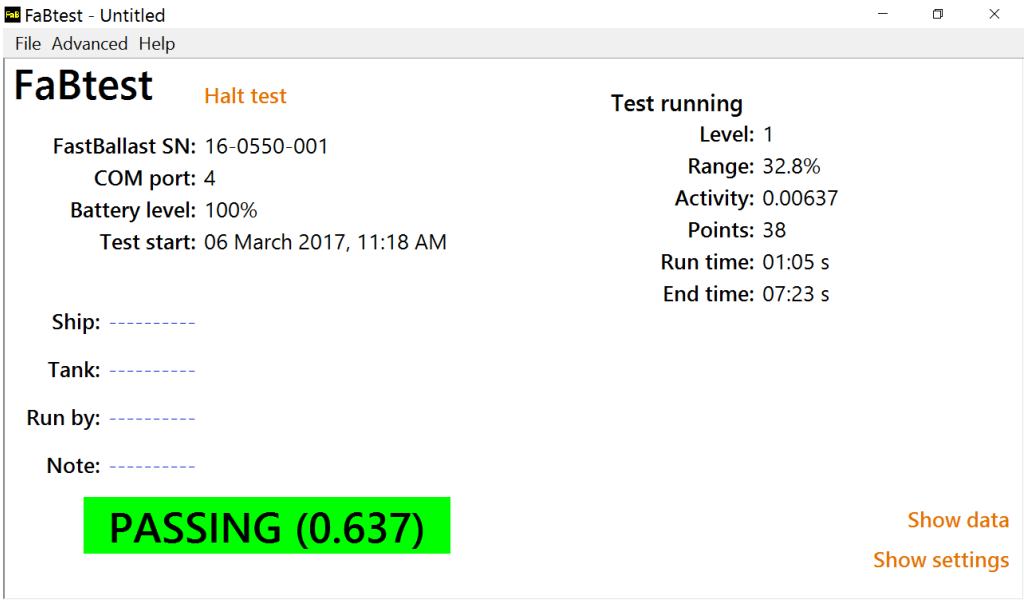
Pressing the Run test button starts a test. Over the first few seconds or tens of seconds, FaBtest will make adjustments to the settings to optimise the test setup.

The **End time** field will initially show the time set for a level 1 test.



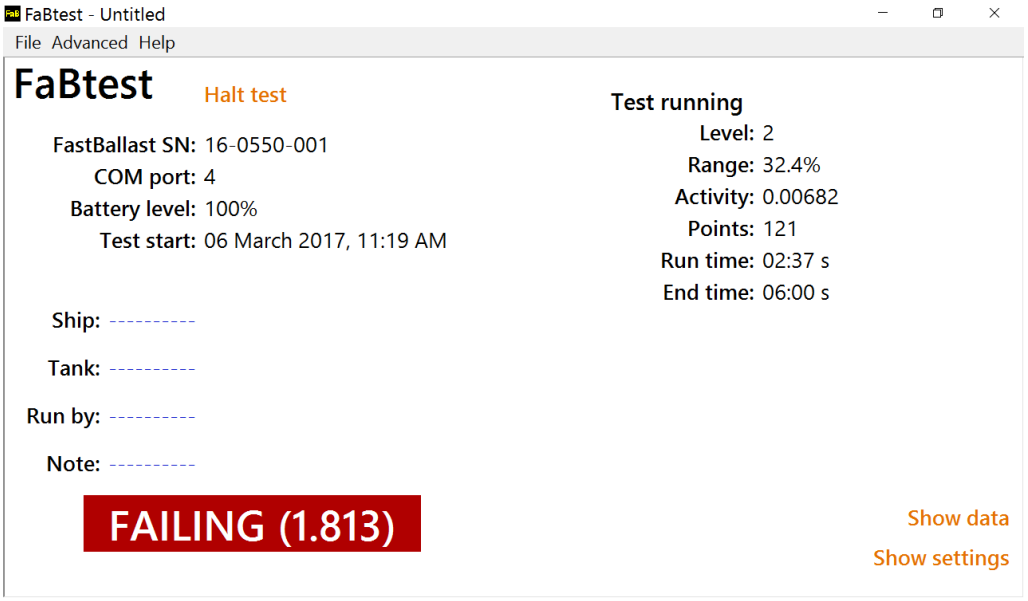
In this example, the test initially shows a PASSING result. However, as noted elsewhere, this initial (level 1) result is a simple function of the **Activity** parameter (variable fluorescence, F_v).

The **End time** field has now shows the level 2 test time. This is because the PASS/FAIL numeric is between 0.04 and 40.0 (see The level 1 test for details).

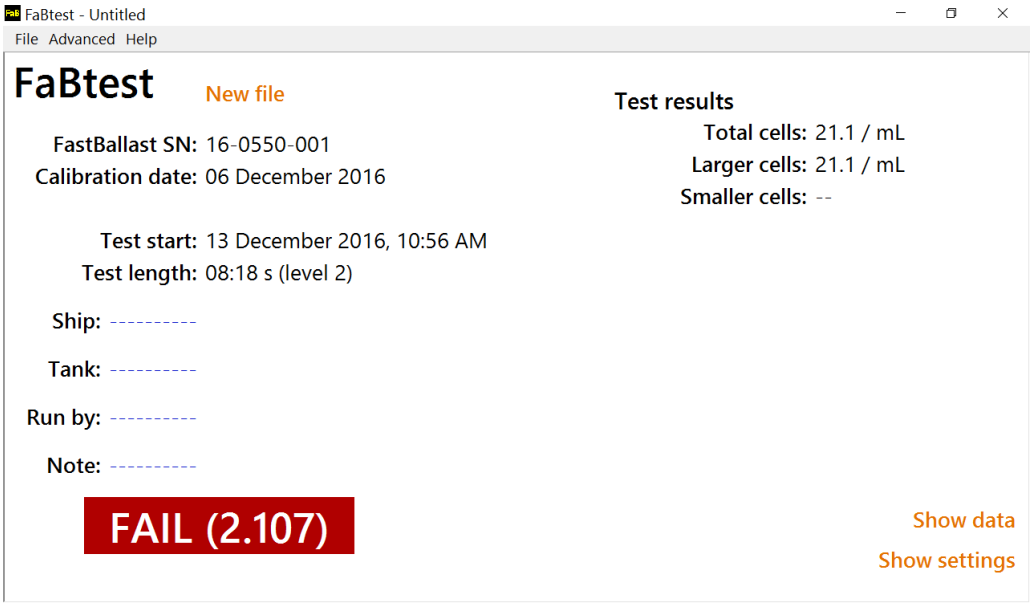


Although the need for a level 2 test can be established within the first few seconds, FaBtest will continue to report at level 1 until the **Points** count reaches 120.

In this case, the switch to level 2 has changed the PASSING to a FAILING and the PASS/FAIL numeric has increased by a factor of almost three.



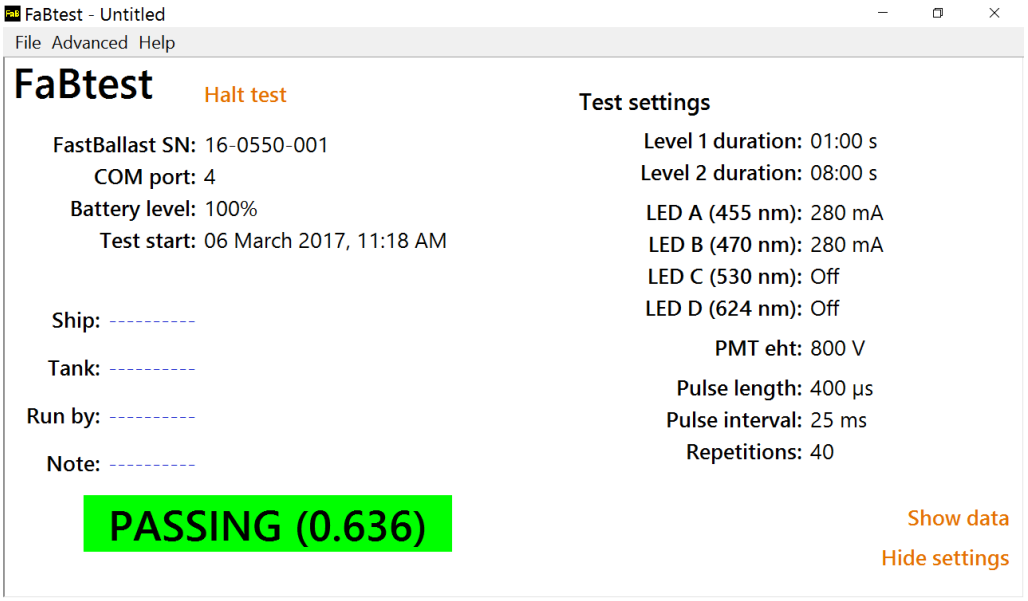
At the end of the test, the FAILING (or PASSING) changes to a FAIL (or PASS). Because this test ran to level 2, an estimate of cell density is also provided. The final Test length: of 08:18 s comprises 480 data points at 1 Hz plus 18 s optimisation time



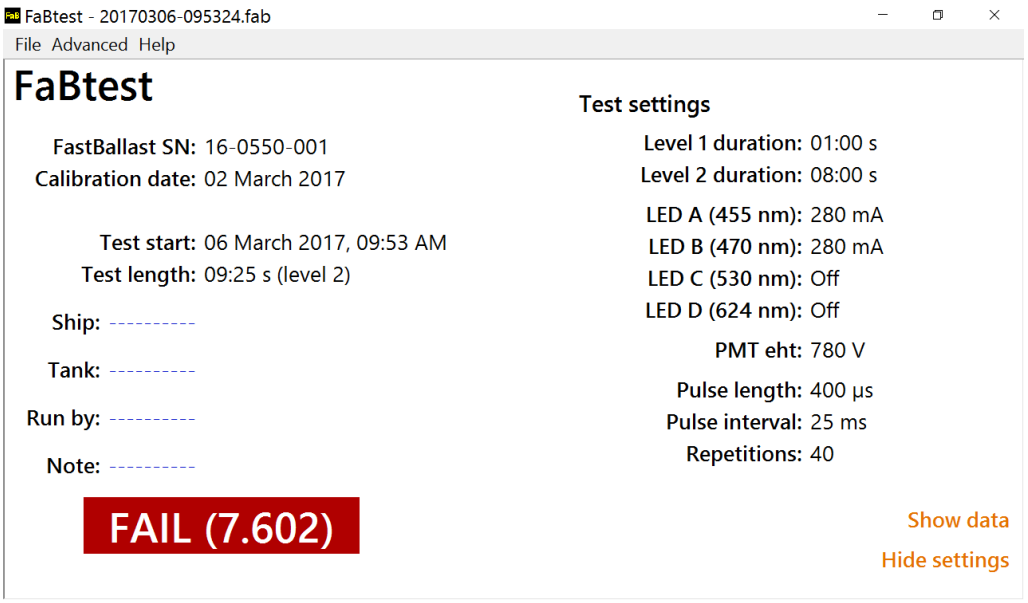
4.1.2 Settings screen

The settings screen can be reached by pressing the Show settings button on the home screen. Only the right of the screen changes, to show the **Test settings**.

The Hide settings button on the settings screen reverts to the home screen.

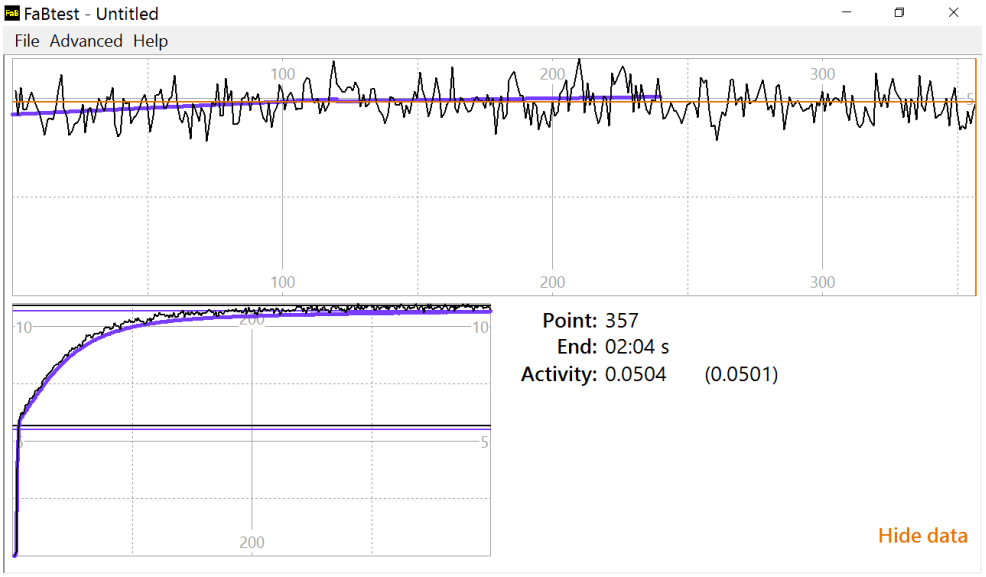


If a test has completed, the **Test settings** section shows the settings used throughout the test. The one setting that can change during the test is the **PMT eht**. The voltage shown is that used at the end of the test.



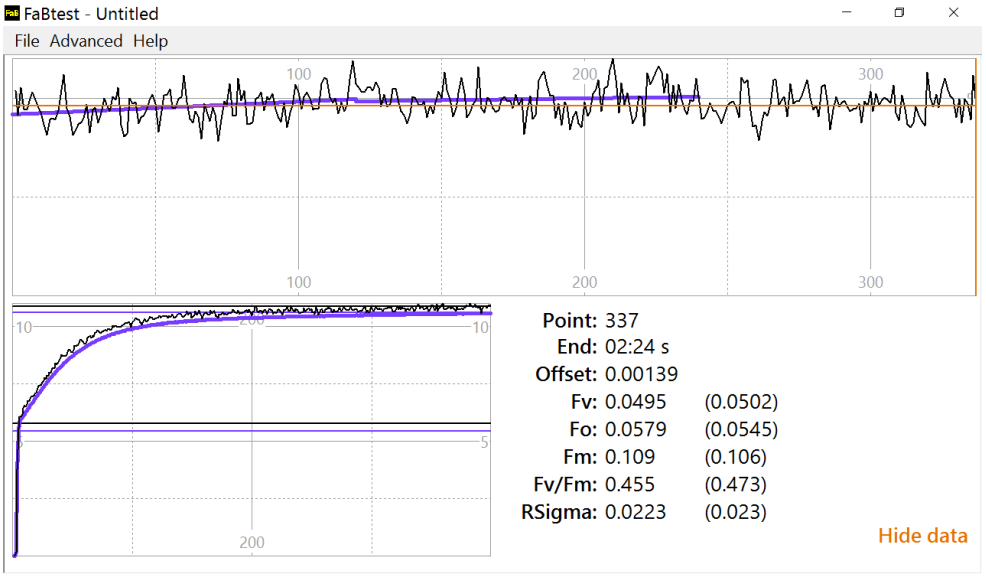
4.1.3 Data screen

The data screen is only available when the file being viewed has ten or more data points (acquisitions). It can be accessed by pressing the Show data button on the main screen or settings screen.



