# Portable LED fluorescence instrumentation for the rapid assessment of potable water quality

J. Bridgeman [a,*], A. Baker [b], D. Brown [a,c], J.B. Boxall [c]

[a] School of Civil Engineering, University of Birmingham, Birmingham, United Kingdom
[b] Connected Waters Initiative Research Centre, University of NSW, Sydney, NSW 2052, Australia
[c] Department of Civil and Structural Engineering, Sir Frederick Mappin Building, Mappin Street, Sheffield S1 3JD, United Kingdom

## HIGHLIGHTS

- LED fluorimeter developed for accurate assessment of potable water quality
- Device offers potential to promote a proactive means of monitoring water quality.
- 4 bacteria isolated from water show a clear and different response between strains.

## ARTICLE INFO

## ABSTRACT

Characterising the organic and microbial matrix of water are key issues in ensuring a safe potable water supply. Current techniques only confirm water quality retrospectively *via* laboratory analysis of discrete samples. Whilst such analysis is required for regulatory purposes, it would be highly beneficial to monitor water quality *in-situ* in real time, enabling rapid water quality assessment and facilitating proactive management of water supply systems.

A novel LED-based instrument, detecting fluorescence peaks C and T (surrogates for organic and microbial matter, respectively), was constructed and performance assessed. Results from over 200 samples taken from source waters through to customer tap from three UK water companies are presented. Excellent correlation was observed between the new device and a research grade spectrophotometer ($r^2 = 0.98$ and 0.77 for peak C and peak T respectively), demonstrating the potential of providing a low cost, portable alternative fluorimeter. The peak C/TOC correlation was very good ($r^2 = 0.75$) at low TOC levels found in drinking water. However, correlations between peak T and regulatory measures of microbial matter (2 day/3 day heterotrophic plate counts (HPC), *E. coli*, and total coliforms) were poor, due to the specific nature of these regulatory measures and the general measure of peak T. A more promising correlation was obtained between peak T and total bacteria using flow cytometry. Assessment of the fluorescence of four individual bacteria isolated from drinking water was also considered and excellent correlations found with peak T (*Sphingobium* sp. ($r^2 = 0.83$); *Methylobacterium* sp. ($r^2 = 1.0$); *Rhodococcus* sp. ($r^2 = 0.86$); *Xenophilus* sp. ($r^2 = 0.96$)). It is notable that each of the bacteria studied exhibited different levels of fluorescence as a function of their number. The scope for LED based instrumentation for *in-situ*, real time assessment of the organic and microbial matrix of potable water is clearly demonstrated.

## 1. Introduction

Regulations place a duty on water companies to supply water that is wholesome at the time and point of supply, with wholesomeness defined by reference to prescribed concentrations assigned to various microbiological, chemical and physical parameters. Prescribed concentrations or values for microbiological parameters rely on proven, but dated indicator organisms, such as coliform bacteria, *E. coli* and colony counts. In addition to meeting standards, water must not contain any micro-organism or parasite at a concentration which would constitute a potential danger to human health.

The challenge to ensure microbial quality is addressed by water companies through disinfection, both during treatment processes and *via* the maintenance of a residual in most distribution systems. The most commonly used disinfectant in the developed world is chlorine. The use of chlorine, ozone, or chlorine dioxide as a disinfectant in

* Corresponding author.
  *E-mail addresses:* j.bridgeman@bham.ac.uk (J. Bridgeman), a.baker@unsw.edu.au (A. Baker), j.b.boxall@sheffield.ac.uk (J.B. Boxall).

water rich in organic matter (OM) (which also acts as a microbial food source) can lead to the occurrence of potentially carcinogenic disinfection byproducts (DBP). Consequently, water companies must manage the competing needs of biological and chemical compliance. Thus, it is imperative that companies monitor the microbial and chemical quality of drinking water at various stages of treated water supply systems, including trunk mains, service reservoirs and the distribution network.

Organic matter is ubiquitous in every water supply system and monitoring of its concentration and attributes is important for issues such as source water ecological health, treatment cost and efficacy, control of disinfection by-products and biological regrowth in distribution. Organic matter concentrations are typically assessed as total organic carbon (TOC) and/or dissolved organic carbon (DOC). Assessment requires complex and time-consuming laboratory procedures such that the data can only be used retrospectively rather than for proactive or pre-emptive management.

Another primary concern to water utilities is to ensure that the drinking water that is supplied does not pose an unacceptable health risk to consumers. As the number and type of different pathogens present in waters is extensive, varied and dependent on a range of environmental factors, it is not feasible to isolate and identify each specific pathogen on a regular basis. Hence, reliance has traditionally been placed on the measurement of total plate counts, as an overall indicator of microbial load and detection of faecal indicator bacteria and other coliform bacteria for contamination. Although these culture based tests give precise enumeration, they can take more than 30 h to perform from sample receipt to results. It is also known that, in the natural environment, often only 1% or less of microbes can be cultured in this way, leading to what has become commonly known as the "great plate count anomaly" (Staley and Konopka, 1985; Amann et al., 1995; Allen et al., 2004).

The measurement of both organic and microbial matter thus currently relies on the collection and transportation of discrete samples that are then analysed by time consuming techniques (taking days). Such approaches are only able retrospectively to confirm (or otherwise) water quality. *In-situ* real time measurement is therefore highly desirable, as it would enable assurance of water quality, optimisation of process control and overall proactive and preventative operation of water supply systems. It would also be of great value to improve spatial and temporal coverage of water supply systems to fully develop and implement proactive management. Hence, there exists a pressing need for the development of novel technologies that will enable real time, *in-situ*, low powered and, ideally, continuous analysis of drinking water quality to improve the robustness of water supply system management. Brock (1987) affirmed the necessity of *in-situ* studies for describing environmental microbiology. Only then is it possible to identify the affinities, symbiosis and interactions with the surrounding environment, which are not applicable with indirect techniques (Souza et al., 2007). Similarly, the measurement of organic matter in an unaltered state makes results directly applicable only to the specific reaction conditions and water (Goslan, 2003).

Water quality is, of course, a function of inorganic pollutants as well as organics. Such inorganic pollutants may include nitrates and phosphates arising from run-off from agricultural land, heavy metals from highway run-off, ammonia from wastewater effluent discharges, arsenic occurring naturally in groundwater, or copper from household plumbing. Whilst the presence of these inorganics in water is recognised, the focus of the work reported here is on the detection of organic and microbial matter.

In the work reported in this paper, the development and deployment of a novel, LED-based instrument, capable of the *in situ* detection of fluorescence peaks T and C (surrogates for organic and microbial matter respectively) in water is described. The novelty of the work (and instrument) is demonstrated *via* the robust correlations observed between instrument results and those provided by well-established analytical methods including, for the first time, a comparison with flow cytometry data and an assessment of the fluorescence response of four individual bacteria isolated from drinking water.

