Flow Cytometry and Food Microbiology: challenges, opportunities and progress to date

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Microbiology is a highly dynamic and important branch of research, quality control and product development for the food industry. In general terms it can be divided into two main areas, (1) detection and monitoring of pathogens and spoilage microorganisms and (2) the usage of beneficial microorganisms for the control and production of fermented foods. For both of the above activities enumeration and identification of bacterial populations are routinely undertaken using a variety of methodologies including viable cell counting on selective and non-selective media, genetic detection of species specific markers using PCR and the use of emerging technologies such as flow cytometry (FCM) and its more advanced formats such as fluorescence activated cell sorting (FACS) and imaging flow cytometry. The scope of this paper will be to consider the main technical challenges, progress to date and future opportunities for the application of flow cytometry to Food Microbiology. In this regard the use of FCM for analysis of both specific pathogens/spoilage bacteria and beneficial Lactic Acid Bacteria (LAB) will be reviewed as case studies. The food industry globally spends much time and expense ensuring that a range of pathogens/spoilage bacteria are excluded as much as possible from their products. Achieving regulatory standards of food safety for consumers ranging from infants to the elderly population cohorts requires the food industry to employ microbiologists in quality control laboratories. This activity is designed to prevent consumer illnesses including those from ingestion from foods containing live bacterial pathogens such as Enterobacteriaecae, *E. coli* 0157:H7 and *Staphylococcus aureus,* or, from ingestion of emetic toxins from spore formers such as *Bacillus cereus.* All of the above species can generate a range of conditions from mild to serious and potentially life threatening. Conversely, a wide variety of fermented foods are consumed which are rendered safe, nutritious and flavoursome by the addition of bacteria such as LAB which stabilise the products by colonisation, acidification and the release of enzymes which generate optimal flavour, texture and aroma. The list of products which rely on LAB activity is extensive and includes natural cheeses, yogurts, fermented milk drinks, fermented meats including salami, fermented vegetables such as pickles or sauerkraut and beverages such as wines and beers.

Limitations of current plate based techniques

Currently, except for a few notable exceptions the food industry is still using quality control analytical techniques based on plating of diluted samples onto media and counting the resultant colonies after incubation under defined conditions of time and temperature. In the case of certain pathogens such as *S. aureus* this process involves plating onto Baird-Parker agar and identifying typical colonies, which can take up to 48 hours. Moreover, further identification of coagulase positive suspects which can prolong the duration before a definitive result can be obtained for up to 4-5 days. Overall this methodology is dependent on a "plate and wait" approach whereby data on product safety is generated after a time lag and in the interim the product may require to be stored before a positive release is obtained. In addition, a cornerstone of this approach is the presence or absence of live cells or those capable of growth under highly selective conditions. However, what is becoming much clearer in modern microbiology is that cells especially in foods that have been processed are present in a multiplicity of physiological states including; live, damaged/permeabilised and dead with varying potential for outgrowth which may render them non-detectable under conventional plate based assays.

Flow Cytometry (FCM) for Food Microbiology: the basics

One emerging technology that may offer the food industry a new approach towards bacterial detection in terms of enumeration, identification and physiological profiling is flow cytometry (FCM). The principles of physical and engineering principles of FCM are very well explained by Shapiro (2015; 2003) and will not be dealt in detail here. However, in terms of microbiological applications FCM involves suspending bacterial cells within a moving liquid

stream which is then interrogated by a laser and gathering of resultant light scattering properties in a Forward angle light scatter (Forward scatter or FSC) or Side angle light scatter (Side scatter or SSC); additionally, fluorescence arising from cells passing thorough the laser beam can be collected at various wavelengths. While FSC and SSC can be used to locate cells on a cytograph profile and give general information on cell size and granularity, the major information on individual cell physiology and structure is obtained by staining of cells with specific fluorescent dyes and collecting the degree of fluorescence from a single or multiple stains applied to cells. The major advantages/potential applications of FCM for food microbiology are: rapid assay times and data generation (1-2 min), high number of cells that can be analysed per sample (10,000 and upwards), minimal sample volume (from 5 µl), potential high throughput, multiplicity of stains available to examine various aspects of cell viability, structure and/or metabolism (multi-parametric), and less labour and space intensive compared with conventional plating techniques (Wilkinson, 2015).