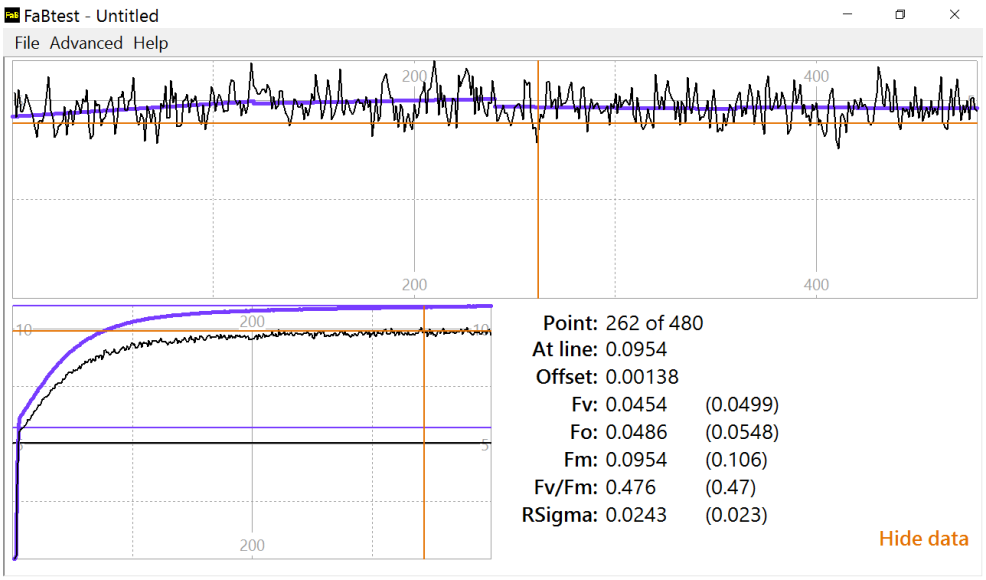
If **Advanced → Data / Show more** is selected from the menu, additional fluorescence parameters are shown on the data screen.

A detailed description of all reported parameters can be found within Additional fluorescence parameters reported by FaBtest.



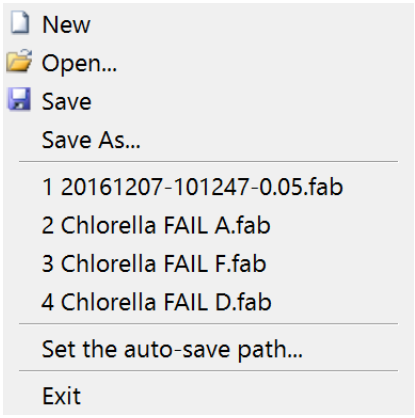
Once the test has completed, an individual acquisition can be selected within the top panel. The lower panel shows the selected acquisition (black line) with a mean of all acquisitions within the test.

See The level 2 test for details of how the F_v data within the top panel are analysed to generate an estimate of cell density.



4.2 Menus

4.2.1 File menu

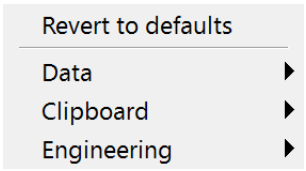


The **File** menu incorporates the basic file options (**New**, **Open…**, **Save** and **Save As…**) plus a list of the most recently opened FaBtest data files.

The **Set the auto-save path…** option allows the end user to change the default path for auto-save files.

The **Exit** option closes FaBtest.

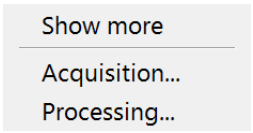
4.2.2 Advanced menu



The **Revert to defaults** option resets all data acquisition and data processing settings to the default values. This option can safely be used to troubleshoot the system during testing.

All other functions within the **Advanced** section are provided for use by systems engineers and specialist users. None of these functions are required for running standard tests.

4.2.2.1 Advanced → Data

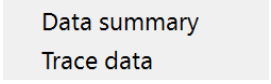


When the **Show more** option is checked, additional parameters are shown on the **Home screen** and **Data screen**.

The **Acquisition…** option opens the Data acquisition dialog.

The **Processing…** option opens the Data processing dialog.

4.2.2.2 Advanced → Clipboard

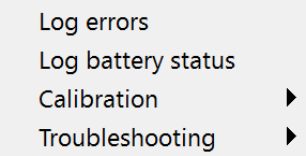


All four functions copy data to the Windows Clipboard in a format that can be pasted directly into a spreadsheet-based application (such as Excel) or text editor (such as Notepad).

The **Data summary** option constructs a very detailed data set, including all settings and calibration values.

The **Trace data** option formats the data points for all acquisitions within the file.

4.2.2.3 Advanced → Engineering



When the **Log errors** option is checked, FaBtest will write errors generated while communicating with the attached FastBallast unit to this logging file:

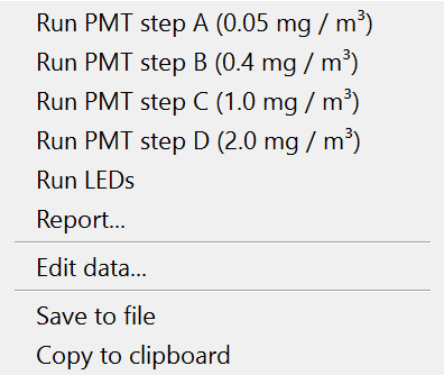
c:\FaBtest\App\FaBtestErrors.csv.

Similarly, when the **Log battery status** option is checked, FaBtest writes the battery status every five minutes to this logging file:

c:\FaBtest\App\FaBtestBatt.csv.

Both files can be opened by a number of applications, including Excel and Notepad.

4.2.2.4 Advanced → Engineering → Calibration

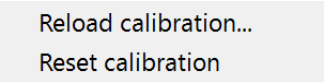


The **Save to file** option generates a backup of the calibration data stored within an attached FastBallast unit. A copy will already have been generated on the Toughpad supplied with the FastBallast unit as part of the factory calibration. This file can be found at the following location:

c:\FaBtest\App\16-0550-001.fbc

Where 16-0550-001 is the serial number of the FastBallast unit. An additional copy of the file can also be found on the USB key supplied with the system.

4.2.2.5 Advanced → Engineering → Troubleshooting



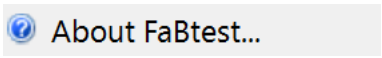
The **Reload calibration…** option can be used to reload calibration data from a file. The name of the calibration file is the same as the serial number of the FastBallast. During factory calibration, a copy of this file is generated at the following location:

c:\FaBtest\App\16-0550-001.fbc

A copy of this file can also be found on the USB key supplied with the FastBallast system.

The **Reset calibration** option will wipe the existing calibration data within an attached FastBallast. It should only be used if the FastBallast becomes unstable or if the FastBallast is in the process of being re-calibrated.

4.2.3 Help menu



About FaBtest… opens the About dialog to provide the installed FaBtest version number.

4.3 Dialogs and functions

4.3.1 Set the auto-save path

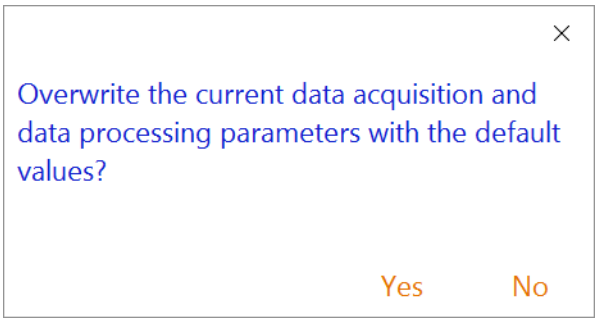
This function can be accessed by selecting **File / Set the auto-save path…** from the menu.

Data are automatically archived at the end of each test. The default location for these data is:
Windows / Users / Public / FaBtest / Auto-saved FastBallast test data
A new sub-folder is generated for each day that data are logged. For example, a test completed on 8th December 2016 would result in the creation of a sub-folder named 20161208 (if this sub-folder didn't already exist). The automatically archived file is saved within this sub-folder.

4.3.2 Revert to defaults

This function can be accessed by selecting **Advanced / Revert to defaults** from the menu.

The default data acquisition and data processing parameters generally provide optimum system performance. If any parameters have been changed, the defaults can be restored by selecting this function. This opens the following dialog…



Press the Yes button to close the dialog and overwrite the existing data acquisition and data processing parameters with the default values.

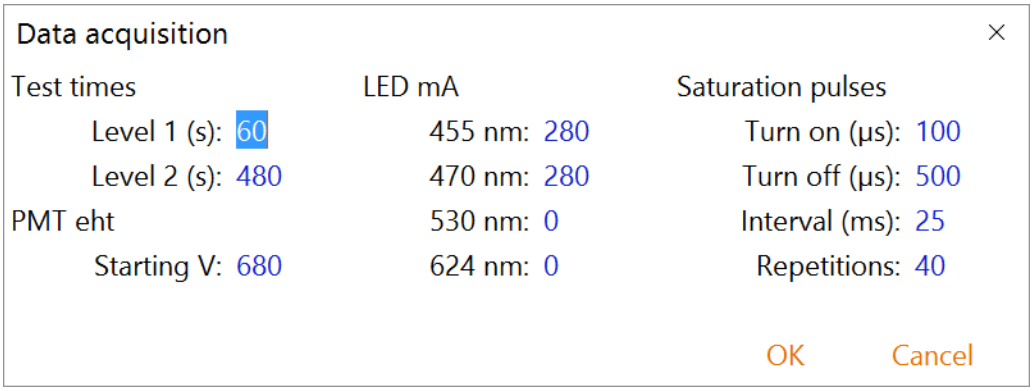
4.3.3 Show more parameters

This function can be switched on and off by selecting **Advanced / Data → Show more** from the menu. It always defaults to off each time FaBtest is started.

When checked, this function increases the number of fluorescence parameters presented on the home screen and data screen. Most of these addition parameters are only provided once a test has completed. This function has no effect on the format of data exported using the clipboard functions (described below). All of the additional parameters are only likely to be of interest to specialist users.

4.3.4 The Data acquisition dialog

Select **Advanced → Settings → Data acquisition…** from the menu.



This dialog allows the user to change the parameters used to define a test protocol. The above example shows the default values. When running standard tests, there is generally no reason to change any of these parameters.

4.3.4.1 Test times

Level 1 (s): The run time for a level 1 test

Level 2 (s): The run time for a level 2 test

Both the level 1 and level 2 test times are the actual data acquisition times and don't include the time required to change the PMT eht (auto gain) to keep the signal from the PMT within range of the data acquisition system.

4.3.4.2 PMT eht

Starting V: The voltage applied to the PMT at the start of a test. If the signal is out of range of the data acquisition system at the start of the test, FaBtest will adjust the voltage to bring it into range (increase the voltage if the signal from the PMT is too low and decrease the voltage if the signal from the PMT is too high).

4.3.4.3 LED mA

455 nm: The drive current applied to the royal blue LEDs (mA)

470 nm: The drive current applied to the standard blue LEDs (mA)

530 nm: The drive current applied to the green LEDs (mA)

624 nm: The drive current applied to the orange-red LEDs (mA)

The default values (280 mA for the royal blue and standard blue and zero current for the green and orange/red) have been selected to maximise the fluorescence signal from phytoplankton groups that incorporate chlorophyll *b* or *c* as the main accessory light-harvesting pigment and minimise the fluorescence signal from phytoplankton groups that don't incorporate chlorophyll *b* or *c* as the main accessory light-harvesting pigment. This is because most phytoplankton cells within the D2 size range (10 to 50 µm in the smallest dimension) do incorporate chlorophyll *b* or *c* as the main accessory light-harvesting pigment, while most phytoplankton cells that don't incorporate chlorophyll *b* or *c* as the main accessory light-harvesting pigment are below 10 µm in the smallest dimension and are, therefore, currently excluded from the D2 regulations.

It should be noted that a wide range of phytoplankton species that do incorporate chlorophyll *b* or *c* as the main accessory light-harvesting pigment are below 10 µm in the smallest dimension and, consequently, these LED settings only provide an imperfect 'screen' of cell size.

Although the green and orange/red channels are not currently used within the default test setup, they will be incorporated within future software updates (as the analysis algorithm is developed). They also effectively future-proof FastBallast against the possible inclusion of cyanobacteria (which are below 10 µm in the smallest dimension and incorporate accessory light-harvesting pigments that are preferentially excited by green and/or orange-red light) within possible updates to the ballast water discharge regulations.

