Using Fast Repetition Rate (FRR) fluorometry to monitor phytoplankton at the IMO D2 standard within ballast water discharge

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FRR and PAM

Within the field of active fluorometry, FRR is often compared with Pulse Amplitude Modulation (PAM). The major differences between these methods arise from their original applications: while the FRR method was specifically developed for working with phytoplankton within an aqueous phase at low optical density, PAM was developed for working with static samples at much higher optical densities, as found within terrestrial plants and benthic autotrophs such as sea grasses, macroalgae and microalgae within biofilms.

Clearly, the system requirements for monitoring phytoplankton within the natural environment are very similar to those for the monitoring of ballast water discharge: extremely high sensitivity, wide dynamic range and fast response time. Conversely, the high optical density of terrestrial plants and benthic autotrophs presents far less of a technical challenge. Consequently, while FRR systems incorporate the 'never waste a photon' approach to active fluorometry, PAM-based systems effectively exclude a large proportion of the available fluorescence signal by using DC light to saturate photosynthesis.

The end result is that while FRR can operate within the sensitivity range required to meet the IMO D2 standard for the detection of viable phytoplankton cells, and work with fast moving water, PAM-based systems are more than an order of magnitude less sensitive and unsuitable for use with moving water.
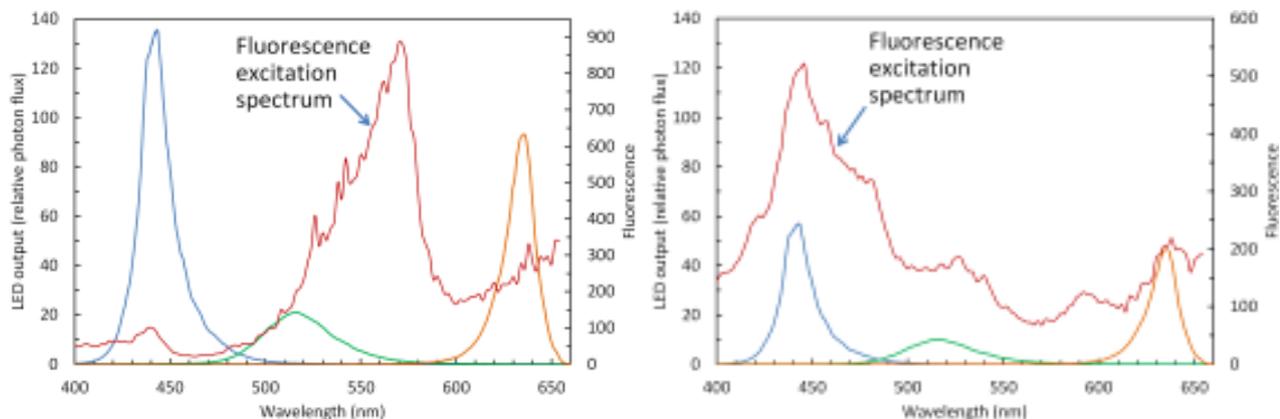
The FRR method

The FastBallast fluorometer, produced by Chelsea Technologies Group, is derived from our established oceanographic fluorometer, FastOcean. FastBallast is specifically designed to monitor ballast water in real time to the IMO D2 standard (10 to 50 µm category), through the monitoring of variable fluorescence (F_v) emitted from viable phytoplankton cells.

Although the relationship between viable cell number and F_v is cell size dependent, FastBallast can resolve the F_v emitted from even the smallest cells specified within the IMO D2 standard for phytoplankton, at the maximum specified viable cell concentration (ten cells per millilitre of sea water, which is roughly equivalent to 0.01 mg m^{-3} chlorophyll *a*). In addition, the large dynamic range of FastBallast allows for the detection of very low levels of F_v on top of a large non-variable 'baseline' fluorescence signal (F_b) emitted from non-viable cells, free chlorophyll *a* and CDOM.

To ensure that phytoplankton from all groups are detected, FRRf3 incorporates three LED channels, with emission peaks at 450, 530 and 624 nm. The plots below show the LED output spectra alongside fluorescence excitation spectra for the cyanobacterium, *Synechococcus* 9903 (left) and the coccolithophore, *Emiliania huxleyi* (right). In both cases, the combination of three LED channels

generated the output required to quantify F_v. Similarly successful tests have been completed with diatoms, chlorophytes dinoflagellates and cryptophytes (data not shown).