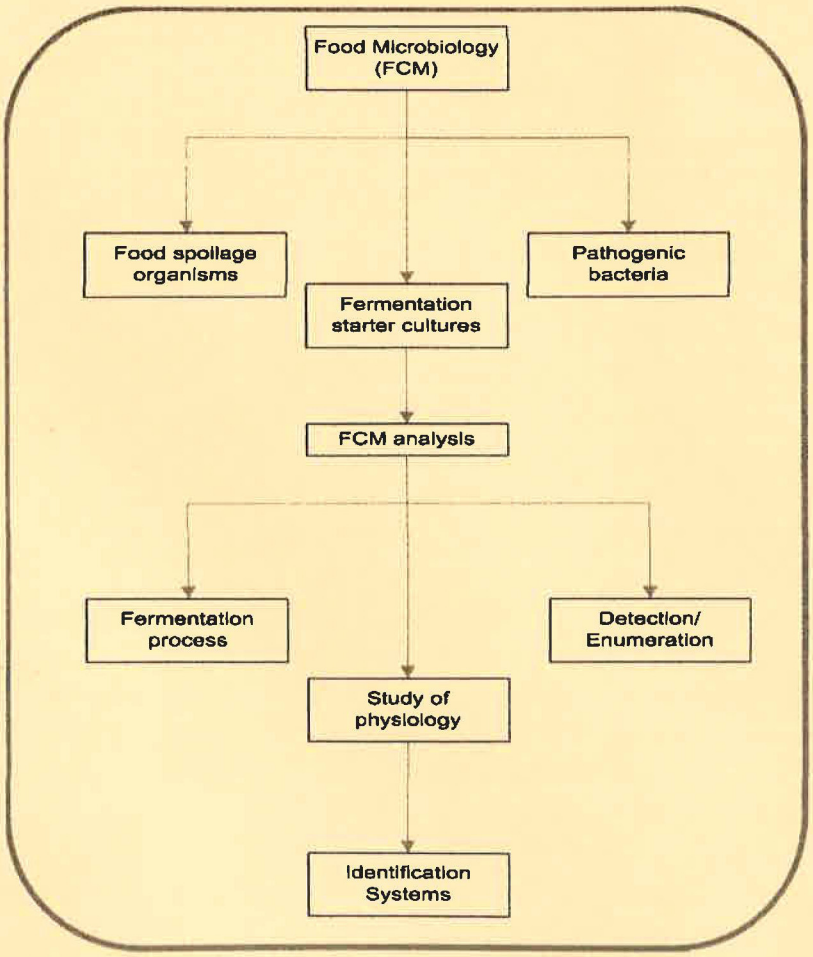

Fig 1. FCM and Potential Applications in Food Microbiology/Food Fermentations (Source: Doolan and Wilkinson, 2016)

FCM: general technical challenges

If the above sounds too good to be true then it can be balanced with a heavy dose of reality! FCM-based assays for cells in foods are still beset by a range of both minor and major technical and perhaps regulatory and mind-set obstacles. Firstly, cells have to be recovered from foods in samples which are essentially free of interfering "bacteria-sized" particles such as debris obtained after homogenisation and stomaching commonly used to release cells for conventional plate analysis. The use of food samples which contain particles which generate auto-fluorescence will interfere with detection of bacterial cells and therefore may require some specialised removal procedures prior to FCM. If analysis using FCM involves complex formulated food samples, such as prepared ready meals, the above problems may exist along with the presence of ingredients containing DNA/RNA

(such as food flavourings) which will non-specifically bind with DNA-based cell viability dyes. In terms of detection limits most cytometers operate best when analysing 10^5-10^6 cells per sample so in general terms you need a good population of cells to get optimal analysis. This means that you either use concentrated samples containing target cells or a carry out a prior enrichment step to increase the low level of target cells to detectable levels. The latter step thus renders the FCM based assay as giving a qualitative "presence or absence" data output. A key drawback for pathogen analysis in foods is the inability of most of the stains used by FCM to enable a high degree of species-specific pathogen identification. However, the use of DNA based techniques such as fluorescent *in situ* hybridisation (FISH) when combined with cytometry (FLOW-FISH) or the use of mono/polyclonal antibodies has certainly made some progress. Overall, it is fair to say that FCM has not yet benefitted from the availability of cheap, reliable and sensitive antibodies with which to target and label particular a pathogen within a mixture of species. However, this paper will focus on the progress and application of antibody labelling of cells as a potential avenue for immuno-cytometric pathogen detection.