4.3.4.4 Saturation pulses

Turn on (µs): The time interval between the start of data acquisition and the start of the LED pulse

Turn off (µs): The time interval between the start of data acquisition and the end of the LED pulse

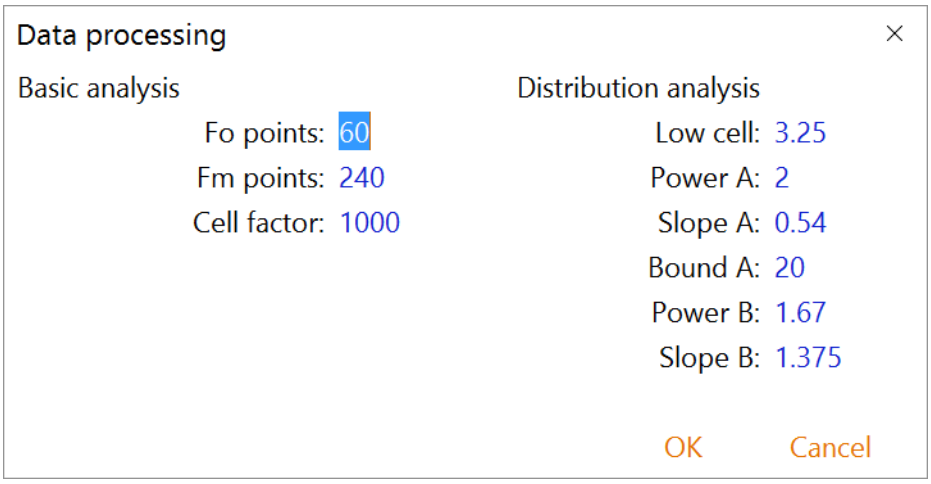
Interval (ms): The time interval between the start of adjacent LED pulses

Repetitions: The number of LED pulses applied during an acquisition

Each data point within a test file is calculated through analysis of the mean of all LED pulse repetitions within an acquisition. In the above example, 40 LED pulses, each of 400 µs duration, are applied on a 25 ms pitch. Consequently, data points are generated at 1 Hz, with each acquisition being formed by averaging the data from the 40 LED pulses.

4.3.5 The Data processing dialog

Select **Advanced → Settings → Data processing…** from the menu.



The parameters set through this dialog are used in the processing of data during level 1 and level 2 tests. The default values have been derived through extensive testing and should only be changed under guidance from a CTG scientist.

4.3.5.1 Fv per cell analysis

Fo slope points: The maximum number of points used to estimate F_o

Fm slope points: The number of points used to estimate F_m

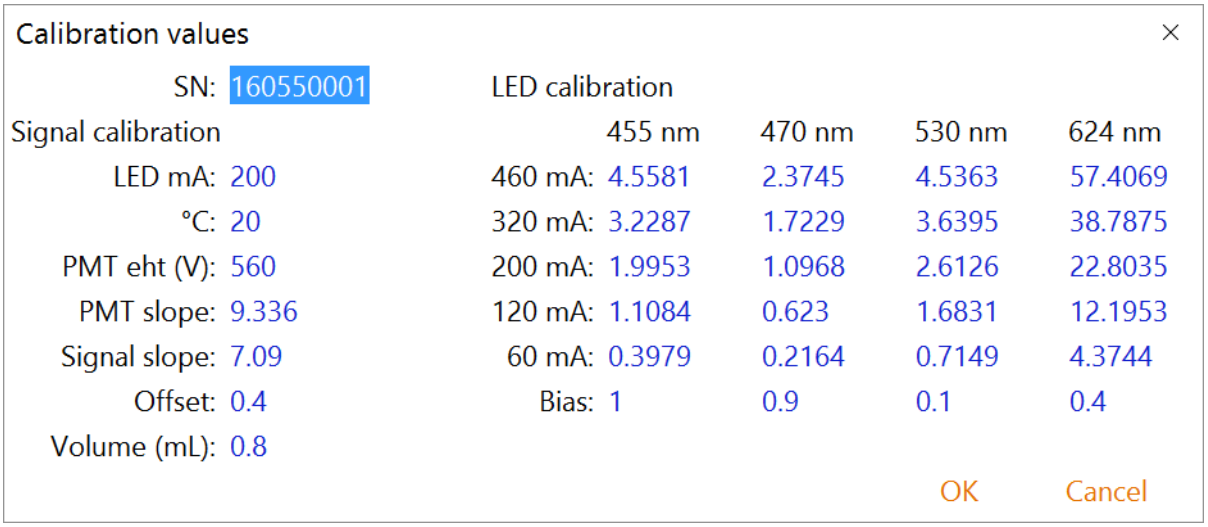
Q factor: A factor used to convert the mean F_v to the PASS/FAIL numeric during a level 1 test

4.3.5.2 Distribution analysis

The parameters within this section are used to analyse the distribution of F_v values within a level 2 test.

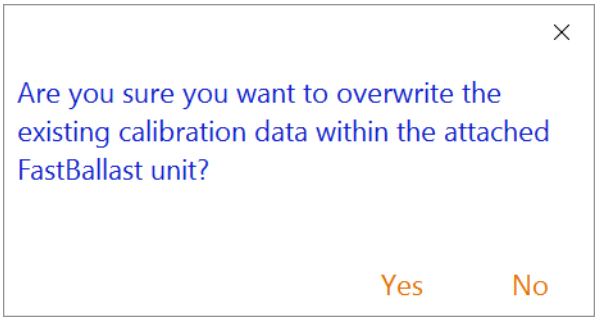
4.3.6 Calibration values dialog

Select **Advanced → Engineering → Calibration → Edit data…** from the menu.

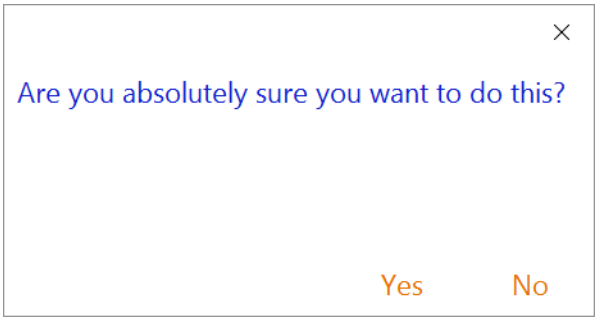


All values within this dialog are loaded from a FastBallast system each time it is attached to FaBtest. There is generally no reason for the end user to change any of these values.

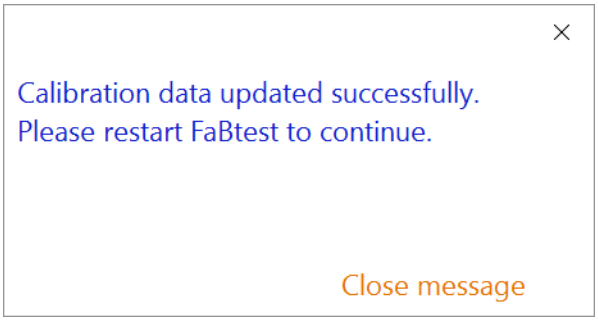
If the OK button is pressed, the following question will appear:



If the Yes button is pressed, the following question will appear:



If the Yes button is pressed, the following message should appear:



If any changes were made, they will have been written to the FastBallast and will be loaded into FaBtest each time it is attached.

4.3.6.1 SN

SN: The serial number of the attached FastBallast unit

The serial number should be entered without dashes. In the above example, the serial number will be shown as 16-0550-001 on the FastBallast unit.

4.3.6.2 PMT calibration

These values are used to normalise the PMT output at the set PMT eht.

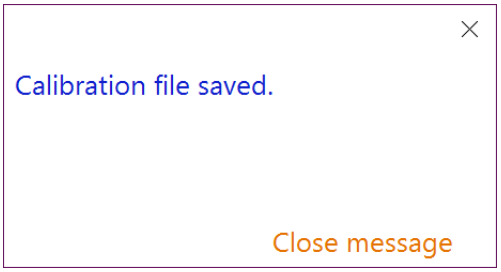
4.3.6.3 LED calibration

These values are used to normalise the output from all four LED channels.

4.3.7 Save calibration values to file

Select **Advanced → Engineering → Calibration → Save to file…** from the menu.

The following message will appear:



The file generated is saved at the following location:

c:\FaBtest\App

The name of the file is the serial number of the attached FastBallast plus an **fbc** extension. For example:

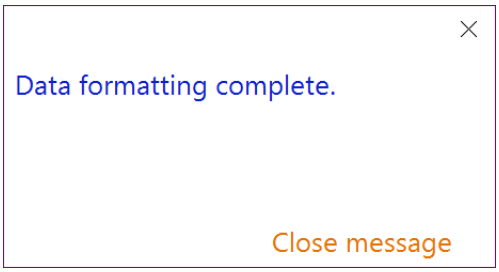
16-0550-001.fcb

A copy of this file is also provided on the USB key supplied as part of the FastBallast system. It can be used to re-install the calibration data within FastBallast if the system becomes unstable (see Troubleshooting).

4.3.8 Copy the calibration data to the clipboard

Select **Advanced → Engineering → Calibration → Copy to clipboard** from the menu.

The following message should appear:

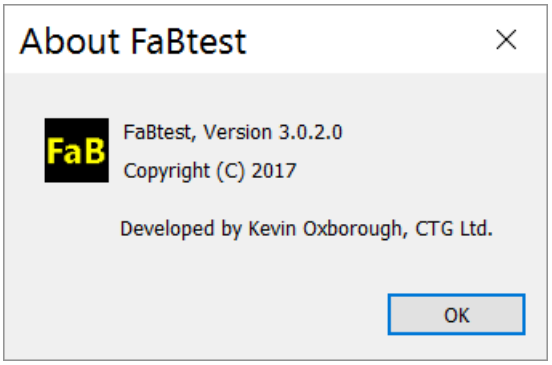


The calibration data can then be pasted into a spreadsheet-based application, such as Excel.

4.3.9 The About dialog

Select **Help → About** from the menu.

This dialog provides the FaBtest version number.



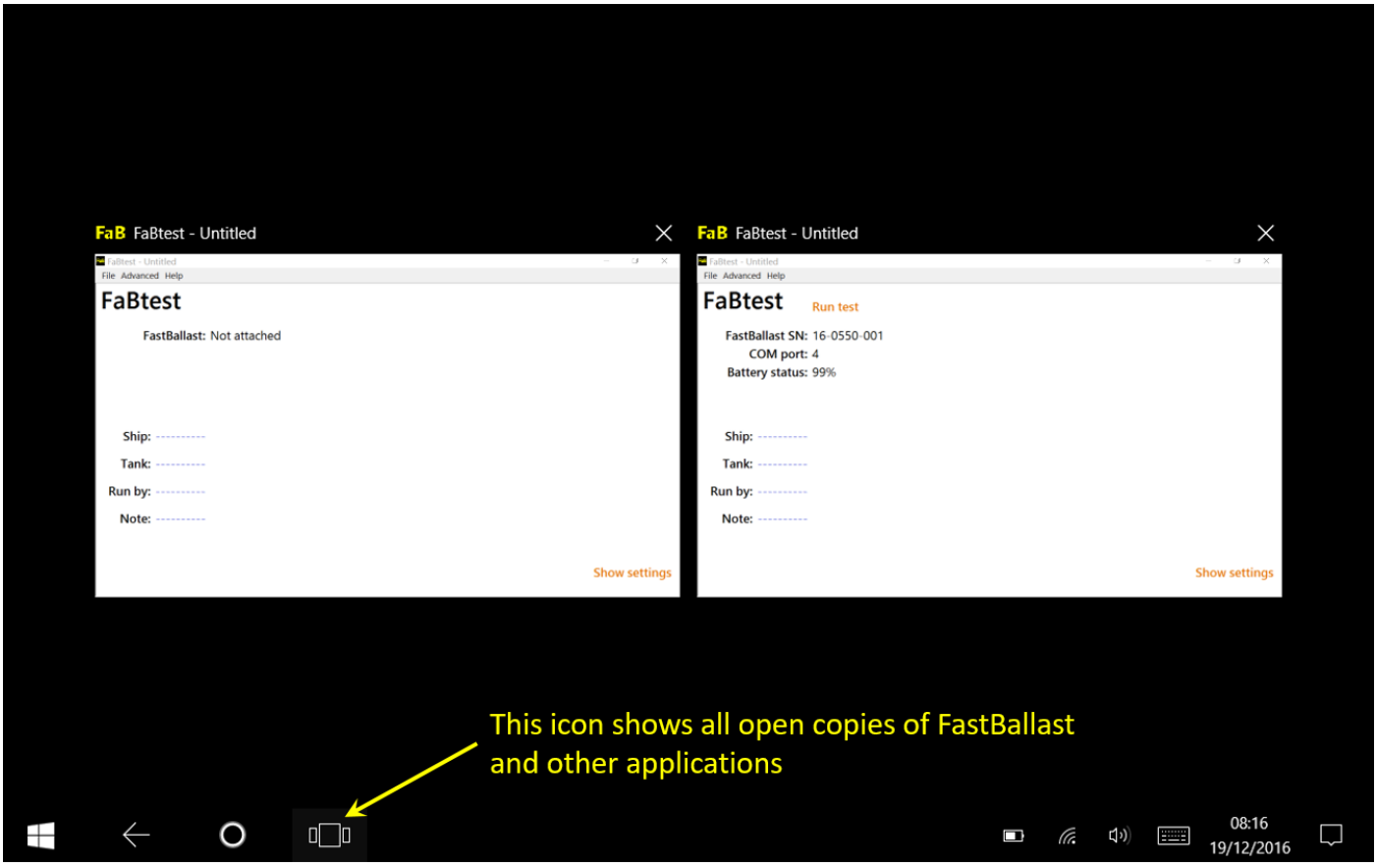
Technical section

5 Troubleshooting

5.1 Bluetooth and other connection problems

5.1.1 Symptom: The FastBallast is turned on but FaBtest just shows **FastBallast:** Not attached

5.1.1.1 Test A: Check that another copy of FaBtest is not running on the ToughPad and already attached to the FastBallast. The ToughPad screenshot below shows two copies of FaBtest running. One copy is already attached to the FastBallast, preventing the second copy from attaching.



5.1.1.2 Test B: If there are multiple FastBallast systems on site, check that the S/N on the back of the ToughPad matches with the S/N inside the lid of the FastBallast.

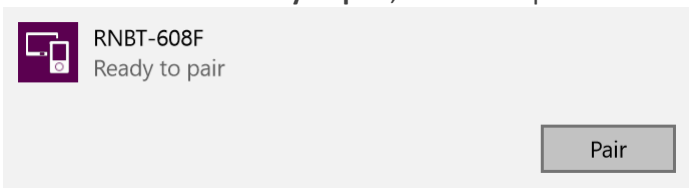
5.1.1.3 Test C: Turn the FastBallast and ToughPad off and on again.

5.1.1.4 Test D: Use the included FastBallast to USB cable (2355-085-PL-A) as an alternative to the Bluetooth connection. The FastBallast should attach to FaBtest automatically.