## 2. Background

### 2.1. Current methods of organic and microbial determination

A range of different techniques have been developed and applied to characterise and quantify the organic and microbial matrix within water. Total organic carbon (TOC) is the most comprehensive and widely adopted operational measure used to quantify the presence of organic carbon atoms in waters. TOC is often synonymous with natural organic matter (NOM) because organic contaminants in naturally sourced water supply systems generally represent an insignificant fraction of the TOC (Leenheer and Croué, 2003). More sophisticated analytical techniques which differentiate physico-chemical properties are also available, including: resin abstraction (used principally to determine hydrophobicity), high performance size exclusion chromatography (HPSEC), gas-chromatography–mass spectrometry (GC–MS) and UV–visible absorbance optical properties (Chin et al., 1994; Visco et al., 2005; Bridgeman et al., 2011). Such techniques are predominantly laboratory-based and labour intensive, requiring extensive sample preparation and analysis, rendering them impractical in studies involving many sampling sites or frequent monitoring.

Standard methods used to test for the presence of heterotrophic bacteria (HB), total coliforms (TC), faecal coliforms (FC) and *E. coli* include, *inter alia*, the multiple tube method (presence of gas and acid at 44 °C), membrane filtration (MF), and colony-forming units (cfu) on agar media (Great Britain Department of Environment, 1983; Cumberland et al., 2011). The genera enumerated by any of these methods are highly variable since the cultivation medium of choice, incubation temperature and time, origin (river, surface water reservoir, treated and disinfected drinking water, *etc.*), season of the year, and age of the water sample all have a significant effect on which genera will grow under these selected conditions (Allen et al., 2004). These factors, in addition to the time taken to acquire results, have stimulated research into more reliable, all-encompassing, more rapid methods of detection, such as the use of flow cytometry (*e.g.* Veal et al., 2000; Hammes et al., 2008; Prest et al., 2013). Flow cytometric methods offer a fast, accurate and quantitative laboratory-based alternative to traditional culture based methods. However the sensitive nature of the equipment, the procedures involved, the necessity for expensive dyes (used for staining samples) and high initial capital cost of equipment, render flow cytometry inappropriate for use in frequent investigatory work in the field.

### 2.2. Applications of fluorescence spectroscopy to water quality assessment

An innovative and rapid way to monitor the quality of water is to exploit the natural fluorescence of DOM. Previous research, conducted predominantly on riverine, marine and waste waters, has identified relationships between fluorescence and organic (*e.g.* Patel-Sorrentino et al., 2002; Baker et al., 2008; Stedmon et al., 2011) and microbial (*e.g.* Determann et al., 1998; Reynolds, 2003; Wu et al., 2003; Elliot et al., 2006) water quality. Fluorescence emitted at 400–480 nm under excitation at 300–360 nm (fulvic-like fluorescence) is indicative of the presence of organic carbon (peak C), whilst fluorescence emitted at 340–370 nm under excitation at 220–240 nm or 270–280 nm (tryptophan-like fluorescence) is indicative of microbial activity (peak T) (Coble, 1996).

Fluorescence analysis is a rapid, reagentless technique that requires only small volumes of sample and little sample preparation. An important advantage of measuring fluorescence in comparison to the more widely adopted optical measurement of $UV_{254}$ absorbance is its sensitivity (Leenheer and Croué, 2003). Fluorescence measurements are typically 10–1000 times more sensitive than those of UV absorption

spectroscopy with single molecule detection possible and discrimination of different sources of organic matter that absorb at similar wavelengths (Skoog et al., 2004; LeBlanc and Dufour, 2002; Henderson et al., 2009). This is particularly relevant for the assessment of treated potable waters which exhibit low DOC, low UV absorbance and therefore low signal to noise ratio. Thus, the increased sensitivity of fluorescence for such analyses represents the optimum technique.

However, fluorescence analysis does have the potential to be confounded when assessing concentrated DOM samples as a result of inner-filtering, where emitted energy is absorbed and re-emitted at longer wavelengths by surrounding molecules.

Fluorescence spectroscopy has generally been reliant on expensive laboratory-based equipment, producing excitation emission matrices which are not readily quantified by the non-expert. These analysis systems typically use a high powered white-light source chosen for its high intensity and broadband spectral output, with appropriate spectral filtering to excite fluorophores in the sample under analysis. For example, a typical xenon arc lamp produces 150 W of output power in a uniform band from 200 to 2600 nm (Moe et al., 2005). Unfortunately, these light sources require high current power supplies and special handling, making them challenging to use outside of a laboratory environment.

Previous research has investigated the use of LEDs in arrays to produce a wider spectrum of light with higher spectral output (*e.g.* Hart and JiJi, 2002; Moe et al., 2005; Dickens et al., 2011). Drawbacks of early LED-based systems include insufficient light output, instability (poor lifetime), expense and, until recently, lack of lower wavelength LEDs, so limiting their application to long wavelength ultra-violet, visible and near infrared excitable fluorescent compounds (Hart and JiJi, 2002). However, recent step change advances in manufacturing processes are now making LEDs an attractive alternative to conventional white-light sources. LEDs offer a highly energy-efficient means of producing monochromatic light. They provide a concentrated, small, cool emitter ideal for miniature analytical devices because of their reduced power of operation, size, and longer lifetime and also provide spectral control without the need for high overhead optics. Recently, the availability and reductions in cost of UV LEDs that emit to 270 nm and below, have broadened the horizons for their use in LED-based fluorescence devices for new applications. Commercially available instruments, such as the Turner Designs Cyclops™ and Chelsea Technologies Group UvLux™, reflect the popularity of LEDs in portable instruments used for targeting a range of fluorophores in a variety of applications.

The sensitivity of fluorescence spectroscopy to all microbial material (rather than the 1% which can be cultured and enumerated as colony forming units, CFU) presents an opportunity to effect a major step change in water quality assessment techniques, moving beyond the dated and limited use of indicator organisms, to enhance security of supply to customers. In this paper, the capability and efficiency of an innovative battery powered, portable, LED-based instrument in detecting peak C and peak T fluorescence at levels potentially found in potable water supply systems is demonstrated. These peaks are of interest as peak C provides a surrogate for the DOC present in the water, whilst peak T will identify any microbial growth which occurs as a result of DOC presence and insufficient residual chlorine concentration in the water. Furthermore, whilst both peaks are quenched by chlorine, a loss of chlorine residual manifests itself *via* an increase in fluorescence.

A comparison of an LED-based instrument with a traditional bench top instrument is presented to assess whether the device provides a suitable, portable, low cost and low powered alternative, before assessing the device's performance against traditional regulatory organic and microbial measures which, as commented upon earlier, are economically and operationally sub-optimal. The feasibility of applying this novel technology in the field to provide real time, *in-situ*, low power and potentially continuous analysis, hence providing an expedient indicator of changes in organic and microbial water quality to improve the robustness of water supply management is discussed.

