Towards developing an ultimate optical method for bacteria detection

Microorganism detection is an essential task for wide range areas of human activities driven by economic and healthcare factors. Total Viable Count (TVC) method is a benchmark in microbiology, which is based on visual observation of the colony forming units (CFU) grown on plate agar. Despite being adopted by regulatory authorities of many countries, TVC method has been known for its variability and limitation e.g.

(a) It is a skewed estimate at best as only the cells able to grow and form colonies under the conditions of the test (incubation time, temperature, agar content *etc.*)

(b) Colony growth requires long incubation time that may vary between day to the week.

(c) The dilution of highly concentrated bacterial suspensions is necessary to visually differentiate and count the CFU, which is a considerable cause for additional variability.

(d) The presence of bacterial growth inhibitors such as preservative materials, chlorine, and solvents makes the method less efficient.

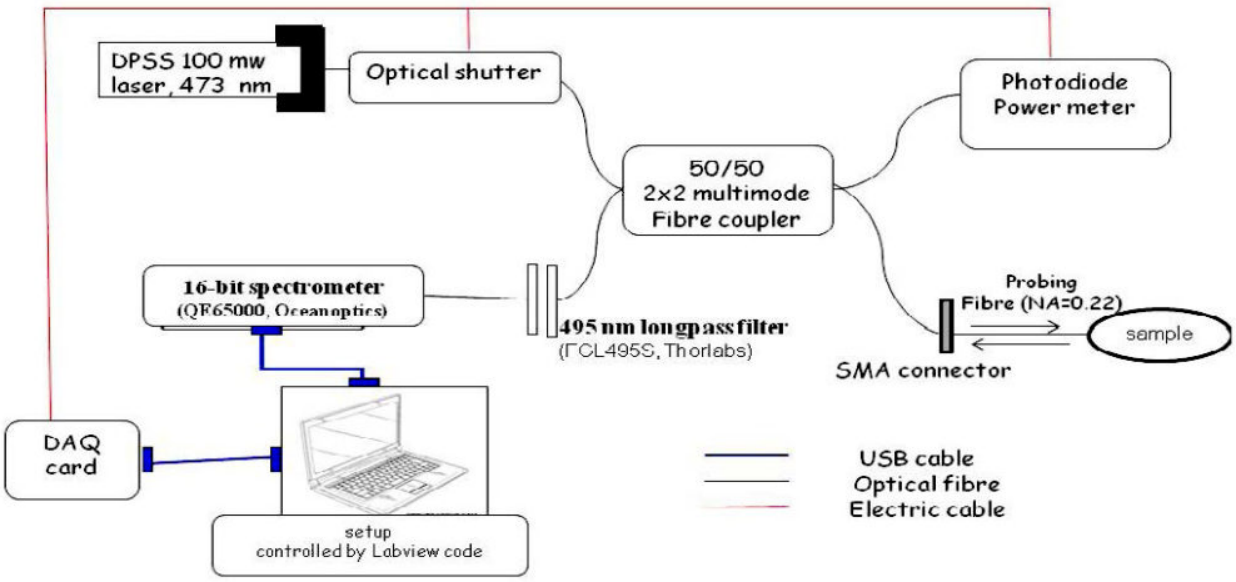

Fig. 1. Schematic diagram of the all-fibre optical system. Light from the DPSS laser with 473 nm wavelength is delivered into a 2x2 multi-mode fibre coupler. Half the excitation light is guided to the sample via one of the output arms of the coupler, while the other half is directed to a photodiode for monitoring the laser power. A fraction of the fluorescence from the illuminated sample is collected

at the fibre tip and returns to fibre-coupled spectrometer via 2x2 multi-mode coupler. The spectrometer is connected via a USB connection to the computer that is used for data acquisition and spectral analysis. A laser shutter is controlled by a data acquisition card (DAQ), which is placed in front of the coupling stage and synchronized the exposure time of the sample to the acquisition time of the spectrometer.

Manifold technologies were developed to mitigate TVC detection specifications, especially to improve

the waiting time for processing bacteria culturing results. Nevertheless, since pioneering work of Robert Koch at the end of 19 century, TVC remains the leading method for bioburdren enumeration due to the inability other techniques to provide in tandem essential detection characteristics such as simplicity, cost effectiveness, specificity, throughput, accuracy and sensitivity etc.