5.1.1.5 Test E: If FastBallast does attach to FaBtest when the USB cable is used (test D), check the Bluetooth connection:

- Go to **Windows Settings / Devices / Bluetooth**
- The FastBallast unit should show up and be **Paired** or **Connected**, rather than **Ready to pair**

- If you are unsure of which listed Bluetooth device is the FastBallast, turn the FastBallast off and see which device disappears from the list
- If the FastBallast is **Ready to pair**, select and press **Pair**



- You may need to re-start the ToughPad and/or the FastBallast for the connection to work

5.2 The Menu Font is too small

Go to **Windows Settings / System / Display / Advanced display settings / Advanced sizing of text and other items**

It is recommended that the **Title bars** and **Menus** options are set to **12** point.

6 FaBtest WARNING and other messages

6.1 WARNING: Calibration data not read from FastBallast

This may be a one-off event. Try closing FaBtest and restarting. If the message persists, you will need to restore the calibration data stored within FastBallast.

7 Maintenance intervals

The FastBallast system should be returned for a factory refit and calibration every two years. The refit includes:

- Replacement of the internal rechargeable battery
- Replacement of all O-ring seals
- Testing of internal electronics
- Instrument re-calibration
- Reset and updating of the Panasonic Toughpad

Background information

8 Constant and variable fluorescence from phytoplankton

8.1 Phytoplankton fluorescence

Phytoplankton contain membrane-bound protein complexes called photosystems which are able to perform photochemistry (absorb and convert light energy into chemical energy, which can then be used for cell growth and maintenance). A small proportion of the light absorbed by the photosystems within viable phytoplankton cells is re-emitted as chlorophyll fluorescence, which can be detected by active chlorophyll fluorometers, such as FastBallast. A significant complication is that the fluorescence signal from ballast water may include contributions from other sources, including dead phytoplankton cells, free chlorophyll and CDOM (coloured, dissolved organic matter). Consequently, the total fluorescence signal detected by FastBallast (or any other active fluorometer) is not a reliable metric for reporting the concentration of viable phytoplankton cells.

There are actually two types of photosystems within phytoplankton: Photosystem II (PSII) and Photosystem I (PSI) – see **Figure 1**. Although fluorescence is emitted from chlorophyll *a* within both PSII and PSI, PSII differs from PSI (and all other contributors to the fluorescence signal detected by FastBallast) in emitting both constant fluorescence and variable fluorescence. All other sources only emit constant fluorescence.

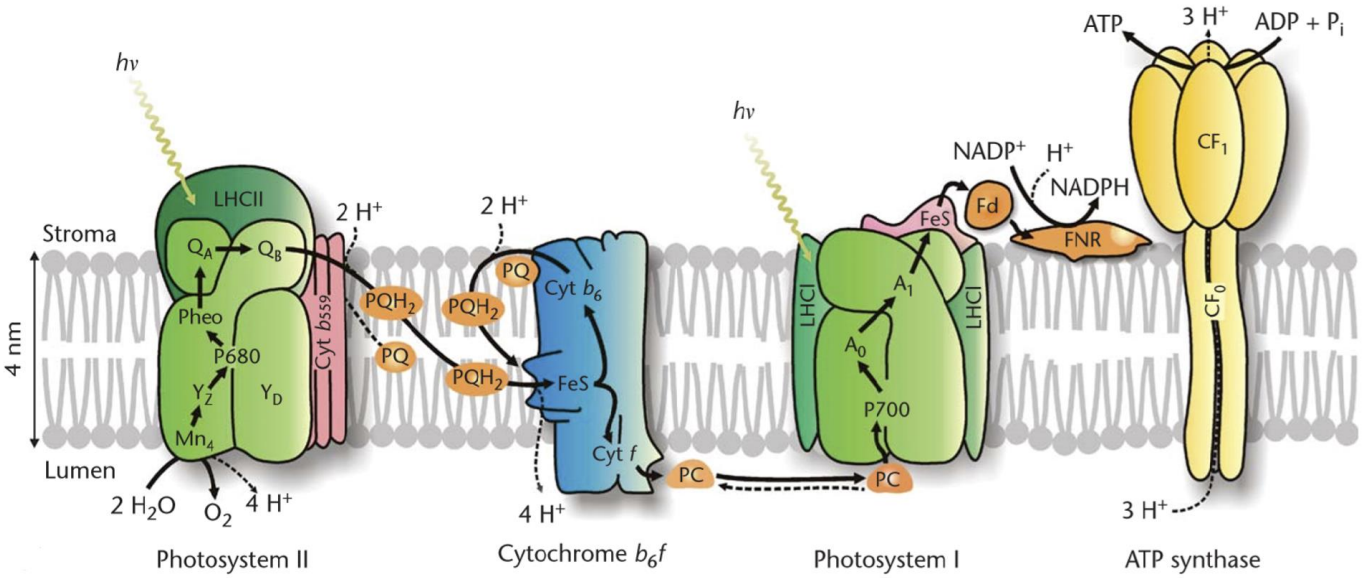


Figure 1: Schematic representation of the protein complexes and cofactors involved in oxygenic photosynthesis. This particular schematic incorporates light harvesting systems (LHCII and LHCI) that are typical of higher plants and green algae. Other phytoplankton groups differ in the structure of their light harvesting systems but are otherwise consistent with this schematic. Taken from Govindjee et al. 2010.

All active chlorophyll fluorometers have the ability to isolate the variable fluorescence originating from PSII complexes (F_v) from the constant fluorescence originating from all other sources. Importantly, F_v is only emitted by PSII complexes that are photochemically active: PSII complexes that are damaged during ballast water treatment lose the variable component of their fluorescence emission and only contribute to constant fluorescence.

8.2 Measurement of F_v

There are two well established methods for the measurement of F_v: the single turnover (ST) method and the multiple

turnover (MT) method. Both ST and MT-based systems generate F_v by applying a short pulse of light to the sample. This pulse is bright enough to saturate photochemistry within a very high proportion of the active PSII complexes within the sample. As PSII photochemistry approaches complete saturation, the yield of fluorescence from these complexes increases from F_o to a maximum level (F_m). F_v is simply the difference between F_o and F_m (see **Figure 2**). It is important to emphasise that, although the measured F_o is likely to include a significant contribution from sources other than active PSII complexes, the measured F_m will include exactly the same contribution from these sources. Consequently, the calculated value of F_v ($F_m - F_o$) is independent of all sources of constant fluorescence.

Within ST-based systems, the saturation pulse is bright enough to saturate PSII photochemistry within 100 to 400 µs. The minimum interval between successive photochemical events within a PSII complex is (on average) about 600 µs. Consequently, only a very small proportion of PSII complexes undergo more than one photochemical event (a single turnover) during each saturation pulse. The impact of a saturating ST pulse is normally dissipated within 25 ms. Consequently, measurements from a static sample can be taken at a frequency of up to 40 Hz.

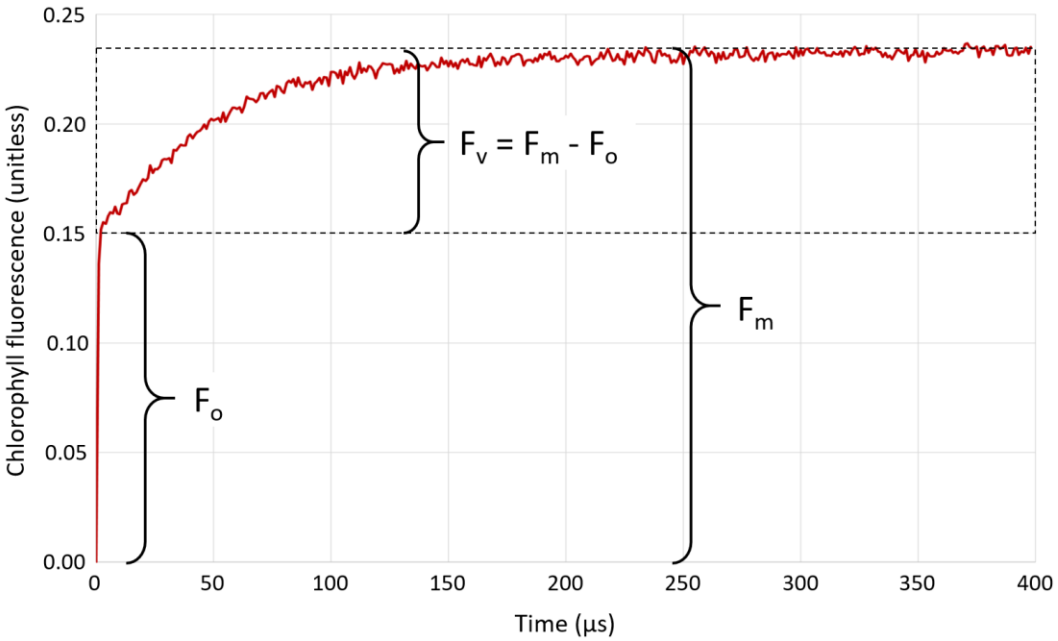


Figure 2: A sample data trace from FastBallast. The trace is an average of 40 ST pulses acquired at 40 Hz, each of 400 points acquired at 1 MHz. The trace therefore represents an acquisition formed from all data collected over 1 s. Additional details are provided within the main text.

The LED output during a FastBallast ST pulse is usually between four and eight times full sunlight, depending on the sample. This is about five times as bright as a MT saturation pulse. However, a MT pulse is up to 1000 times longer than a ST pulse. It follows that a single MT pulse delivers about 200 times as many photons as a single ST pulse. This is because the MT-method relies on multiple photochemical events (multiple turnovers) at each active PSII complex within the sample being induced during each saturation pulse. An inevitable consequence of the multiple turnovers is that a very long time interval (tens of seconds) is required to dissipate the impact of each saturation pulse.

Parameter	**Single turnover**	**Multiple turnover**
Duration of measurement pulse	400 µs	40 – 1200 ms
Recovery time between successive pulses	25 ms	10 – 100 s
Maximum measurement frequency with a static sample	40 Hz	0.1 Hz
Maximum linear flow rate (10% sample exchange)	2 m s^{-1}	< 0.01 m s^{-1}

Table 1: Summary of performance differences between single turnover (ST) and multiple turnover (MT) systems.

The low maximum linear flow rate imposed by the MT method effectively rules out MT measurements being made on a moving sample. In addition, the very long interval required between MT saturation pulses severely limits the signal to noise ratio (S:N) and, ultimately, the detection limit of MT-based systems.

The fluorescence traces within **Figure 3** provide a graphic illustration of the main differences between ST and MT measurements. Both traces were generated from the same suspension of cultured cells of *Chlorella* sp. A FastOcean Fast Repetition Rate fluorometer (FRRf) was used to collect these examples as this type of sensor can easily be programmed to generate alternate ST and MT measurements.

The red bar within the MT panel of **Figure 3** provides a graphic illustration of the time difference between ST and MT pulses. The difference in measurement frequency is almost as dramatic: whereas a pulse interval of 100 ms is more than sufficient for ST measurements, at least 30 s is required between successive MT pulses.

The amplitudes of F_v generated during the ST and MT measurements are shown within **Figure 3**. Also included are values for F_v/F_m. This parameter is widely used, outside the field of ballast water discharge testing, as an indicator of photosynthetic efficiency within phytoplankton cells. Typically, ST values for F_v/F_m are 15 – 20% lower than MT values, within healthy cultures under nutrient replete conditions (as is the case here). The extent of this difference is well documented (e.g. Kolber et al. 1998) and is consistent across a wide range of phytoplankton species. Within the example shown in **Figure 3**, it is clear that the lower value of F_v/F_m from the ST measurement is due to a lower value for F_v (the amplitude of the fluorescence increase induced by the pulse). Again, this difference is well documented and consistent among phytoplankton species.

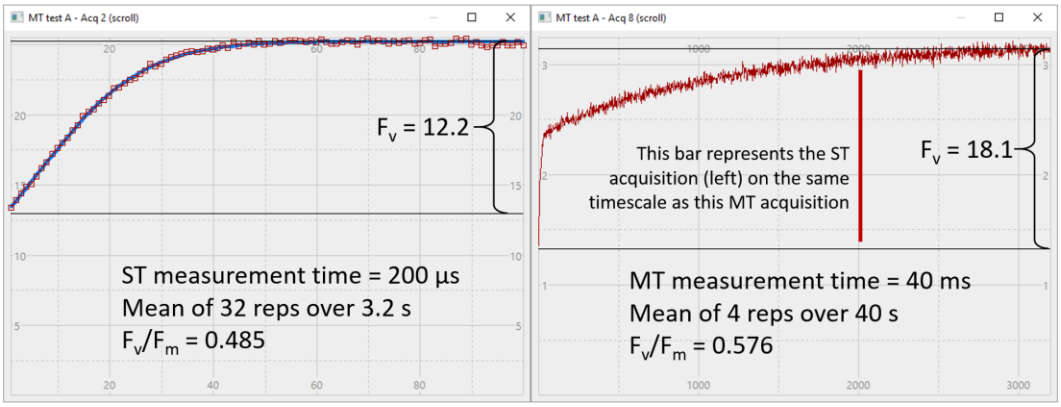


Figure 3: Data collected from a single sample of *Chlorella* sp., using a FastOcean sensor, to illustrate some the differences between the ST method employed within FastBallast and the more commonly used MT method.

8.3 F_V and cell viability

Phytoplankton cell viability is closely linked to their capacity for PSII photochemistry. A robust quantitative relationship between the fluorescence emitted by active PSII complexes and the capacity for photochemistry, within a wide range of phytoplankton species, has been demonstrated by Oxborough et al. (2012) and Silsbe et al. (2015). The data presented within these studies indicate that the constant component of the fluorescence emission arising from active PSII complexes (F_o) is better correlated with the capacity for photochemistry than F_v, when working with healthy phytoplankton cultures under nutrient-replete conditions. However, it has always been clear that the relationship between F_o and the capacity for photochemistry breaks down in situations where constant fluorescence from sources other than active PSII complexes becomes significant.

In contrast to F_o, F_v is unaffected by additional sources of constant fluorescence. Consequently, there are some situations where it is better to work with the relatively low variability between F_v and the capacity for photochemistry than to ignore the potentially much larger errors introduced by the contribution of constant fluorescence from sources other than active PSII complexes. This is certainly the case with ballast water discharge, where such contributions can be extremely high.