## 3. Methodology

### 3.1. Instrument design

The prototype portable device has been designed to house all optomechanics, electronic circuitry and rechargeable battery in an enclosed toughened plastic case type structure. The case also provides space for a notepad computer *via* which the software for device control is operated. Only a small area for the sample holder is openly visible, capable of accommodating both discrete, and flow-through, standard 10 mm quartz cuvettes. When closed, the dimensions of the device are approximately 425 (l) × 300 (w) × 225 (d) mm, with a weight of approximately 3.5 kg. The battery life for routine operation of the software is dependent on the usage of the notepad computer, but is estimated at approximately 8–9 h. As this is the first prototype, further size optimization is likely. Further, it is anticipated that the notebook computer will be replaced by a data logger and simple four number display. Two LEDs, pulsed only when sampling and fired sequentially with a 2-s delay, are used to excite the water sample in the peak C and peak T regions. Emission is detected by two corresponding photomultiplier tubes (PMTs) for each excitation wavelength, measuring at the peak and the Raman emission wavelengths, respectively. The PMTs are located at the centre points of the fluorescence signal at right angles to their respective LEDs in a "T" shaped formation (Fig. 1). This formation is identical for both channels, with the peak C set being layered vertically over the peak T set, thus enclosing the cuvette when it is present. Concave collection mirrors opposite the LEDs are used to refocus the LED light into the sample, thus maximising the potential output fluorescence signal density on the active area of each PMT.

The case also provides space for a notepad computer, which runs the software interface developed specifically to control the prototype device. The software user interface displays six values: *viz.* T and C fluorescence, the Raman signal of each channel (each read directly from separate PMTs), and the ratios of the T and C fluorescence signals over the corresponding Raman signal. The excitation wavelength for the T channel is 280 nm, with emission being recorded at 310 nm for the Raman signal and 350 nm for the peak T signal. For the C channel, the
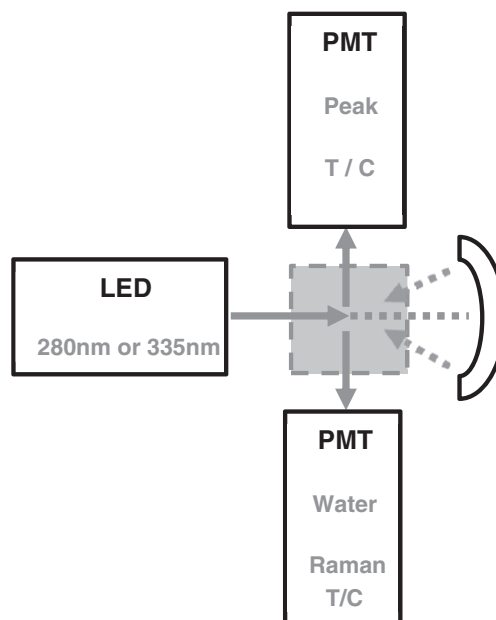


**Fig. 1.** Plan view of arrangement of LED and PMTs in device.

excitation wavelength is 335 nm and the emission is recorded at a wavelength of 380 nm for the Raman and 425 nm for the peak C signal. The emission wavelengths were selected based on findings from a previous work (Bridgeman et al., 2011). This configuration therefore permits the measurement of the ratio between the Raman signal and the corresponding emission signal and so allows the T and C peaks to be measured as a ratio of Raman intensity rather than as an absolute measurement. Normalisation of the fluorescence intensity in this way provides a useful internal standard and also means that potentially confounding effects of external variables, such as temperature fluctuations, are minimised.

Digital gain software on the instrument was incorporated to allow for the gain on the four photomultiplier tubes to be adjusted and measurement settings to be clearly defined, allowing for accurate comparison between groups of test results and to also allow for different measurement sensitivity ranges to be established, appropriate to the quality of water being inspected.

### 3.2. Experimental design

Laboratory investigations were conducted to (a) explore the device's sensitivity and confirm relevant settings suitable for potable water supply systems; (b) demonstrate if the LED device could match, or improve upon, a bench top device performance; and (c) compare measurement accuracy to alternative measures of organics and microbial determination.

Initial tests were undertaken on six samples of water from the Sheffield locality to optimise the instrument settings for the expected range of organic and microbial material within potable water supply systems. These six waters were selected to provide a representative spread of the types of water quality that would be expected in water supply systems from raw waters through to customer taps. Settings were adjusted to ensure that the viable measurement range was as broad as possible. (Full details are presented in the Supplementary information.) All results reported herein had consistent instrument settings. Results are presented as averages of three real repeats on each water sample which had three connected technical repeats (total of nine readings).

Validation of the sensitivity and accuracy of the portable device was demonstrated *via* a comparison of fluorescence intensities recorded on the portable device with measurements at equivalent peak C and T excitation and emission wavelengths on a bench top spectrophotometer (FluoroSENS, Gilden). For this purpose, 209 samples were collected through the three collaborative water company water supply systems, from source waters through to customer taps. The organic and bacterial content of the majority of these water samples was also assessed in the water company's (UKAS) accredited laboratories by the traditional regulatory measures of TOC, heterotrophic plate counts (HPC) at 22 °C (48 h) and 37 °C (24 h), *E. coli* and total coliforms.

Due to the previously described shortcomings of traditional bacteriological determinations, further tests were conducted on a selection of the collected water samples to compare the performance of the portable device against more selective bacteriological measures. Total bacteria counts of 48 of the samples were made using flow cytometric staining with a mixture of SYTO 9™ and propidium iodide dyes. To explore further possible bacterial specific florescence response, four distinct bacterial isolates were tested at a range of concentrations.

### 3.3. Laboratory methods

Fluorescence was measured in a 10 mm pathlength quartz cuvette (Starna Scientific Ltd), on both the prototype portable instrument and a bench top instrument. Emission scans were taken on the bench top device at the same peak T (280 nm) and peak C (335 nm) excitation wavelengths as the portable device, with the emitted fluorescence detected in 2 nm steps between 290–400 nm and 345–450 nm,

respectively, and excitation and emission slit widths both set to 5 nm. Sets of three repeat analyses were made for each sample on both devices, with each repeat being subjected to three technical repeats. Results presented are therefore an average of nine readings. Between each test, the cuvette was thoroughly rinsed with distilled water. To maintain consistency of measurement conditions, blank scans using a sealed cell containing deionised water were run systematically to measure the intensity of the Raman line of water at 348 nm excitation wavelength. The mean Raman value during the study period was 412 intensity units. Before and after each set of tests a reading was taken on the portable and bench top device with distilled water in a sealed cuvette to check machine stability. All tests were conducted in temperature controlled laboratories at 20 °C. All fluorescence data are presented as the ratio of measured fluorescence to Raman intensity and are referred to as Raman-normalised fluorescence intensity.

Analysis of all regulatory organic and microbial measures took place at water company UKAS (United Kingdom Accreditation Service) laboratories. The majority of corresponding fluorescence measurements on the portable device were also made on-site, coupled with repeat analysis at the University of Sheffield laboratories when samples were tested again on the bench top fluorescence device. TOC was measured using an Aurora 1030 W analyser, using the non-purgeable method in which orthophosphoric acid is used to convert the total inorganic carbon to carbonic acid which is subsequently removed in a nitrogen carrier gas along with the purgeable organic carbon. The non-purgeable organic carbon (NPOC) is then oxidised using acidified sodium persulphate using heat (1030 W) as an initiator, before being sparged with nitrogen carrier gas for detection by a non-dispersive infrared detector. Results are presented as the mean of three valid measurements.

