ENVIRONMENTAL MONITORING

A Novel System for the Assessment of Quality of Water Courses

Chelsea Technologies Group FAST*tracka* II - 2nd Generation Fast Repetition Rate Fluorimeter

- **Provides baseline on algae activity from which effects of pollutants and fertilizers can be monitored.**
- **Suitable for both investigative and operational monitoring.**
- **Simple interconnectivity for network building.**

Monitoring agencies have in recent years seen an increased demand for a better understanding of watercourses from their source to the seas. This demand has been heralded by such drivers as the Water Framework Directive within the European Union.

Although within these programmes there is a clear call and defined structure for an efficient use of resources (classification of and within catchments), there will inevitably be a requirement for increased monitoring. Compounding this pull on resources, within these programmes there exists the requirement to detect many individual pollutants and naturally occurring toxins.

From the instrument manufacturers perspective this equates to a market requirement for inexpensive sensors that can be flexibly deployed. However, it is also recognised that a demand would exist for a broad-spectrum sensor that would detect an incident without necessarily determining the specific pollutant. Such sensors can be adopted within sentry stations for operational monitoring systems, and provide alarms to trigger investigative monitoring to determine cause.

The FAST*tracka* II fluorimeter (Fast Repetition Rate Fluorimeter (FRRF)) provides such a tool for monitoring biological elements to assess ecological status. The technique, licensed by Chelsea Instruments from Brookhaven National Laboratory, USA, provides measurements of dynamic fluorescence parameters in real time and *in-situ*.

Open watercourses are vulnerable to spills from farms and factories, where the quality of the water course can be degraded by destruction from herbicide, or excessive growth due to increased levels of nutrients from fertilizers.

Work at Oakridge National Laboratory, USA has found that flourescence from water bourne algae is affected by toxic chemicals. The fluorescence signal from algae in chemically polluted water is impaired compared to signals from algae in clean water, whereas the signal is increased when nutrients are added. In a monitoring situation, algorithms based on rate of change of fluorescent signal are more appropriate than an absolute fluorescent measurement, as variation in algae concentration and ambient light levels will effect the absolute signal.

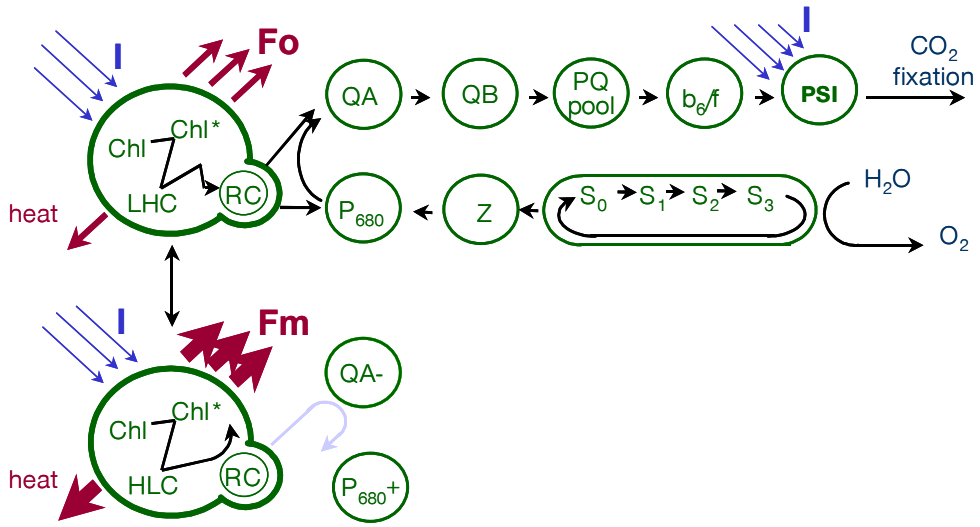
NOVEL SYSTEM FOR CONTAMINANT DETECTION

The approach is to measure a parameter that can be related directly to the physiology of the algae independent of environmental variations. The FRRF measurement achieves this by measuring a time dependent fluorescence signal that arises solely from chlorophyll that is active in photosynthesis. A standard fluorescence measurement on the other hand cannot discriminate between chlorophyll that is free in the water column, present in dead algae and in physiologically active organisms.

The FRRF response is real-time (sub-second) and concentration independent. The only factor limiting the response is the detection limit of the FRRF fluorimeter, as will also be the case with any fluorimeter making standard fluorescence measurements. The FRRF fluorimeter is actually probing the photochemistry of chlorophyll photosynthesis, one of the metabolic processes within algae that is known to be affected by both nutrients and pollutants.