The FastBallast data presented within **Figure 4** illustrate the impact of constant fluorescence from sources other than active PSII complexes. Both traces are from cultured cells of the marine chlorophyte, *Dunaliella tertiolecta*. The difference is that the sample on the left was prepared by diluting the culture with a cold tea infusion (which contains significant levels of CDOM and some free chlorophyll *a*) prepared with F/2 medium (artificial sea water) while the sample on the right was prepared by diluting the culture with F/2 medium alone.

One very obvious feature of these data is that diluting with the tea infusion has added a high background signal. Because this background fluorescence contributes to the measured values of F_o and F_m, but not to the calculated value of F_v, the calculated value of F_v/F_m is much lower (less than 4% of the value from the sample diluted with artificial sea water).

If an F_v/F_m this low was recorded from a pure (single species) cell culture that had been subjected to stress, it might reasonably be concluded that the culture was unlikely to recover, even if the source of stress was removed. However, ballast water discharge could be very different from a pure cell culture and a more cautious approach to the interpretation of low F_v/F_m values is required. This is because, as already discussed, the constant component of the fluorescence recorded by FastBallast can include a significant contribution from non-viable phytoplankton cells, including species with low tolerance of ballast water treatment and/or the environment within ballast water tanks, in addition to free chlorophyll and CDOM. It follows that the total contribution from these sources could generate a low measured F_v/F_m even if the sample also contains a significant number of viable phytoplankton cells (i.e. cells that have a high tolerance of ballast water treatment and the environment within ballast water tanks).

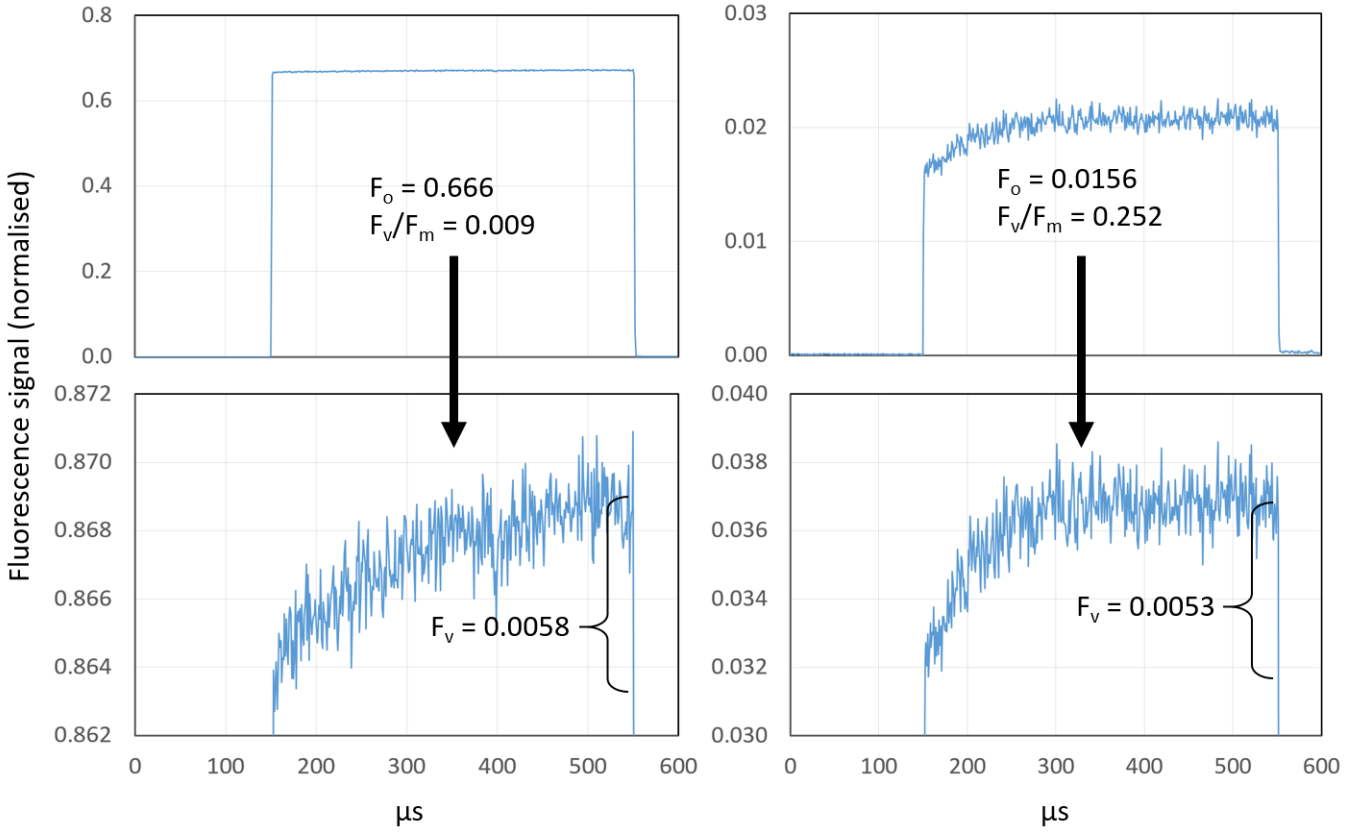


Figure 4: Sample test data from FastBallast. The top panels show traces from a suspension of cultured cells of *Dunaliella tertiolecta*. The bottom panels show detail from the top panels. The cell suspension used to generate the left trace was prepared by diluting a more concentrated culture, using a tea infusion prepared with F/2 medium. The cells suspension used to generate the right trace was prepare by diluting cells with F/2 medium alone. Both traces are the mean of 16 sequences acquired at 0.1 Hz. Further details are provided within the main text.

8.4 F_v per cell

The accuracy of any estimate of cell density based purely on F_v clearly depends on the variability of F_v generated by phytoplankton cells. Table 1 provides data from our own programme of testing. Measurements of F_v at known cell densities were made using calibrated FastOcean and FastBallast systems. Cell volumes were determined through microscope-based measurements. The number of PSII complexes per cell was estimated using a flash-O_2 system, incorporating an Act2 control unit and Hansatech Oxygraph system. A similar combination was used by both

Oxborough et al. (2012) and Silsbe et al. (2015).

Within the list of species used, there is one coccolithophore (*Emiliania huxleyi*), four diatoms (*Phaeodactylum tricornutum*, *Thalassiosira pseudonana, Thalassiosira weissflogii* and *Thalassiosira punctigera*) and three chlorophytes (*Chlorella vulgaris*, *Dunaliella tertiolecta* and *Dunaliella salina*). This range of species incorporates a number of cell shapes plus wide ranges of cell volume (four orders of magnitude), F_v per cell (two orders of magnitude) and PSII complexes per cell (two orders of magnitude).

Species	**Shape**	**Smallest dimension (µm)**	**Volume (µm^3)**	**F_v per cell**	**PSII complexes per cell**
Emiliania huxleyi	Spherical	4 – 5	30 – 55	0.00022	9.11 x 10^5
Chlorella vulgaris	Spherical	2 – 3	7 – 15	0.000032	6.53 x 10^4
Phaeodactylum tricornutum	Fusiform – Triradiate and Ovoid	2 – 3	60 – 150	0.00019	1.25 x 10^6
Dunaliella tertiolecta	Ellipsoid	8 – 12	480 – 1350	0.00022	9.69 x 10^5
Dunaliella salina	Ellipsoid	10 – 14	690 – 1500	0.00018	6.45 x 10^5
Thalassiosira weissflogii	Cylindrical	16 – 18	880 – 5500	0.00075	2.11 x 10^6
Thalassiosira pseudonana	Cylinder	7 – 8	200 – 300	0.0003	1.55 x 10^6
Thalassiosira punctigera	Capsule	30 – 55	63500 – 240000	0.016	5.58 x 10^7

Table 2: Summary of data from our in-house PASS/FAIL boundary study. All values are based on a minimum of three independent measurements. All values are based on at least ten independent measurements.

8.5 The level 1 test

From the data within Table 1, it is clear that the extreme variability of F_v per cell prevents accurate estimation of cell density based on an assumed F_v per cell, there are situations where the F_v measured from a ballast water discharge sample is so low or so high that a PASS or FAIL, respectively, becomes inevitable. In these situations, the level 1 analysis applied by FaBtest can provide a result in under two minutes.

The level 1 test applied by FaBtest generates a numeric that is normalised to the fluorescence signal generated by 1 mg chlorophyll *a* dissolved in 1 m^3 of 90% acetone when excited by the 455 nm LED waveband.

Figure 5 uses the data provided from Table 1 to place all eight species on the level 1 numeric scale. What this plot shows is that all but one of the tested species fall within an order of magnitude of the PASS/FAIL boundary on the PASS side. However, the one species on the FAIL side (*Thalassiosira punctigera*) is more than an order of magnitude away from the PASS/FAIL boundary, with a level 1 numeric of 15.8. Consequently, even for the narrow range of species incorporated within this study, the total range of F_v per cell values covers two orders of magnitude.

Based purely on the data presented within **Figure 5**, a PASS could perhaps be considered reasonable where the level 1 numeric is below 0.1 and a FAIL where the level 1 numeric is above 20. The actual values applied by FaBtest are more conservative: a PASS is only reported where the level 1 numeric is below 0.04 and a FAIL where the level is greater than 40. To put these thresholds in perspective, even one healthy cell / mL of *D. salina* would generate more than four times the low numeric of 0.04 and approximately 25 healthy cells / mL of *T. punctigera* would be required to exceed the high numeric of 4.0.

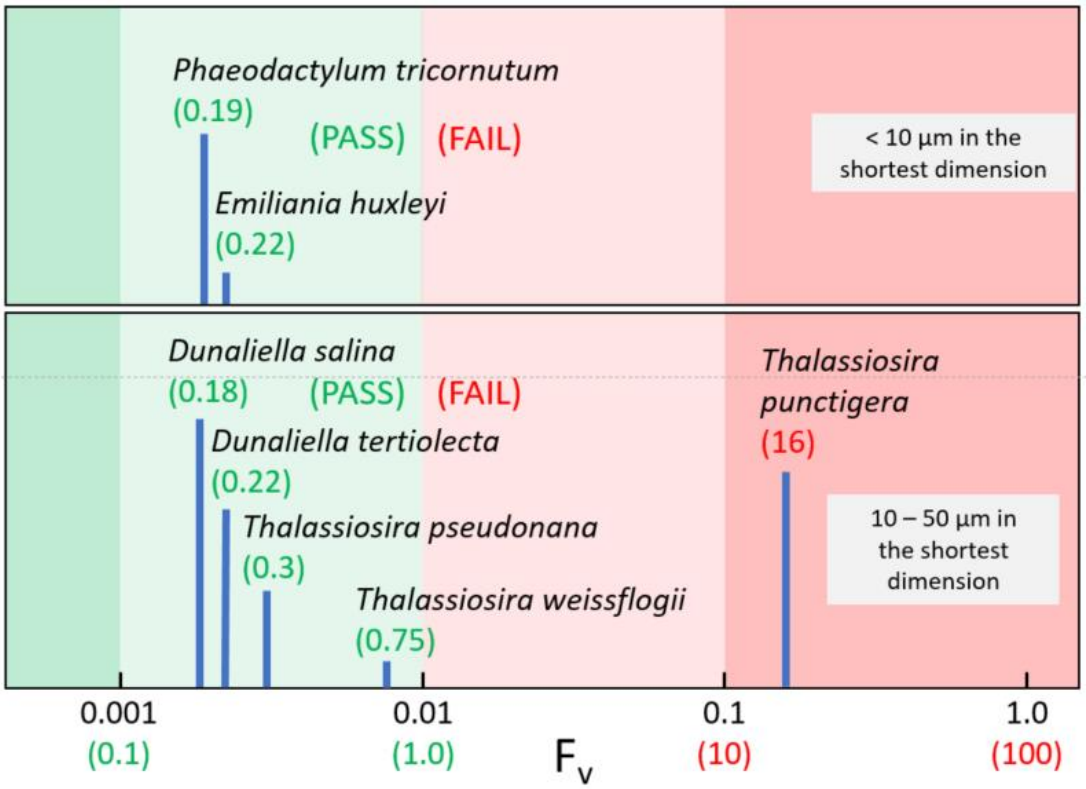


Figure 5: Summary of data from our in-house PASS/FAIL threshold study. All values are based on a minimum of ten independent measurements.

Please note that the F_v values on the x-axis are on a $\log_{10}$ scale and are for 10 cells / mL.

The numbers in brackets against each species are the level 1 numerics generated at 10 cells / mL.

8.6 The level 2 test

The sensitivity of FastBallast is such that F_v can be measured accurately from a 0.5 mL sample containing a single phytoplankton cell (even at the low F_v per cell levels of *C. vulgaris* and *D. salina*). In principle, this level of sensitivity allows for interrogation of the Poisson distribution of cells at low density within ballast water. This type of analysis has a major advantage over the level 1 test because the estimate of cell density is derived from the distribution of F_v values within a series of discrete measurements, rather than the amplitude of F_v from a single measurement.

At the simplest level, a Poisson-based test could involve a single measurements being made from a number of low volume sub-samples, taken from a larger well-mixed volume of ballast water. The data presented within **Figure 6** show the anticipated Poisson distribution of cell densities within 0.5 mL sub-samples, at different overall cell densities within the larger volume. An important feature of these distributions is that the spread of values, relative to the mean value, becomes narrower with increasing cell density.