Standard methods were followed for the enumeration of both HPCs and the identification of *E. coli* and total coliforms. Heterotrophic bacteria numbers were derived from 1 ml samples cultured with 20 ml of sterile molten yeast extract agar in sterile plastic Petri dishes. Colony-forming units (CFU) were counted after incubating at either 22 °C (for 48 h) or 37 °C (for 24 h). Results are presented as CFU per 100 ml with all tests being performed in triplicate. *E. coli* and total coliforms were determined by the membrane filtration/plate count (MF/PC) method, whereby 100 ml of the sample was filtered through a sterile 0.45 μm cellulose nitrate filter (Millipore) and cultured on a sterile pad soaked in membrane lauryl sulphate broth (OXOID) and incubated for 4 h at 30 °C followed by 14 h at 37 °C. After incubation the dishes were examined for yellow and green colonies, signalling the presence of presumptive coliforms and *E. coli*, respectively. All green colonies were counted as confirmed *E. coli* and did not require any additional testing. All yellow colonies were counted as presumptive coliforms and were confirmed by sub-culturing onto two sets of TYEA confirmation plates to which an ONPG disc was added. In addition, one set of McConkey agar plates and one set of TSA agar plates were prepared. One set of TYEA plates were incubated at 44 °C, whilst all other plates are incubated at 37 °C. All tests were performed in triplicate and results presented as the mean CFU per 100 ml.

Flow cytometer analysis was performed using a BDTM LSRII Flow Cytometer System (BD Biosciences, UK). Samples were transported in sterile 50 ml falcon tubes in the dark and refrigerated at 4 °C until being analysed (within 6 h of collection). Water samples were stained with LIVE/DEAD® BacLight™ Kit (Invitrogen, UK) following the manufacturer's instructions. BacLight staining contains two nucleic acid-binding stains: SYTO 9™ and propidium iodide (PI). SYTO 9™ can penetrate all bacterial membranes, bind to nucleic acids and stain the cells green, whereas propidium iodide (PI) only enters cells with damaged membranes and the combination of the two stains yields red fluorescent cells. 1.5 μl of component A (SYTO 9 dye, 1.67 mM/PI, 1.67 mM) and 1.5 μl of Component B (SYTO 9 dye, 1.67 mM/PI, 18.3 mM) were added to 1 ml of the sample and incubated in the dark at room temperature for 20 min prior to measurement. Both dyes were excited by a blue 488 nm laser. SYTO 9 was detected by a 505 nm long-pass and a

530/30 nm band-pass filter set and PI by a 655 nm long-pass and a 660/20 nm band-pass filter set. Data were processed and analysed using the BDFACSDiva™ software (BD Biosciences, UK), and results presented as the average of triplicate measurements.

Isolation of bacteria from tap water was undertaken following Ramalingam et al. (2013). From the pure cultures selected, a small quantity of each of the bacteria was suspended in a liquid medium and grown at 25 °C for 72 h with agitation at 150 rpm. The suspended cultures were then centrifuged at 5000 rpm for 10 min, before being washed in a small volume of distilled water and centrifuged again. This cleaning process was repeated another two times, before the pellet was removed and suspended in 30 ml of distilled water and adjusted to give an optical density of 1 (at 595 nm) for each strain. Subsequently, the bacteria suspensions were diluted to different strengths (1:2, 1:4, 1:8 and 1:12) and then tested for their fluorescence response. Additional counts of total bacteria in each dilution were also made using flow cytometry by applying the same methodology as described earlier.

## 4. Results

### 4.1. Instrument comparisons

209 individual samples were collected at various locations through multiple water supply systems. Peak C and peak T intensities were determined for each sample using both the new portable instrument and a traditional bench top device. Figs. 2 and 3 show excellent correlation between the two instruments, with $r^2$ values of 0.98 and 0.77 for the full data sets for both peak C and peak T, respectively (each point displays the averages of nine readings with $\pm 1$ s.d.). Data in Figs. 2 and 3 have been broken down into different sample types and correlations for each subset are shown in Table 1. These comparisons indicate the portable device's ability to record the two peaks' intensities for a broad range of fluorophore concentrations typically expected in raw, partially treated and potable water samples, in a fraction of the time, cost and power used by the bench top device.

### 4.2. Comparison of peak C with total organic carbon

Peak C fluorescence intensity has been shown to correlate with TOC in a range of water types, from marine waters (Vodacek et al., 1995; Ferrari et al., 1996), a collection of surface waters (Smart et al., 1976), partially-treated waters (Bieroza et al., 2009, 2010), sewage and industrial effluents (Hudson et al., 2008), to ground waters (Stedmon et al., 2011). As the majority of waters tested as part of this study were randomly selected samples collected as part of routine water company regulatory analyses, a variety of raw (surface and ground water), partially treated, and treated waters are represented in our comparison of TOC concentrations and portable device peak C intensities (Fig. 4). At the lower TOC concentrations (i.e. 2.5 mg/l and below), the relationship between peak C fluorescence and TOC approximates to a linear one. However, as the TOC concentration increases, it is noticeable that the relationship is non-linear. This is to be expected and is a result of inner-filter effects confounding the measurements at higher TOC concentrations. The relationship between fluorescence and measured TOC across the full data range can best be described using a power function, such that peak C = $260.30 \times [TOC]^{0.32}$.

As the majority of TOC samples were not taken in the distribution system, as regulatory requirements do not necessitate it, there is a large spread in TOC concentrations, ranging from 0.16 to 17.1 mg/l. Analysis of those TOC values typically found in potable water distribution systems (i.e. TOC < 2.5 mg/l, $n = 107$) yielded a linear correlation between fluorescence and TOC ($r^2 = 0.75$). This is encouraging considering that sensitivity settings were set for a wider spread of waters whilst performing these tests (i.e. the instrument was not optimised for low TOC waters).

### 4.3. Comparison of peak T with regulatory measures of bacteria

Work was undertaken to consider whether comparison of regulatory microbial measures with readings from the portable device would yield robust correlations between culturable microbial presence and water fluorescence, thus demonstrating the future potential use of the device as a more expedient microbial indicator than current traditional methods. However, analysis of 190 samples found no discernible relationships between peak T intensity and 2-day (37 °C) plate counts ($r^2 = 0.01$), between peak T intensity and 3-day (22 °C) plate counts ($r^2 = 0.08$), or between peak T intensity and either presumptive coliforms or E. coli ($r^2 = 0.11$ and 0.08 respectively). Whereas the waters selected for peak C comparison with TOC were taken primarily from raw, partially treated and treated samples, the majority of samples tested for regulatory microbial measures were taken in distribution systems. Hence, many of the samples analysed display zero counts, albeit still with a fluorescence response. However, it is known that living and dead cellular material, non microbial compounds, and possibly exudates can all generate a peak T fluorescence response, and it is known that 1% or less of environmental microbes can be cultured by the HPC methods,
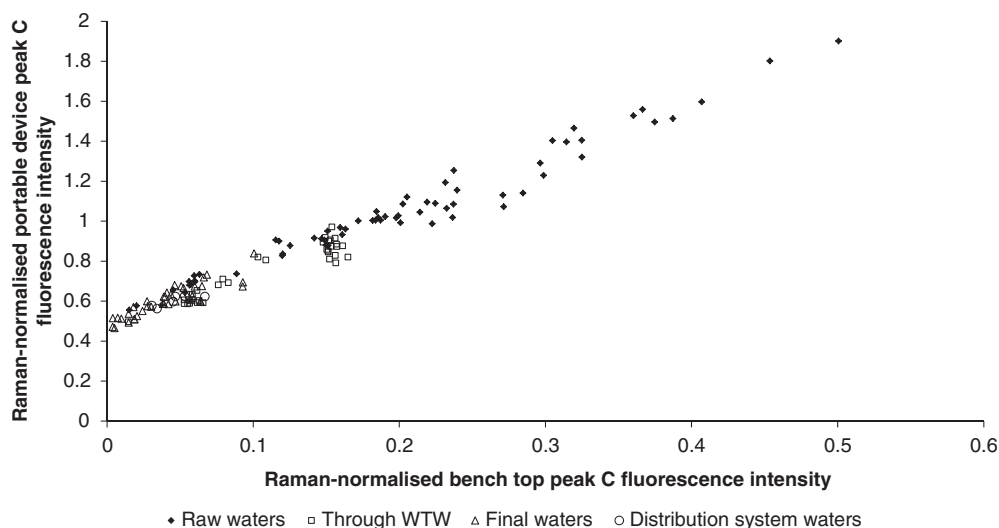