To address these challenges, the all fibre software embedded optical system was introduced (Fig. 1). Specific protocols based on nucleic acid staining (SYTO 9 dye), fluorescence acquisition parameters and multivariate signal processing approach were developed. This optical method offers the following advantages for microorganism detection:

(a) Sensitivity: this is due to the efficient light delivery of 10 mW and *in situ* fluorescence excitation and signal detection as well as the use of a sensitive CCD spectrometer.

(b) Accuracy: by monitoring the laser power, we take into account the laser power fluctuations while measuring the fluorescence signal. Also a synchronized laser shutter allows us to acquire a signal with a high signal to noise ratio (SNR) and with minimal integration time, thereby reducing the photobleaching effect. Computerized data processing and multivariate analysis further decrease the measurement variance.

(d) Near real time data acquisition.

(e) Ability to detect a wide range of bacterial concentrations without using dilution or filtration.

Figure 2 demonstrates an example of laboratory grown bacteria results: Escherichia coli (*E.coli*, ATCC 25922 grown on Horse blood agar of Fort Richard). A strong correlation (Pearson correlation coefficient is 0.997) between predicted bacteria concentrations using optical method and Partial Least Squares analysis (for PLS, Wold’s iteration method was used) and TVC method was observed. The concentration with 100 CFU/ml was verified by plate count other concentrations were extrapolated based on their dilutions.

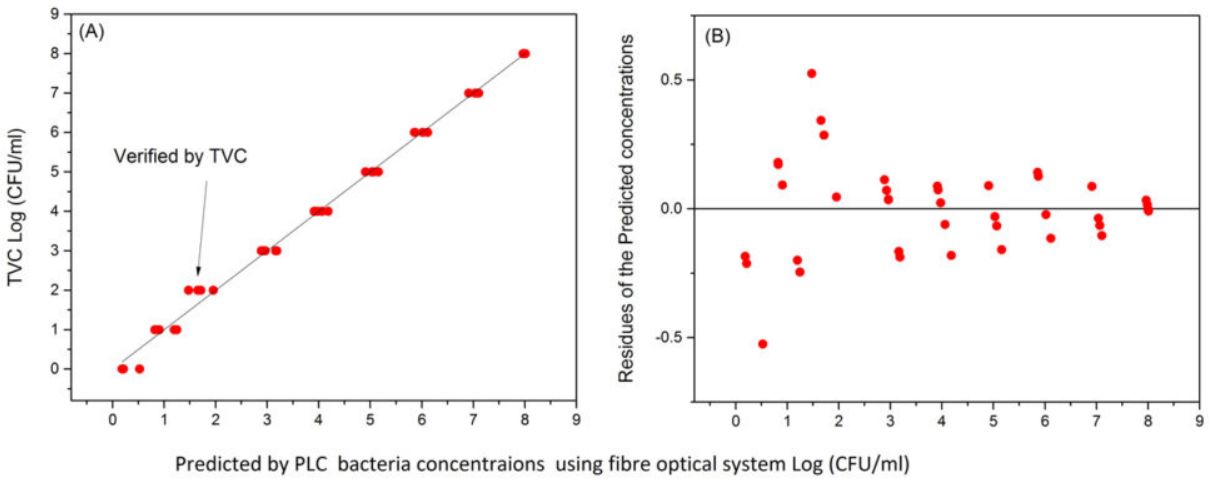

Fig. 2. Example of *E.coli* ATCC 25922 results.(A) linear regression between two methods: TVC concentrations versus PLS predicted concentrations of *E.coli*. (B) Residues of the *E.coli* predicted concentrations. To calculate PLS coefficients a short nucleic acid fluorescence range was used.

In summary, substantial progress has been made in incorporating advanced optical technology as a computational method for microorganism quantification. This optical system has a promising performance compared to other counterparts methods. There is still a requirement for pilot studies to ensure the applicability of such system for industrial purposes. The main experimental ambiguity comes from system sensitivity that cause a variability in fluorescence signal, especially deviation from linearity at low concentrations, defining standard operating procedures and multivariate optimization approaches may significantly improve accuracy of the prediction.

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Publications

Correction: Total viable bacterial count using a real time all-fibre spectroscopic system.
Bogomolny E
Analyst. 2016 Aug 7

Total viable bacterial count using a real time all-fibre spectroscopic system.
Bogomolny E, Swift S, Vanholsbeeck F
Analyst. 2013 Jul 21