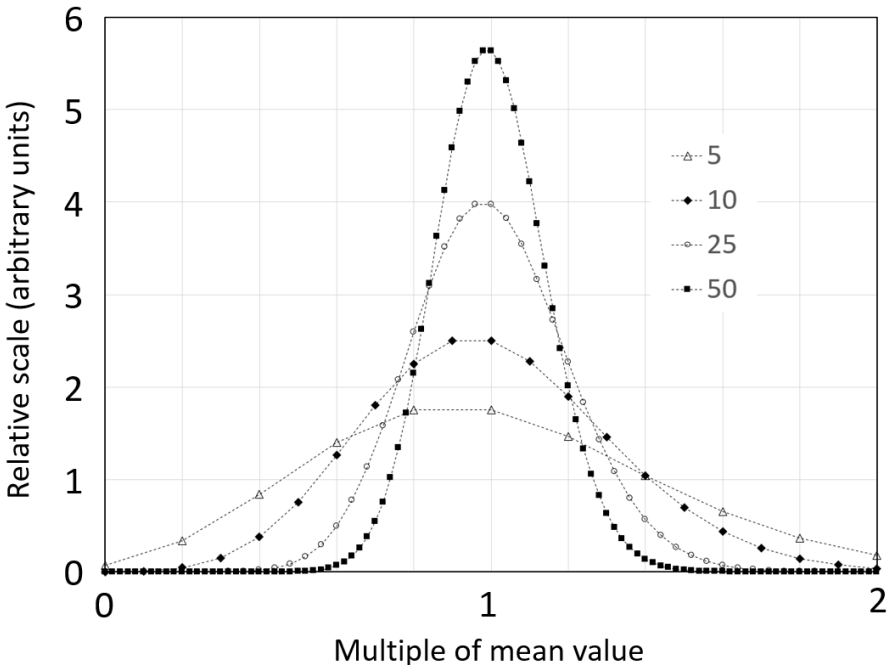


Figure 6: The interrogated volume within the FastBallast sample chamber is 0.5 mL. These plots show the expected Poisson distribution of cell counts within a large number of discrete 0.5 mL samples taken from a larger volume of ballast water containing 10, 20, 50 or 100 cells / mL.

The legend shows mean cells / 0.5 mL sample. The connecting lines are there to guide the eye.

The test method that is actually implemented by FaBtest is a variation on this approach. Instead of taking measurements of F_v from a series of discrete samples, F_v data are acquired at 1 Hz by analysing an average of 40 ST sequences collected at 40 Hz. A full level 2 test acquires 1 Hz data for eight minutes, generating an F_v data set of 480 points. The interrogated volume of 0.5 mL represents 2.5% of the 20 mL bulk sample volume. The sample is stirred at a speed that provides an optimum rate of sample exchange within the interrogated volume: slow enough to provide minimal exchange during each 1 s acquisition but fast enough to ensure that the entire sample is interrogated over the eight minute test period.

Because the interrogated volume is continuous with the total sample volume, it allows for fractions of cells. From basic theory, it follows that the level 2 F_v data set should fit to the approximation of a Poisson distribution generated by a normal distribution with a standard deviation (SD) equal to the square root of the mean ($\sqrt{\sigma}$). The algorithm used to process level 2 data was actually derived using a mathematical simulation that incorporates this assumption. The data presented within **Figure 7** illustrate how effective the real-time, non-iterative data processing algorithm embedded within FaBtest is in estimating cell density from the simulated data.

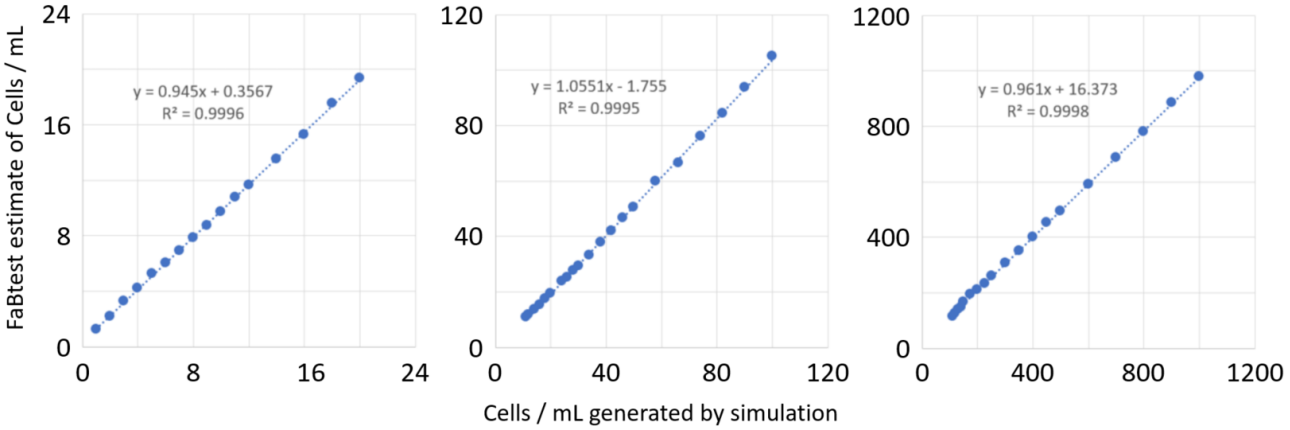


Figure 7: The x-axis values are the means of 64 test simulations, each of 480 F_v values. The y-axis values are means generated from the same 64 x 480 F_v values using the real-time data processing algorithm embedded within FaBtest. SD values for the FaBtest estimates are below 1 cell / mL up to 10 cells / mL and below 10% of the mean above 10 cells / mL.

The F_v distribution data presented within **Figure 8** provide comparisons between actual test data (using cultured cells of the chlorophyte, *Dunaliella salina*) and simulations of the same nominal cell densities (27 and 126 cells / mL).

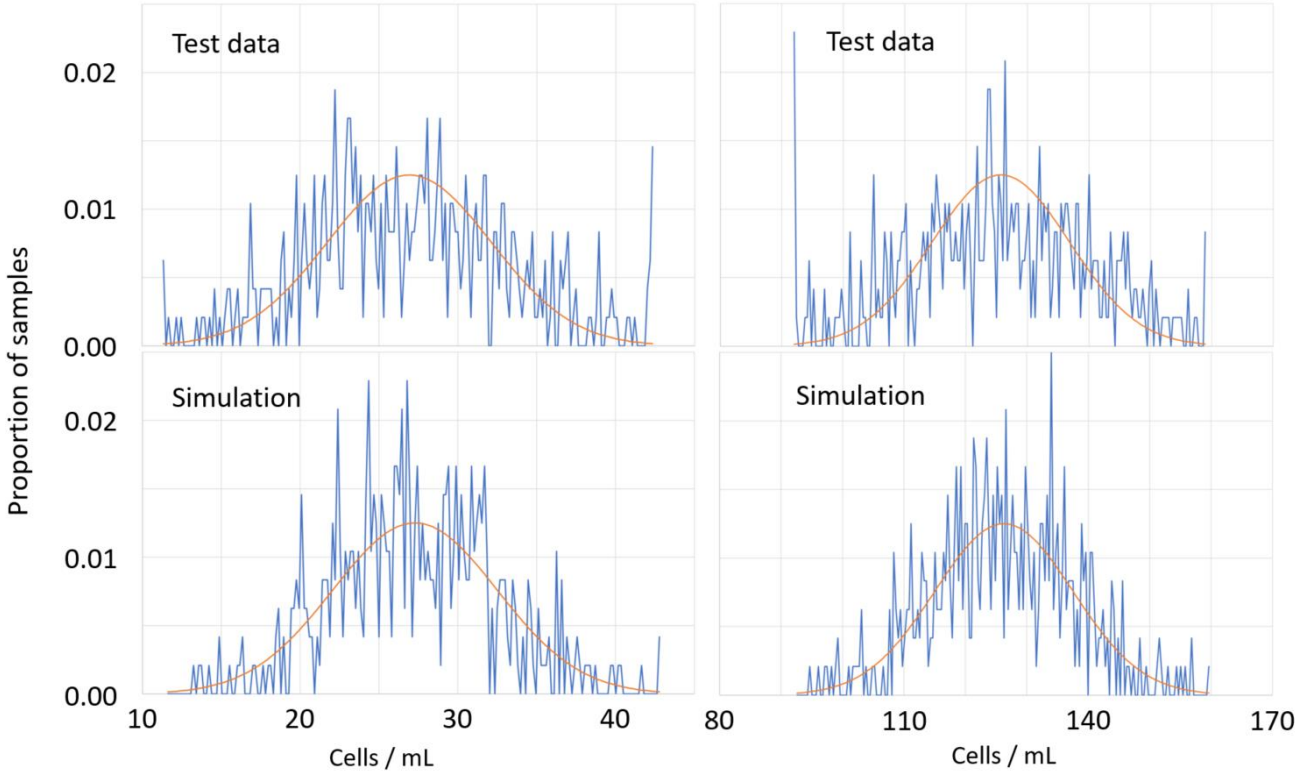


Figure 8: Sample curves providing comparisons of the distribution of F_v data (blue lines) from FastBallast tests using cultured cells of the chlorophyte, *Dunaliella salina* and simulations of the same nominal mean cell densities (27 and 126 cells / mL). The orange curves show normal distributions generated by making the SD equal to the square root of the mean ($\sqrt{\sigma}$). Both curves extend to ±3 x SD. Points that fall outside this range are included within the first and last data values.

The data presented in **Figure 9** show estimates of cell densities derived from microscope-based cell counts (x-axis) against level 1 and level 2 test data. The species included within these plots are highlighted in green in Table 1. The solid purple lines define the 1:1 relationship between microscope-based estimates of cell density and level 1 or level 2 test results.

The red-filled circles within **Figure 9** use the default threshold value for the PASS/FAIL boundary (see **Figure 5**). This has generated a number of level 1 false positive results, which are all derived from tests on pure cultures of *T. punctigera*. This data set also includes a number of level 1 test false negatives, generated by the species with lowest F_v per cell values. The green-filled circles use a level 1 threshold value that is 15.8 times the default. This makes it equal to the level 1 numeric generated by *T. punctigera* (see **Figure 5**). As a result, the false positives generated by *T. punctigera* at the default threshold value now lie within the green zone (i.e. are correctly reported as PASS values). However, the 15.8 times increase in the PASS/FAIL threshold has, inevitably, pushed samples with low F_v / cell even further into the false negatives zone, to the point where some samples with several hundred cells / mL are reported as a PASS.

One thing that is clear from the data within **Figure 9** is that the level 2 data points (open squares) are generally much closer to the 1:1 line than the level 1 data points (green or red filled circles). The only exceptions to this rule are the red level 1 points associated with *T. weissflogii* and the green level 1 points associated with *T. punctigera*, which are all very close to the 1:1 line. As noted above, the default PASS/FAIL threshold for the red points is very close to the F_v / cell of *T. weissflogii* while the PASS/FAIL threshold for the green level 1 data points was specifically optimised for *T. punctigera*. Consequently, the fact that these points are so close to the 1:1 line is expected and simply demonstrates the consistency of FastBallast measurements.

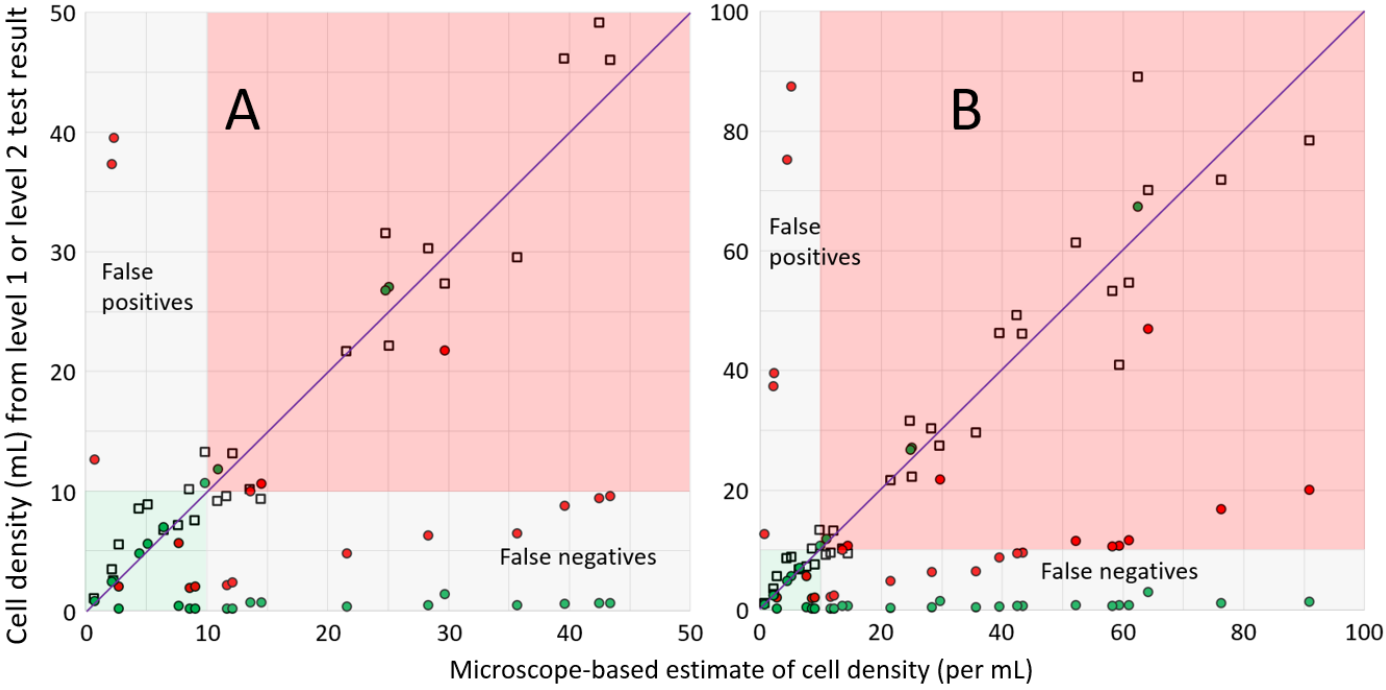


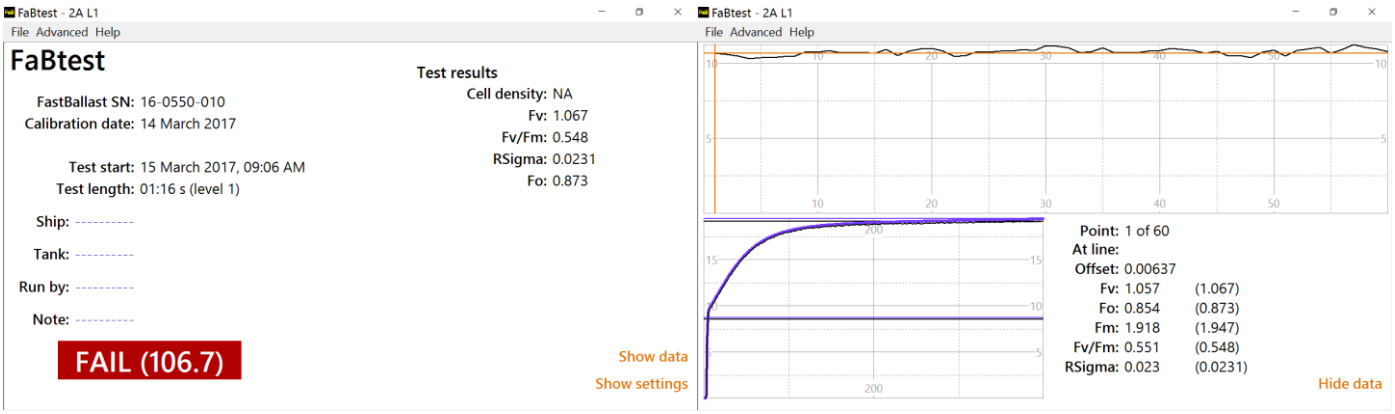
Figure 9: Circles represent level 1 data, squares represent level 2 data. The red-filled circles use the default level 1 threshold value for the PASS/FAIL boundary (see **Figure 5**). The green-filled circles use a threshold value that is 15.8 times the default value (equal to the level 1 numeric generated by *T. punctigera*). The only difference between **A** and **B** is the data range on the axes. Points within the green or red zones indicate a match between the microscope-based estimate and the level 1 or level 2 test result (green for a PASS and red for a FAIL). The grey zones within indicate false positives (against the y-axes) or false negatives (against the x-axes). Additional details are provided within the main text.