◆ Raw waters    □ Through WTW    △ Final waters    ○ Distribution system waters

**Fig. 2.** Peak C intensity comparison between new portable and traditional bench top devices.
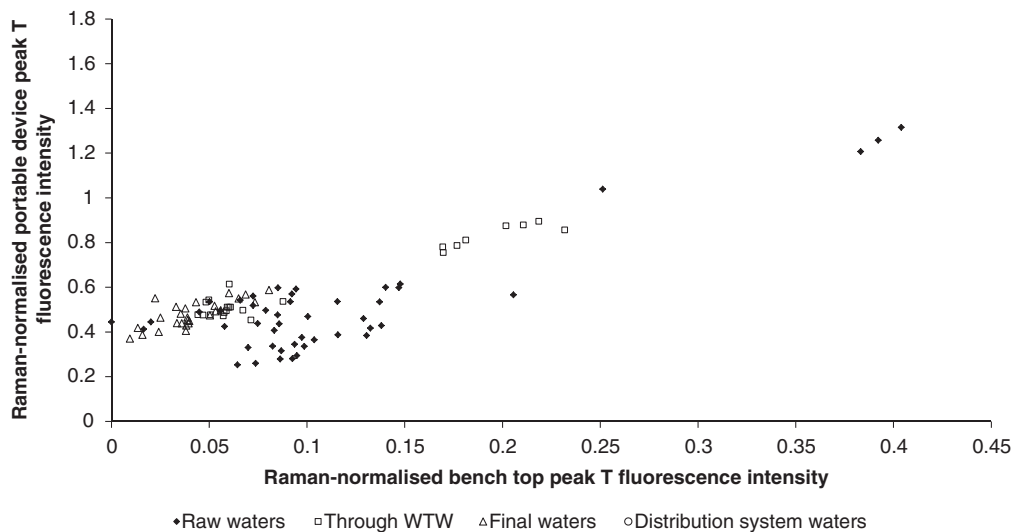
**Fig. 3.** Peak T intensity comparison between new portable and traditional bench top devices.

the "great plate count anomaly". Hence the lack of correlation is perhaps unsurprising. Consequently, the decision was taken to investigate peak T fluorescence in relation to flow cytometry and specific bacterial isolates.

### 4.4. Comparison of peak T with flow cytometer counts

Although large discrepancies between total bacterial concentrations and cultivable bacterial cell concentrations have been identified in previous studies (Staley and Konopka, 1985; Hammes et al., 2008), the former is still not included in routine regulatory monitoring, chiefly due to the lack of appropriate analytical equipment to make it resource and economically viable. Recently, flow cytometry coupled with fluorescent staining has been demonstrated for the rapid assessment of drinking water (Hammes et al., 2008; Lautenschlager et al., 2010; Ho et al., 2012; Prest et al., 2013), capable of providing a quantitative and accurate measure of information such as total cell concentration, bacterial viability and characteristics.

48 of the samples collected from collaborating water company distribution systems and tested using standard methods were also tested using flow cytometry. Total cell count results obtained reflect typical concentrations observed in previous studies on drinking water, in the order of $10^4$ to $10^5$ per ml of diverse microbial populations (Hoefel et al., 2003). Comparison of the portable device peak T intensity and these flow cytometry data, Fig. 5, reveal a reasonable correlation ($r^2 = 0.56$) and certainly an improvement in comparison to the regulatory microbial measures. It is interesting to note that the error bars associated with technical repeats of flow cytometry are generally greater than those associated with the new fluorescence device. The improved correlation reflects the benefits of flow cytometry identifying all bacteria in a sample rather than the culturable bacteria only. Hoefel et al. (2003) demonstrated that HPC counts in a mix of raw and potable waters were generally 2–4 log fold less than

the numbers of total bacteria reported using the same stains as the BacLight™ Kit in this study. With peak T fluorescence capable of picking up an area of identifiable bacterial activity, a correlation would therefore be anticipated.

### 4.5. Fluorescence response of individual bacteria

As an initial assessment of the portable device's potential for picking up the fluorescence response of individual bacteria which are found in potable water, four isolates used in a drinking water distribution related project at the University of Sheffield were assessed. Initial reasoning behind the selection of these bacterial isolates was based on their ubiquity (*Sphingobium* sp. and *Methylobacterium* sp.) in water distribution systems worldwide and also for their less common or unique presence (*Rhodococcus* sp. and *Xenophilus* sp.) in the chosen domestic drinking water (Ramalingam et al., 2013).

Each of the 4 strains of bacteria showed a strong and clear peak T fluorescence response on the portable device. A clear and different response to the dilutions (expressed at flow cytometry counts) of each of the bacteria with good correlation was observed (Fig. 6), with the two more commonly observed bacteria in distribution systems (*Sphingobium* sp. and *Methylobacterium* sp.) showing the greatest peak T intensities. It is noteworthy that the strains with the highest bacteria counts (*e.g. Rhodococcus* sp.) did not necessarily give the greatest fluorescence response, in keeping with the observations by LeBlanc and Dufour (2002) between different bacteria types. These results confirm that at least some of the peak T fluorescence which is observed in potable water can have a direct bacterial origin, but that the association is not trivial.