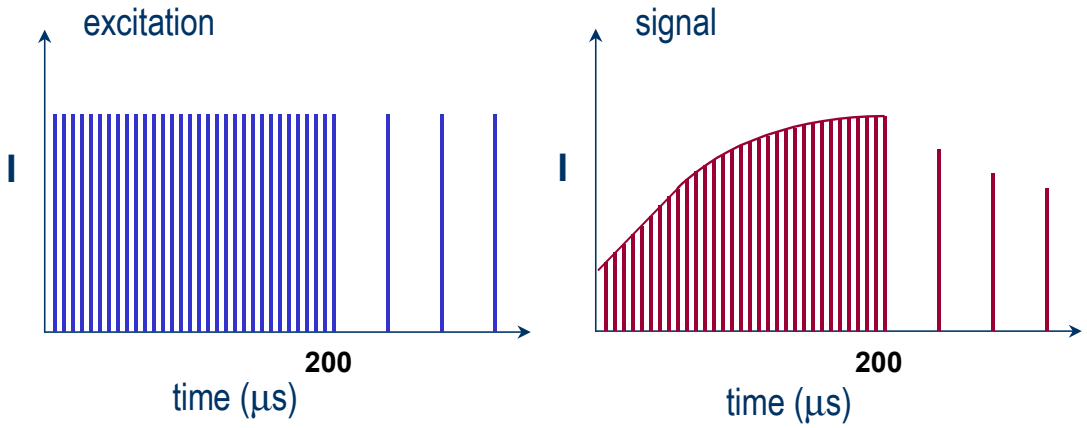
SCIENTIFIC PRINCIPLES

A schematic of the photosythesis process that occurs in aquatic microorganisms is illustrated schematically below:

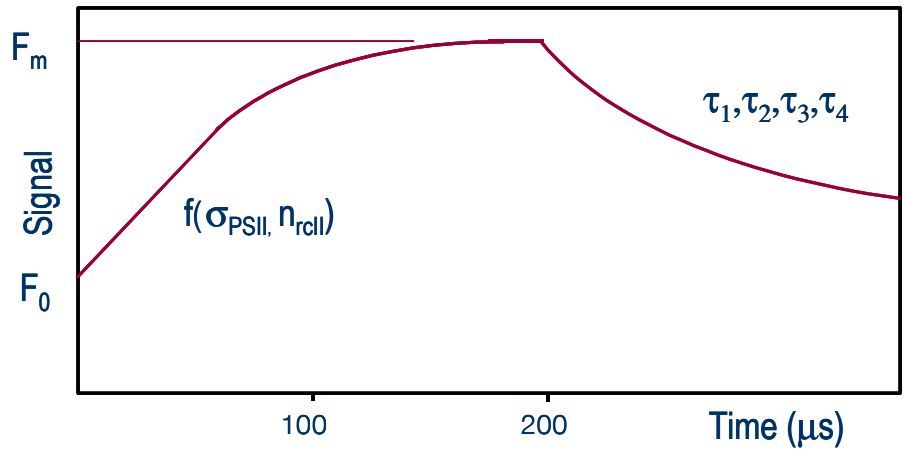


The light-harvesting complex (LHC) first absorbs ambient light (I) and the energy is transferred to the reaction centre (RC) through via a number of fluorescence energy transfer steps between chlorophyll molecules. On absorbing this energy the reaction centre initiates a cycle of photosynthesis, which takes approximately 200µSecs to complete. During this time the reaction centre remains 'closed' and is unable to absorb any more energy from the light-harvesting complex, this excess energy is then dissipated as heat or additional fluorescence.

In FRRF a rapid series of 1µSec light pulses, typically 100 pulses spaced 1µSec apart, is directed into the water sample. Each pulse closes a proportion of the reaction centres and the detected fluorescence signal increases from an initial value (F_0) to a maximum value (F_m) when all the reaction centres have been closed. This time-dependent fluorescence signal can only arise from organisms that are photosynthetically active; chlorophyll that is either free in solution or present in dead organisms may change the value of F_0 but will not produce this so-called 'variable fluorescence'.



The magnitude and shape of the response (see diagram below) can be used to derive a number of important parameters that relate to the health of these organisms and their photosynthetic activity and because of this any chemical pollutants that affect the photosynthetic process are likely to be detected using FRRF.



$(F_m - F_0)/ F_m$: photochemical quantum efficiency (F_v/F_m)
σ_{PSII}: efficiency of light harvesting (m^2 photon^{-1})
n_{rcII}: concentration of active reaction centres
τ_i: rate constants of electron transfer from PSII to PSI

The most important parameter obtained from FRRF is the variable fluorescence expressed as a fraction of the maximum signal, known as Fv/Fm. This parameter is related to the photochemical quantum efficiency and can vary from very low values for organisms in a poor environment to a maximum value of around 0.65 for those in an optimal one. It is likely that pollutants or toxins will have a direct impact on this parameter and importantly, because the value is expressed as a ratio, it is independent of chlorophyll concentration.

Other parameters that can be obtained from the variable fluorescence response are the concentration of reaction centres (n_{rcII}) and the absorbance cross-section of the light harvesting complexes (σ_{PSII}). For example, in nutrient rich environments where there is plenty of light, the light harvesting complexes will undergo conformational changes that optimise their light collection efficiency and the absorbance cross-section will increase.

Again one might expect toxins to have an influence on this parameter as well as the more obvious reaction centre concentration, which will be related to the number of living organisms in the sample.

Pollutants are also known to affect the electron transfer processes from QA to PSI (see schematic above). These occur on a longer timescale than the excitation response and can be assessed by probing the system using single light pulses spaced by approximately 40µSecs to monitor the drop in fluorescence signal as the system relaxes back to its 'ground state'. It may then be possible to detect the influence of pollutants or toxins through a change in the time constants associated with this process.

In all cases one would be looking for a step change in any of these parameters as an indication of contamination compared to what is likely to be a gradual drift due to weather or seasonal factors.

FAST*tracka* II fluorimeter shown with dark chamber

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