8.7 Test details

The sample data presented within this section are all from a dilution series of healthy, cultured cells of *Dunaliella tertiolecta*. Each step dilution was approximately one in four. All of the screenshots presented within section have been taken with the **Advanced → Data → Show more** menu option activated.

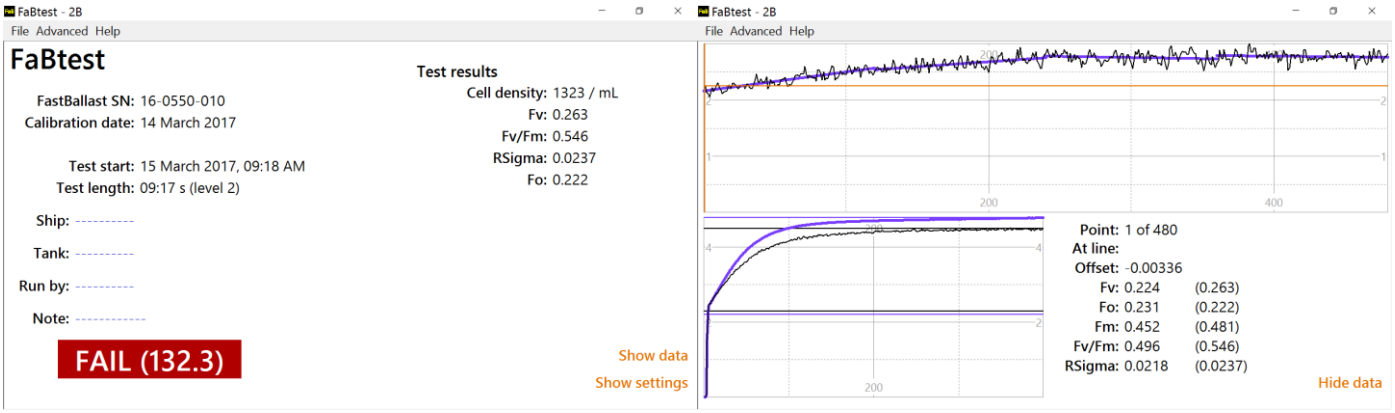
8.7.1 Test A

The series starts at a high enough cell density to generate a level 1 test numeric of more than 40 (106.7). At this level, the test is halted once the set number of points for a level 1 test (default of 60) has been reached. Because there can be an order of magnitude difference between the PASS/FAIL numeric generated by a level 1 test and the density of cells within the sample, an estimate of cell density is not provided for this test (see The level 1 test for a discussion).



8.7.2 Test B

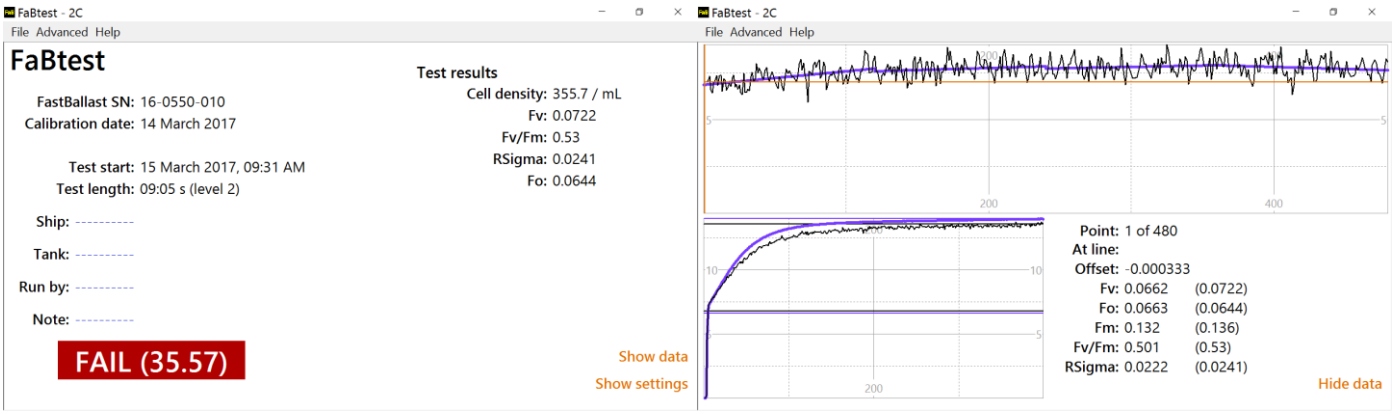
The first dilution generated a level 1 numeric of 26.3 (roughly 25% of the previous test). Because this is below the value of 40 required to stop the test at level 1, the test automatically ran straight into level 2. The test numeric from the level 2 is actually higher than test numeric for Test A, even though the Test B sample is a one in four dilution from the Test A sample. This result is very much in line with expectations because the F_v emitted by each cell of *D. tertiolecta* is 0.22 times the level 1 normalised value (see **Table 2** and **Figure 5**).

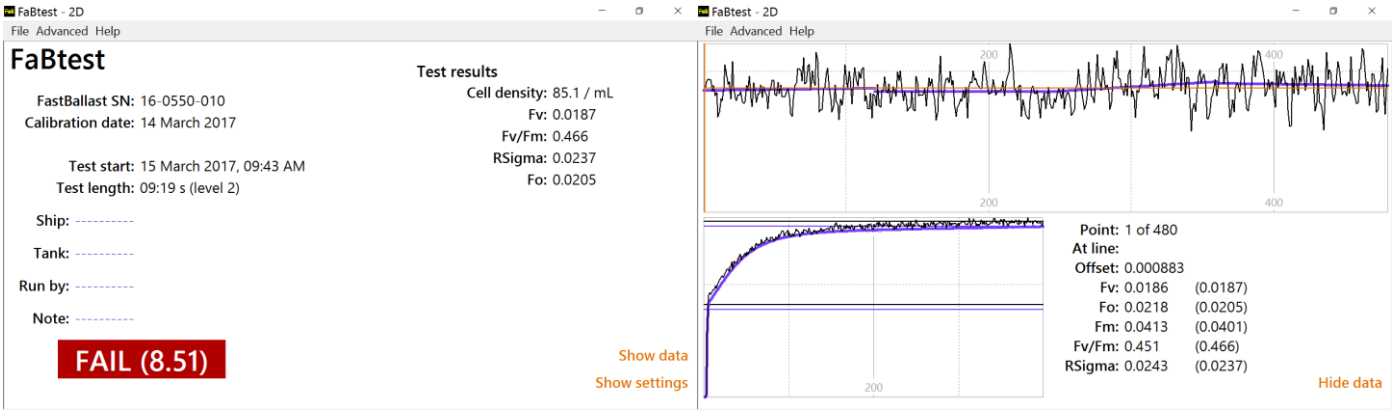


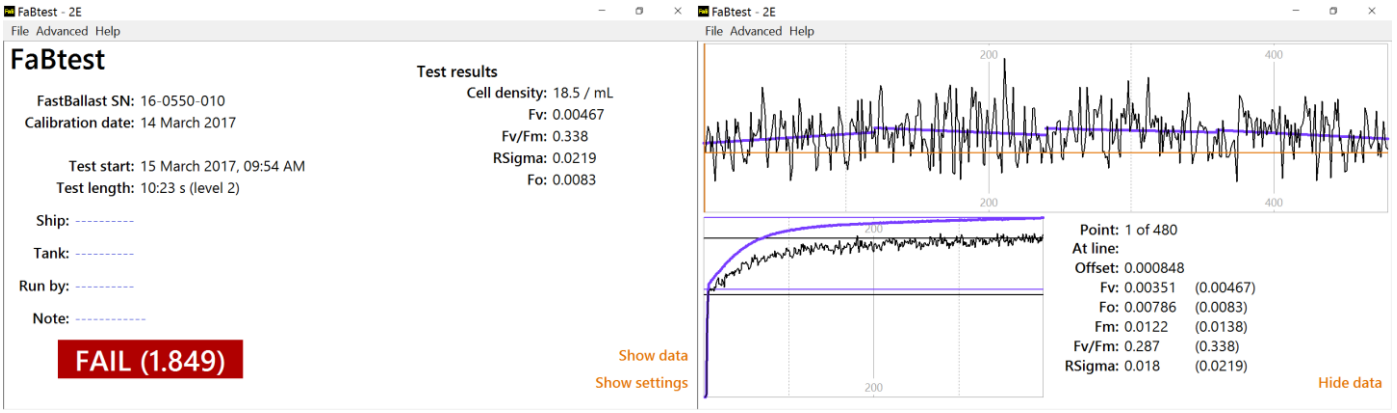
The F_v values within the top panel of the data screen for Test B show a significant increase over the first 200 points or so. This is likely to be due to a combination of dark adaptation and the cells adapting to a slight increase in temperature within the sample chamber. FaBtest corrects for such changes in amplitude over time by generating regression lines through the data set and building the F_v distribution as deviations from these lines, rather than deviations from the overall mean F_v value for the test (which would tend to underestimate cell density). A new regression line is started every 120 points. Consequently, the default level 2 data set incorporates four regression lines.

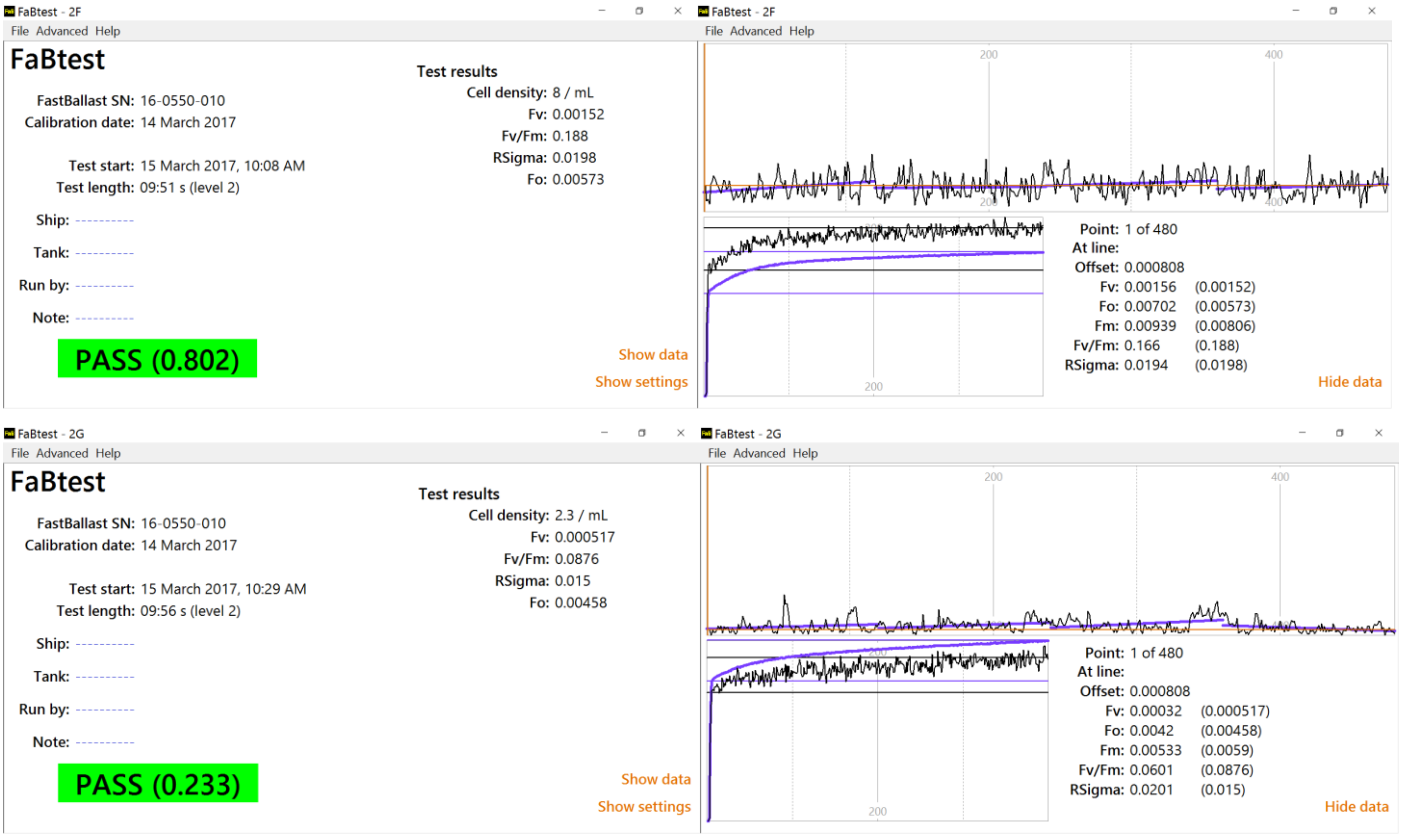
8.7.3 Tests C, D, E, F and G

The decrease in test numeric for the subsequent dilutions is always consistent with the approximate one in four dilution at each step. One point to note is that the F_v/F_m value starts to decrease significantly after Test C – to below 0.1 by Test G. This decrease can largely be attributed to the optical filtering within FastBallast. The filters located in front of the LEDs used to excite chlorophyll fluorescence and the PMT used to detect chlorophyll fluorescence have been selected to maximise the proportion of F_v detected. As a result, a very small proportion of the excitation LED light breaks through the filters and reaches the PMT. In this case, the proportion of the signal generated by this LED breakthrough is roughly equivalent to the F_o signal generated by five cells of *D. tertiolecta*. It is important to understand that this does not impact on the test results, simply because both the level 1 and level 2 tests only use the values of F_v, which are unaffected by LED breakthrough.