## 5. Discussion

Common methods used to measure the microbial quality of drinking water are based on spot samples, followed by analysis *via* time-consuming laboratory tests. However, contamination events in distribution systems are typically characterised as being stochastic, short-termed and difficult to detect (Stedmon et al., 2011). Therefore the probability of spot samples, typically performed (at most) on a weekly basis on small quantities of water, picking up a contamination event before it reaches consumers is low. Of the failures that are captured by regulatory sampling, few may be attributed to known or quantified events, as associations could be hidden by the potential for

**Table 1**
Peak C and peak T correlations between portable and bench top devices ($r^2$ values shown, $p$ value <1%).

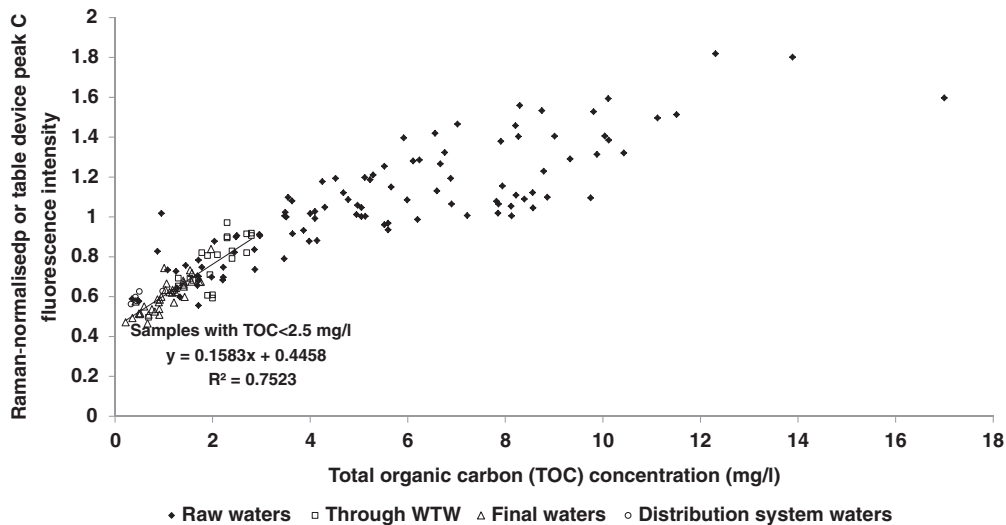|                     | Peak C | Peak T |
|---------------------|--------|--------|
| All data            | 0.98   | 0.68   |
| Raw                 | 0.96   | 0.74   |
| Through WTW         | 0.90   | 0.94   |
| Final water         | 0.79   | 0.58   |
| Distribution system | 0.72   | nd     |

**Fig. 4.** TOC concentration *versus* peak C intensity on portable device. (Raw water n = 107, through WTW n = 35, final waters n = 37, distribution system n = 6.)

bacteriological contamination to survive for long periods and even multiply if disinfection residuals are low (LeChevallier and Shaw, 1996).

Although fluorescence does not provide a direct measure of the presence or concentration of pathogens in a water supply, it does offer a potentially sensitive and online approach to detect sudden changes such as those brought about by the intrusion of contaminated water potentially bearing pathogens (Stedmon et al., 2011). Therefore, in a similar manner to how HPC tests are currently applied to understand changes in drinking water systems over time, online monitoring, using a device such as the one developed here, could be applied to alert operators to increases in general microbial activity. In contrast to HPC tests however, online monitoring provides the opportunity to build up a comprehensive picture of the range in water quality at any given location, to which any deterioration or sudden change can highlight the need for further investigation. Spot samples can only indicate that, at any given time, certain bacteria (not necessarily harmful to health) did or did not grow under the laboratory conditions and tests to which they were subjected. Further work is required to determine the device's capabilities as a continuous monitor and the potential issues which may arise when applying it in this way, such as optical fouling

and the determination of appropriate base levels from which fluctuations are determined.

Although uncertainty exists as to what the peak T results in this study could be correlated with, the potential for the device to be applied as a means of assessing contamination events, such as ingress into service reservoirs or cross water connections, is reinforced by employing a combined measurement of both peak C and peak T fluorescence. The majority of bacteria associated with water supply systems, including all human pathogens, are heterotrophs. As heterotrophic bacteria require carbon, nitrogen, and phosphorus in a ratio of approximately 100:10:1 (C:N:P), organic carbon may often be a growth-limiting nutrient in drinking water (LeChevallier et al., 1991). Assessment of peak C in addition to peak T, at defined sensitivities according to water type, therefore provides a valid tool for discrimination of water sources and assessing abnormal fluctuations in water quality to give operators an indication of the need for further investigation.

In the environmental literature, peak T has been linked with measures such as biological oxygen demand (BOD) (for example; Hudson et al., 2008; Bridgeman et al., 2013), which, acting as a measure of oxygen uptake in a water, acts itself as a surrogate for bacterial activity.
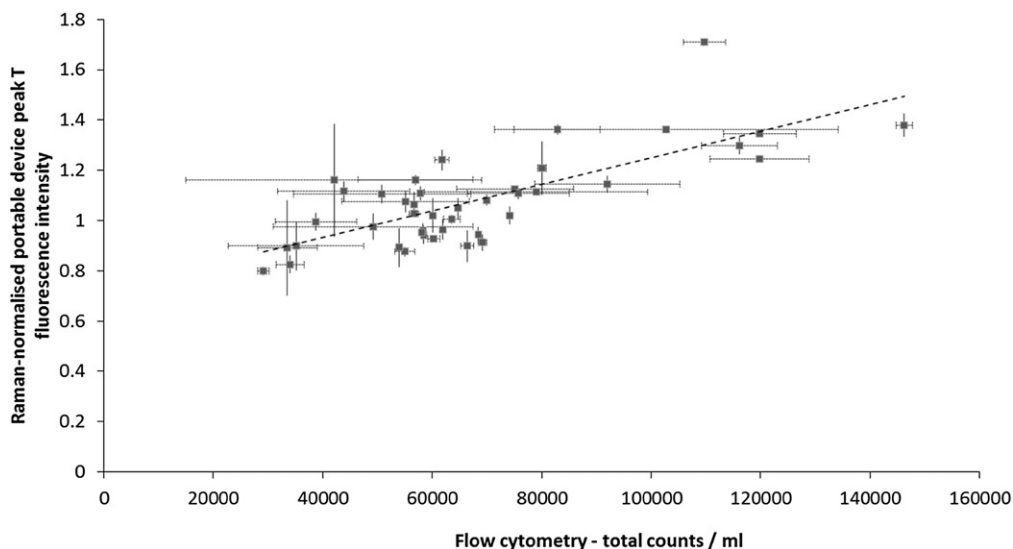


**Fig. 5.** Comparison of flow cytometry counts of 48 samples with peak T intensity measured on the portable device.
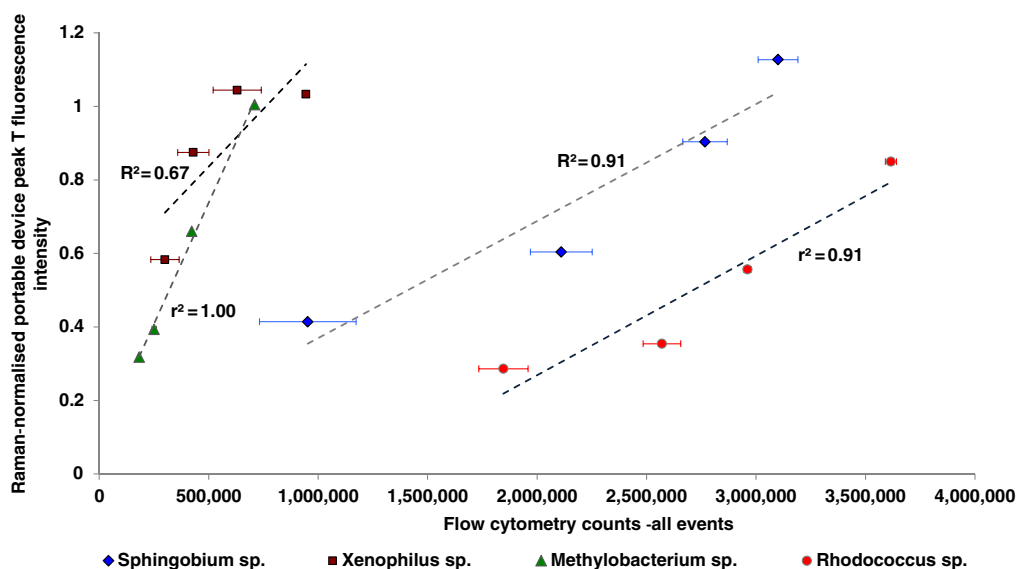
**Fig. 6.** Portable device peak T intensities *versus* counts of dilutions of four bacteria isolates.