One important practical feature of the FRRf method is that each measurement takes 200 μs. This means that data can be collected from fast moving water (up to two metres of linear flow per second), without any significant change of sample during the measurement.

FRR data

The image shown right is an annotated screen shot from the FastPro8 graphic user interface (GUI), which incorporates real time processing of the data stream from a FastBallast sensor. This involves application of a fully automated, iterative algorithm, which generates the curve fit to the FRR data (shown as the solid blue line through the data points within the image). The required value of F_v is calculated, as part of the automated process, as the difference between the extrapolated value at the zeroth flashlet (F_o) and the asymptote of the curve (F_m).
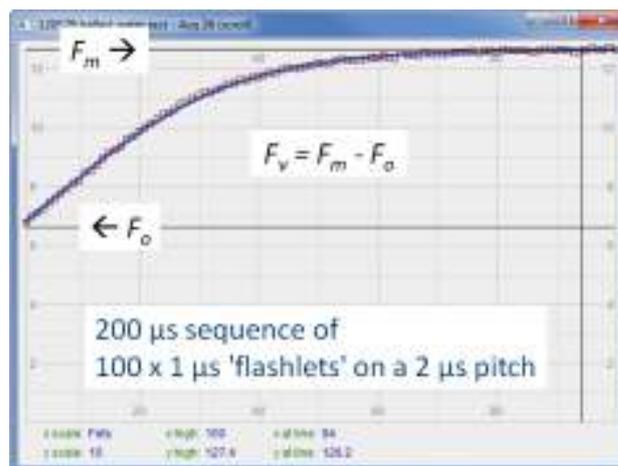

To develop an effective online system for testing to the IMO D2 standard for phytoplankton, we have worked to the following requirements specification:

1) Resolve F_v as at below 5% of the total fluorescence signal, with the remaining fluorescence signal coming from non-viable cells

2) Resolve F_v at the equivalent of 0.01 mg m^{-3} chlorophyll a in acetone from all phytoplankton groups

3) Operate over prolonged periods (several weeks) without human intervention

4) Process data in real time, to report compliance as a red light / green light

The data presented below illustrate how FastBallast can satisfy requirements 1) and 2). The first figure shows the F_v emitted from 2% viable cells when combined with 98% non-viable cells. The relative amplitude of the fluorescence from the viable and non-viable components is consistent with the non-viable cells emitting at F_m. Additional measurements (data not shown) support this interpretation. An important consequence of the high non-viable to viable cell ratio is that the reported value of F_v/F_m is 0.02, while the F_v/F_m from the viable cells (verified separately) is very much higher, at around 0.5. This is