The data from Tests B to G are summarised within Figure 10. Test A is excluded because there is no level 2 result from this test.

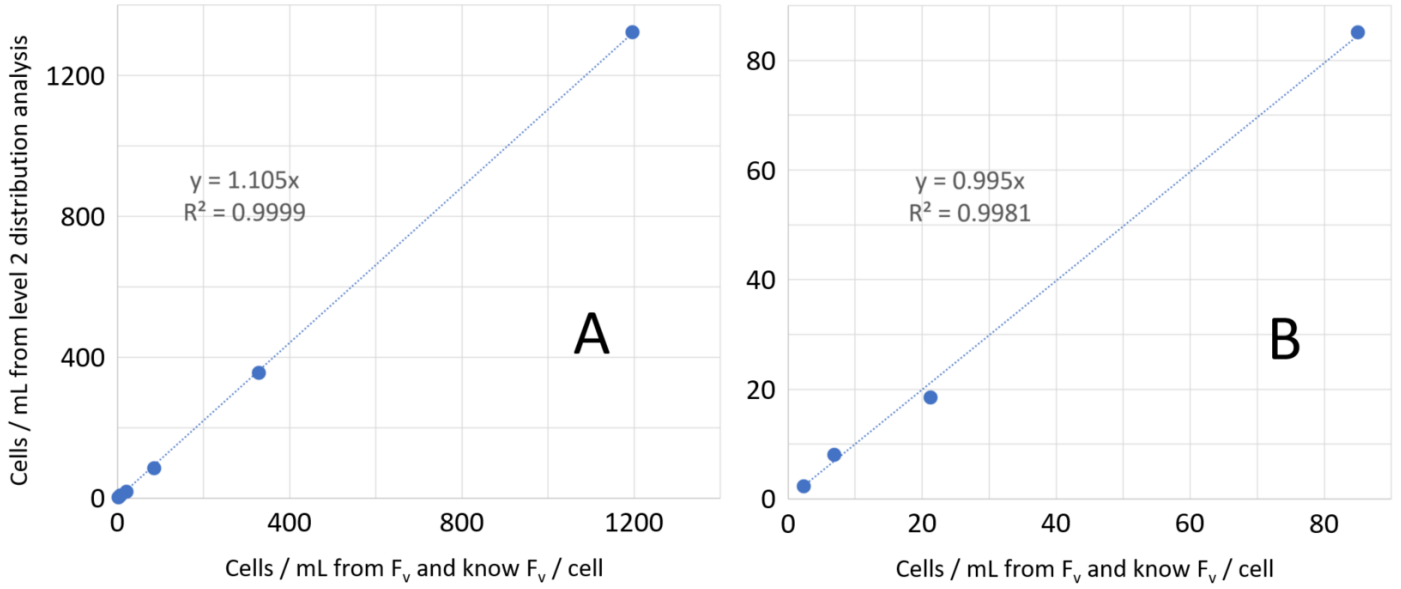


Figure 10: Estimates of cell density derived from F_v and known F_v per cell values and the level 2 distribution analysis. All values are from the primary data from a dilution series of healthy, cultured cells of *D. tertiolecta*, presented within the above screenshots (Test B to Test G). **A** shows values from Tests B to G, **B** shows values from Tests D to G.

Technical information

9 Instrument calibration

During a test, chlorophyll fluorescence from phytoplankton is excited by some combination the four LED wavebands within FastBallast and detected by a photomultiplier tube (PMT). A short pass filter located between the LED arrays and sample, in combination with a bandpass filter located between the sample and PMT, minimises filter breakthrough (light from the LEDs reaching the detection window of the PMT).

Instrument calibration comprises:

1. Standardisation of the output from the PMT over a range of gain settings
2. Measurement of the output from each LED waveband over a range of drive currents
3. Normalisation of all four LED wavebands

The PMT calibration allows the potential between

9.1 PMT calibration

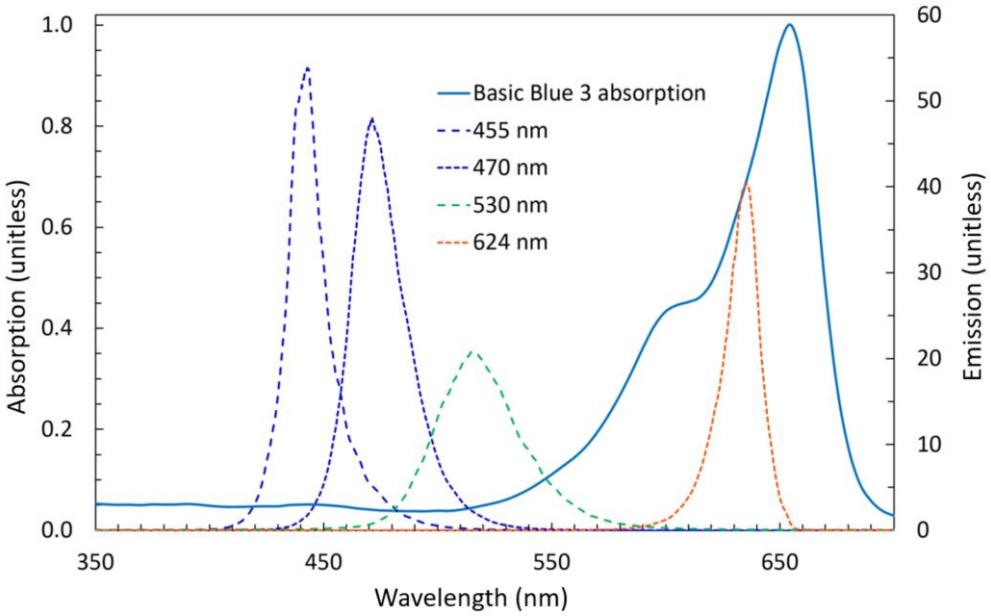
The FastBallast PMT is calibrated using four concentrations of a fluorophore called Basic Blue 3 (BB3) dissolved in MilliQ water. As shown below, the absorption spectrum of BB3 is relatively flat over the emission spectra of the 455 nm and 470 nm waveband LEDs. The PMT calibration only used the 455 nm LED waveband to excite BB3 fluorescence. The emission spectrum of BB3 overlaps very strongly with the transmission spectrum of the bandpass filter in front of the PMT, providing an easily detectable fluorescence signal.

For historical reasons, the four concentrations of BB3 are selected to provide the same fluorescence signal as chlorophyll *a* dissolved in 90% acetone to final concentrations of 0.05, 0.4, 1.0 and 2.0 mg m^{-3} when fluorescence is excited by the 455 nm waveband with FastBallast. Two parameters are derived from the PMT calibration, which quantify the change in PMT sensitivity with eht (V) and the signal slope at the reference PMT eht (560 V). These values are stored within FastBallast and are used to normalise the fluorescence signal in real time. This approach allows for the PMT eht to be changed at any point during a test. An auto-PMT function is integrated within FaBtest to optimise the PMT eht throughout the test. Out of range data and data acquired during auto-optimisation of the PMT eht are automatically rejected.

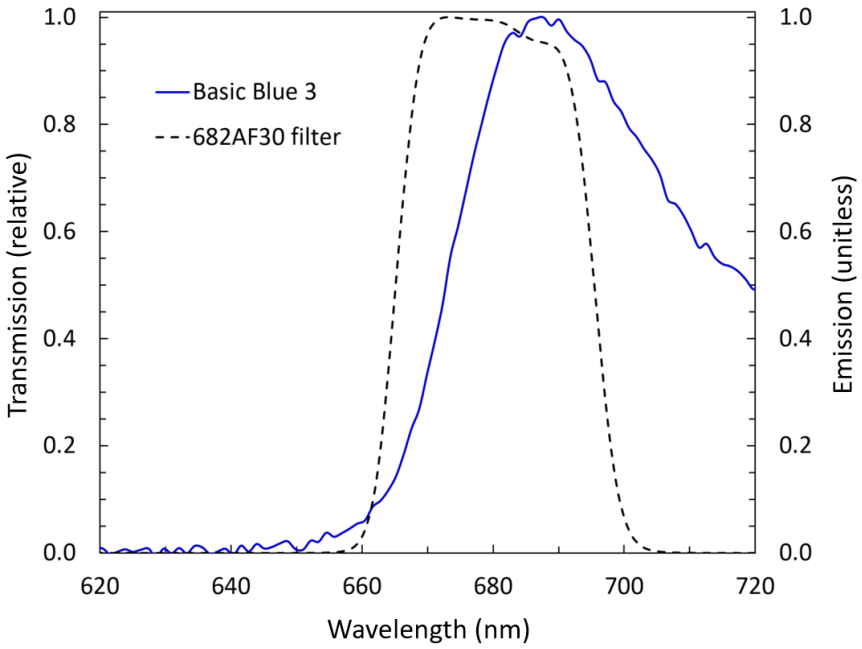
9.2 LED calibration

As with the PMT calibration procedure, the LED calibration procedure uses BB3 dissolved in MilliQ water to generate a fluorescence signal that can be detected by the PMT.

The LED output is recorded at each of five drive currents for each of the four LED wavebands. During real time data processing, an additional bias parameter, derived from one-off measurements from a range of phytoplankton cultures, is used to normalise LED channels against each other. The LED outputs at each drive current and the bias parameters are stored within FastBallast.



Absorption spectrum of Basic Blue 3 dissolved in MilliQ water plus the emission spectra for the four LED wavebands within FastBallast.



Emission spectrum of Basic Blue 3 dissolved in MilliQ water plus the transmission spectrum for the 682 nm bandpass filter located in front of the PMT in FastBallast.

Glossary

Term	Definition
Active fluorescence	The increase in the normalised fluorescence signal that is induced by a saturating pulse (see F_v).
Activity	Shorthand for Active fluorescence (F_v).
ADC	Analog to Digital Convertor. The value presented within FaBtest is percent of full scale. FaBtest automatically adjusts the eht voltage on the PMT to keep this value optimised.
Baseline fluorescence	Fluorescence signal arising from sources other than active PSII complexes.
F_o	The 'origin' of variable fluorescence (F_v). F_o can include fluorescence from sources other than photochemically active PSII complexes.
F_m	The 'maximum' fluorescence, assessed as the asymptote of the fluorescence signal induced by a saturating pulse.
F_v	Variable (active) fluorescence, calculated as $F_m - F_o$ Active fluorescence (F_v) originates from photochemically active (functional) PSII complexes. Photochemistry provides the energy required for cell growth and maintenance.
F_v/F_m	The proportion of the total fluorescence signal that is active. In the absence of Baseline fluorescence, this parameter provides an estimate of PSII photochemical efficiency.
Offset	A mathematical offset applied to the reported Activity (F_v).
PMT	Photo-multiplier tube. This is the highly sensitive device used within FastBallast to detect the fluorescence signal.
PSII	Photosystem II. Active fluorescence (F_v) originates from PSII complexes.
RSigma	RSigma provides the rate at which the fluorescence signal increases between F_o and F_m during each measurement pulse.
Saturating pulse	A short, bright pulse of light applied to a ballast water sample. This pulse increases the fluorescence signal from F_o to F_m by transiently saturating PSII photochemistry. The saturating pulse applied by FastBallast is 400 µs duration at several times full sunlight.

Cited and other useful references

Cullen, J.J. and MacIntyre, H. L. (2015) On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment.
J. Appl. Phycol. DOI: 10.1007/s10811-015-0601-x

Govindjee, Kern, J.F., Messinger, J. and Whitmarsh, J. 2010. Photosystem II. In: Encyclopedia of Life Sciences (ELS). Jhon Wiley and Sons, Ltd: Chichester. DIO: 10.1002/9780470015902.a0000669.pub2.

Kolber, Z. S., Prásîl, O. and Falkowski, P. G. 1998. Measurements of variable chlorophyll fluorescence using fast-repetition rate techniques: defining methodology and experimental protocols. Biochim. Biophys. Acta 1367:88–106.

MacIntyre, H. L. and Cullen, J.J. (2016) Classification of phytoplankton cells as live or dead using the vital stains fluorescein diacetate and 5-chloromethylfluorescein diacetate. J. Phycol. 52:572–589

Oxborough, K. Moore, C.M., Suggett, D.J., Lawson, T., Chan, H.G. and Geider, R.G. 2012 Direct estimation of functional PSII reaction centre concentration and PSII electron flux on a volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data. Limnol. Oceanogr.: Methods 10:142–154.

Silsbe, G.M., Oxborough, K., Suggett, D.J., Forster, R.M., Ihnken, S., Komárek, O., Lawrenz, E. Prášil, O., Röttgers, R., Šicner, M., Simis, S.G.H., VanDijk M.A. and Kromkamp, J.C. 2015. Toward autonomous measurements of photosynthetic electron transport rates: An evaluation of active fluorescence-based measurements of photochemistry. Limnol. Oceanogr.: Methods 13:138–155.

Steinberg, M., First, M., Lemieux, E., Drake, L., Nelson B., Kulis D., Anderson D., Welschmeyer, N. and Herring, P. (2012) Comparison of techniques used to count single-celled viable phytoplankton. J. Appl. Phycol. 24:751–758. DOI:10.1007/s10811-011-9694-z

Spares and replacement parts

From Panasonic:

http://panasonic.net/avc/pc/

Item	Part number
Panasonic Lithium Ion 2 Cell Battery Pack	FZ-VZSU94W
Panasonic 4-Bay Battery Charger	FZ-VCBM11U

From CTG Ltd:

http://www.chelsea.co.uk/

sales@chelsea.co.uk

Item	Part number
FastBallast stir unit	2355-138-PL-A
Panasonic ToughPad with FaBtest installed	2355-149-PL-A
FastBallast charger with 15 V at 1.2 A output	2355-137-PL-A
FastBallast to USB cable	2355-085-PL-A
FastBallast Ethernet cable	2355-086-PL-A