It has been demonstrated with these initial findings that at least some of the peak T fluorescence which is observed in potable water can have a direct bacterial origin. Similar to a study of the bacteria *Pseudomonas aeruginosa*, isolated from an urban river (Elliot et al., 2006), a peak T response has been observed on the new device that is directly attributable to the type and quantity (albeit large) of four bacteria found in potable water. Further work is required to assess further strains of potable water bacteria to compare the peak T response of different species, to allow for a more comprehensive understanding of the root cause behind this area of fluorescence in environmental rather than laboratory cultured waters.

Thus, the foregoing demonstrates the development of a new approach for the simultaneous *in-situ*, real-time monitoring of both organic and microbial matter, with correlations with TOC ($r^2 = 0.75$) and total microbial counts ($r^2 = 0.56$). No other technology currently exists which can make both measurements simultaneously, although TOC can be measured on-line using automated TOC analysers and also determined indirectly by ultraviolet absorbance (for a review of all TOC analysis methods see Matilainen et al., 2011). The observed correlation between fluorescence and TOC shown here, although strong, is lower than that typically reported for the UV absorbance–DOC/TOC relationship, which typically has $r^2 > 0.9$ (Matilainen et al., 2002; Spencer et al., 2009; Carter et al., 2012), but has the advantage of also providing information on total microbial counts. On-line microbial monitoring has yet to be established; the first feasibility study has recently been reported (Besmer et al., 2014) but the authors note the challenges of instrument cost and the handling of large datasets. Advantages of the fluorescence instrumentation, in addition to the ability to measure both organic and microbial matter, include low cost, small size and portability, low power requirements and a simple data output.

## 6. Conclusions

A fully operational portable LED-based device has been developed and shown to provide an accurate *in situ* assessment of the peak C and peak T response for a broad range of fluorophore concentrations typically found in potable water treatment and supply samples. The systematic validation of the results achieved by the new device and those provided by well-established analytical methods clearly demonstrate that this device offers, in a fraction of the time and cost of a traditional bench top device, the potential to promote a more proactive means of monitoring water quality. Unlike a traditional bench top device, the portability, low powered nature and ruggedness of the device allows for its use outside of laboratory conditions, and through its adjustable sensitivity, it is possible to apply the device to a range of water qualities. The device has been used in a discrete manner to measure the quality of a range of water company samples. Although correlating well with regulatory organic surrogates, the device did not provide any notable correlation with regulatory microbial measures. This highlights the difficulties in comparing the small percentage of the bacteria in a water sample which are culturable to a fluorescence response which is an area of identifiable general bacterial activity, regardless of the type or strain of bacteria.

Flow cytometer tests showed peak T intensity to correlate with an increase of total live and dead bacteria numbers in a sub-set of the samples. Tests with four bacteria isolated from a potable water tap sample showed a clear and different response between strains, although the strains with the highest bacteria counts did not necessarily give the greatest peak T fluorescence response.

The results presented here demonstrate that the novel fluorescence-based instrumentation that has been developed to assess treated water quality in real-time, *in-situ*, based on new developments in fundamental science, and innovative engineering design and deployment, represents a low cost, rugged, monitoring instrument which can be deployed extensively in supply systems to provide early indication of the need for active management of water quality. The significant scientific and engineering developments derived from this work can be used to facilitate a step change in quality assessment for the water industry with the potential for a much-needed move beyond the use of indicator organisms, and beyond measurement and reporting purely to satisfy regulatory requirements. Furthermore, the new instrument has deployment applications in the real-time determination of the microbial health and biochemical oxygen demand of surface and ground waters that will assist with enhancing understanding and improving water quality as required under the Water Framework Directive. Whilst initially focussed on the water industry, it is believed that this new capability could lead to the development and widespread adoption of a new class of fluorescence analysis tools with applications in many other academic disciplines.

laboratory support provided by Esther Karunakaran, Isabel Douterelo and David Gaskell.

## Appendix A. Supplementary data

## References

Allen, M.J., Edberg, S.C., Reasoner, D.J., 2004. Heterotrophic plate count bacteria—what is their significance in drinking water? Int. J. Food Microbiol. 92, 265–274.

Amann, R.I., Ludwig, W., Schleifer, K.H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59 (1), 143–169.

Baker, A., Tipping, E., Thacker, S.A., Gondar, D., 2008. Relating dissolved organic matter fluorescence and functional properties. Chemosphere 73, 1765–1772.

Besmer, M.D., Weissbrodt, D.G., Kratochvil, B.E., Sigrist, J.A., Weyland, M.S., Hammes, F., 2014. The feasibility of automated online flow cytometry for in-situ monitoring of microbial dynamics in aquatic ecosystems. Front. Microbiol. 5, 265. http://dx.doi.org/10.3389/fmicb.2014.00265.

Bieroza, M., Baker, A., Bridgeman, J., 2009. Relating freshwater organic matter fluorescence to organic carbon removal efficiency in drinking water treatment. Sci. Total Environ. 407, 1765–1774.

Bieroza, M., Baker, A., Bridgeman, J., 2010. Fluorescence spectroscopy as a tool for determination of organic matter removal efficiency at water treatment works. Drinking Water Eng. Sci. 3, 63–70.

Bridgeman, J., Bieroza, M., Baker, A., 2011. The application of fluorescence spectroscopy to organic matter characterisation in drinking water treatment. Rev. Environ. Sci. Biotechnol. 10, 277–290.

Bridgeman, J., Baker, A., Carliell-Marquet, C.M., Carstea, E., 2013. Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy. Environ. Technol. 34 (23), 3069–3077.

Brock, T.D., 1987. The study of microorganisms in situ: progress and problems. Symposium of the Society for General Microbiology, pp. 1–17.

Carter, H.T., Tipping, E., Koprivnjak, J.-F., Miller, M.P., Cookson, B., Hamilton-Taylor, J., 2012. Freshwater DOM quantity and quality from a two-component model of UV absorbance. Water Res. 46, 4532–4542.

Chin, Y.P., Aiken, G., O'Loughlin, E., 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol. 28, 1853–1858.

Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. Mar. Chem. 51 (4), 325–346.