important because F_v/F_m (which provides an estimate of PSII photochemical yield) is widely used as a proxy of cell viability. In this instance, a simple analysis of these data could lead to the conclusion that the sample contains a very high number of non-viable cells (and is therefore compliant with the IMO D2 standard) whereas, in reality, the sample contains a much smaller number of viable cells, which is still roughly 100 times too high to satisfy the IMO D2 standard.
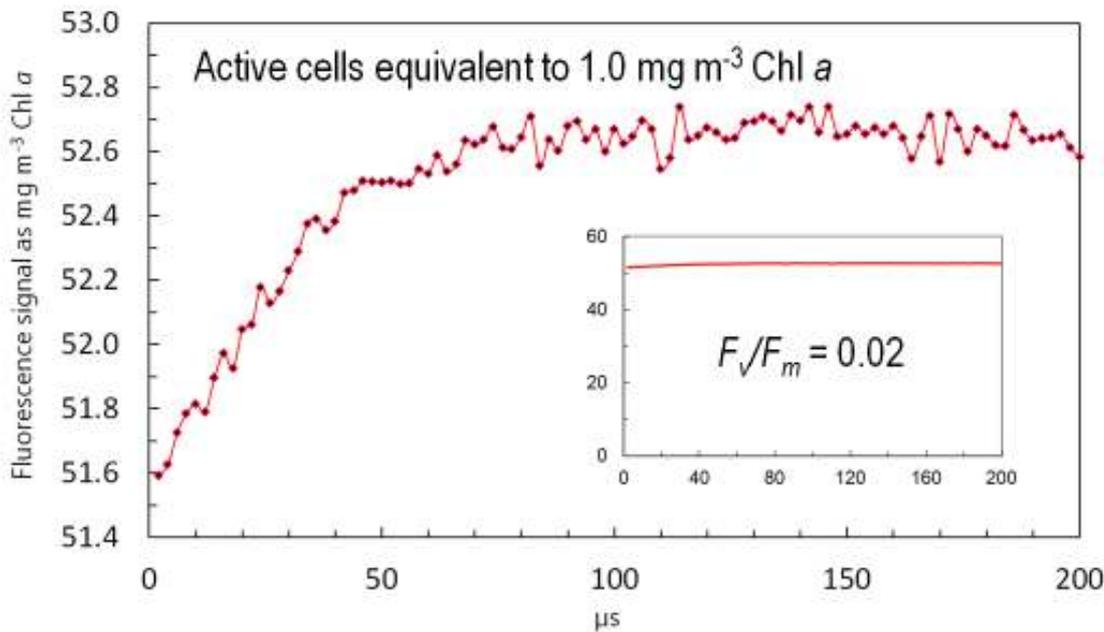
The data presented in the images below illustrate the ability of FastBallast to satisfy point 2) of the requirement specification, by clearly resolving F_v, even at the equivalent of 0.01 mg m^{-3} chlorophyll *a*. As within the earlier image from FastPro8, the solid blue lines through the data points are curve fits, generated in real time using a well established iterative algorithm.
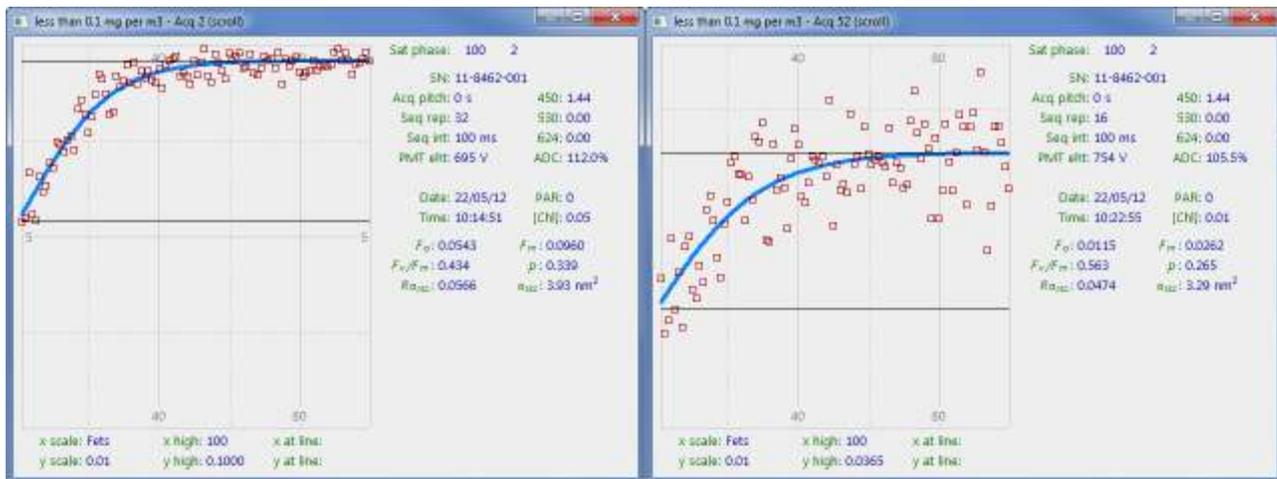
Physical installation

The FastBallast fluorometer is designed to operate within a marine environment. As such, it uses high grade titanium for the external housing and A4 stainless steel fixings. It is pressure rated to 60 bar. Data are streamed to FastPro8 for real time data processing, whilst simultaneously being logged internally as backup. A number of options are available for transferring data between the installed FastBallast and FastPro8.

With regard to point 3) of the requirements specification, FastBallast is designed to require virtually no maintenance between biannual calibrations. In addition, Chelsea Technologies Group have considerable expertise in the design of manifolds and sample chamber that are effective in using flowing water to keep the optical surface clear of sediment and biofouling, should this prove necessary.

At the time of writing, a red light / green light facility, as detailed within point 4) within the requirements specification, has not been activated within FastPro8. However, all of the required underlying code is in place to ensure that this aspect of the system can be implemented very quickly, once the thresholds for triggering a red light event have been established.

The image below left shows two FastBallast fluorometers ready for in-water testing. The image below right shows the proposed setup for FastBallast, to provide continuous monitoring of ballast water during discharge. The four connectors on the end plate provide a wide range of options for powering the sensor and transferring data to FastPro8 in real time.

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