Cumberland, S., Bridgeman, J., Baker, A., Sterling, M., Ward, D., 2011. Fluorescence spectroscopy as a tool for determining microbial quality in potable water applications. Environ. Technol. 33 (6), 687–693.

Determann, S., Lobbes, J.M., Reuter, R., Rullkotter, J., 1998. Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. Mar. Chem. 62, 137–156.

Dickens, J.E., Vaughn, M.S., Taylor, M., Ponstingl, M., 2011. An LED array-based light induced fluorescence sensor for real time process and field monitoring. Sensors Actuators B 158, 35–42.

Elliot, S., Lead, J.R., Baker, A., 2006. Characterisation of the fluorescence from freshwater, planktonic bacteria. Water Res. 40, 2075–2083.

Ferrari, G.M., Dowell, M.D., Grossi, S., Targa, C., 1996. Relationship between the optical properties of chromophoric dissolved organic matter and total concentration of dissolved organic carbon in the southern Baltic Sea region. Marine Chemistry 55 (3-4), 299–316.

Goslan, E.H., 2003. Natural organic mater character and reactivity: assessing seasonal variation in a moorland water. (EngD Thesis). University of Cranfield.

Great Britain Department of Environment, 1983. The Bacterial Examination of Drinking Water Supplies 1982: Methods for the Examination of Waters and Associated Materials — Reports on Public Health and Medical Subjects. HMSO, London.

Hammes, F., Berney, M., Yingying, W., Vital, M., Koster, O., Thomas, E., 2008. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. Water Res. 42, 269–277.

Hart, S.J., JiJi, R.D., 2002. Light emitting diode excitation emission matrix fluorescence spectroscopy. Analyst 127, 1693–1699.

Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M., Khan, S.J., 2009. Fluorescence as a potential monitoring tool for recycled water systems: a review. Water Res. 43, 863–881.

Ho, L., Braun, K., Fabris, R., Hoefel, D., Morran, J., Monis, P., Drikas, M., 2012. Comparison of drinking water treatment process streams for optimal bacteriological water quality. Water Res. 46, 3934–3942.

Hoefel, D., Groobya, W.L., Monisa, P.T., Andrews, S., Sainta, C.P., 2003. Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques. J. Microbiol. Methods 55, 585–597.

Hudson, N., Baker, A., Ward, D., Reynolds, D.M., Brunsdon, C., Carliell-Marquet, C., Browning, S., 2008. Can fluorescence spectrometry be used as a surrogate for the biochemical oxygen demand (BOD) test in water quality assessment? An example from South West England. Sci. Total Environ. 391, 149–158.

Lautenschlager, K., Boon, N., Wang, Y., Egli, T., Hammes, F., 2010. Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. Water Res. 44, 4868–4877.

LeBlanc, L., Dufour, E., 2002. Monitoring the identity of bacteria using their intrinsic fluorescence. FEMS Microbiol. Lett. 211, 147–153.

LeChevallier, M.W., Shaw, N., 1996. Factors Limiting Microbial Growth in Distribution Systems: Full Scale. American Water Works Association, Denver, Colorado.

LeChevallier, M.W., Schulz, W., Lee, R.G., 1991. Bacterial nutrients in drinking water. Appl. Environ. Microbiol. 1991, 857–862 (March).

Leenheer, J.A., Croué, J.-P., 2003. Aquatic organic matter: understanding the unknown structures is key to better treatment of drinking water. Environ. Sci. Technol. 19A (January 1, 2003).

Matilainen, A., Lindqvist, N., Korhonen, S., Tuhkanen, T., 2002. Removal of NOM in the different stages of the water treatment process. Environ. Int. 28, 457–465.

Matilainen, A., Gjessing, E.T., Lahtinen, T., Hed, L., Bhatnagar, A., Sillanpää, M., 2011. An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. Chemosphere 83, 1431–1442.

Moe, A.E., Marx, S., Banani, N., Liu, M., Marquardt, B., Wilson, D.M., 2005. Improvements in LED-based fluorescence analysis systems. Sensors Actuators B 111–112, 230–241.

Patel-Sorrentino, N., Mounier, S., Benaim, J.Y., 2002. Excitation–emission fluorescence matrix to study pH influence on organic matter fluorescence in the Amazon basin rivers. Water Res. 36, 2571–2581.

Prest, E.I., Hammes, F., Kotzschb, S., van Loosdrecht, M.C.M., Vrowenvelder, J.S., 2013. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. Water Res. 47, 7131–7142.

Ramalingam, B., Sekar, R., Boxall, J.B., Biggs, C.A., 2013. Aggregation and biofilm formation of bacteria isolated from domestic drinking water. Water Sci. Technol.: Water Supply 13 (4), 1016–1023.

Reynolds, D., 2003. Rapid and direct determination of tryptophan in water using synchronous fluorescence spectroscopy. Water Res. 37, 3055–3060.

Skoog, D.A., West, D.M., Holler, F.J., Crouch, S.R., 2004. Fundamentals of Analytical Chemistry. Brooks/Cole Thomson Learning, Belmont, CA, USA.

Smart, P.L., Finlayson, B.L., Rylands, W.D., Ball, C.M., 1976. The relation of fluorescence to dissolved organic carbon in surface waters. Water Research 10, 805–811.

Souza, J.V.B., da Silva, R.M., Koshikene, D., Silva, E.S., 2007. Applications of fluorescent in situ hybridization (FISH) in environmental microbiology. J. Food Agric. Environ. 5 (3&4), 408–411.

Spencer, R.G.M., Aiken, G.R., Butler, K.D., Dornblaser, M.M., Striegl, R.G., Hernes, P.J., 2009. Utilizing chromophoric dissolved organic matter measurements to derive export and reactivity of dissolved organic carbon exported to the Arctic Ocean: a case study of the Yukon River, Alaska. Geophys. Res. Lett. 36, L06401. http://dx.doi.org/10.1029/2008GL036831.

Staley, J.T., Konopka, A., 1985. Measurement of in situ activities of non-photosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. 39, 321–346.

Stedmon, C.A., Seredynska-Sobecka, B., Boe-Hansen, R., Le Tallec, N., Waul, C.K., Arvin, E., 2011. A potential approach for monitoring drinking water quality from groundwater systems using organic matter fluorescence as an early warning for contamination events. Water Res. 45, 6030–6038.

Veal, D.A., Deere, D., Ferrari, B., Piper, J., Attfield, P.V., 2000. Fluorescence staining and flow cytometry for monitoring microbial cells. J. Immunol. Methods 243, 191–210.

Visco, G., Campanella, L., Nobili, V., 2005. Organic carbons and TOC in waters: an overview of international norm for its measurements. Microchem. J. 79, 185–191.

Vodacek, A., Hoge, F.E., Swift, R.N., Yungel, J.K., Peltzer, E.T., Blough, N.V., 1995. The use of in situ airborne fluorescence measurements to determine UV absorption coefficients and DOC concentrations in surface waters. Limnol. Oceanogr 40 (2), 411.

Wu, F.C., Evans, R.D., Dillon, P.J., 2003. Separation and characterization of NOM by high-performance liquid chromatography and on-line three-dimensional excitation emission matrix fluorescence detection. Environ Sci Technol 3716, 3687.