FCM assay development

To consider progress in FCM for Food Microbiology it is necessary to follow a typical assay development route then to compare and contrast it with conventional techniques. Firstly, in terms of release and recovery of cells in foods for FCM analysis the procedure follows quite common initial routes used for traditional plating analysis including: dilution of sample, mixing via stomaching or homogenisation and final production of a suspension of food particles and bacterial cells. Thereafter, the necessity for the use of specific clean up procedures for FCM analysis is encountered. These, and other procedures typically used in bacterial sample preparation methods have been reviewed in detail by Dwivedi and Jaykus (2011). Recovery of cells may involve an initial low speed centrifugation (3,000 x g for 10 min) to obtain a cell pellet, followed by resuspension of the pellet in buffer and thereafter filtration through various particle exclusion membranes such as 15, 10, or 5µm to reduce non-cellular particulates while a final centrifugation step may often be needed to concentrate cells. Thereafter, the cell pellet is either stained directly or may be antibody-labelled to detect a specific population followed by differential staining. If the population of target cells is below the sensitivity of the cytometer or fails to produce a signal well beyond background "noise" then a further enrichment step is often necessary to enable growth of detectable levels of cells for analysis by FCM. A single step method we use in our laboratory to both recover and concentrate LAB cells from semi-hard Cheddar cheese for FCM analysis involves a 3 hr extraction to express the aqueous phase ("juice") from 300 g of grated cheese sample at 320MPa of hydraulic pressure to yield a relatively clear solution which can be centrifuged and contains from 10^4-10^9 cells per ml (Wilkinson *et al.*, 1994; Sheehan *et al.*, 2005; Yanachkina *et al.*, 2016). This particular sample is highly versatile and can be analysed simultaneously for FCM profiling alongside the determination of released peptidolytic enzymes and peptide/amino acid ripening products. As Cheddar cheese is a semi-solid having a moisture content of 38-40% it may well be possible to apply this system, or some modifications thereof, to other food types as a potential integrated cell recovery/concentration procedure. However, at this juncture it is reasonable to suggest that for FCM analysis additional sample preparation steps are required when compared to current ISO-accredited methodologies used for viable plate counting of pathogens and other microorganisms. However, method development is proceeding apace for FCM and it should be possible in the medium term to identify agreed validated procedures for sample preparation and cell extraction from various food groups. Regarding sample volume, FCM can certainly claim to be more flexible in this aspect compared with plate counting (traditionally requiring 1 ml for pour plating) as volumes from 5 µl up to 100µl can easily be analysed in the cytometer based on initial cell numbers and flow rate manipulation. Analytical speed for FCM is quite amazing and tens of thousands of cells (known as "events" in FCM-speak) can be analysed and their data obtained within 2-3 mins per sample. Contrasting that with conventional plate counting where a range of dilutions must be prepared, plated and then incubated, following which, those giving from 30 to 300 colonies per plate are selected as having statistically valid data.

Dyes/Stains useful for FCM in Food Microbiology

Assuming that a good particle free sample has been obtained with sufficient cell concentrations, what can FCM offer in terms of analysis? The methodology used for microbial analysis is based on fluorescent staining of cells, this can involve, at its simplest, a single stain for enumeration of the entire population or combinations of stains where multiple cellular characteristics are analysed. However, a wide range of stains are commercially available and must be chosen on the basis of analytical data required, non-toxicity to the cell and suitability for detection by the laser in the particular cytometer along with other factors such as spectral over-lap with other stains (Leonard *et al.*, 2016). Single viability stains, which are designed to bind to the DNA/RNA of the cell population such as SYTO9 or Thiazole Orange (TO), allow a general estimation of bacterial biomass based on the fact that these stains can enter all cells and bind to the nucleic acid of both live (intact), injured or dead cells. However, this data is of limited use for obvious reason, hence the general approach is to use combinations of stains to measure various aspects of cell viability and metabolism. The Live/Dead BacLight stain combination from Invitrogen (USA) is comprised of SYT09 and propidium iodide (PI)—a green and a red fluorescent dye combination that has been very useful in determination of cell viability and is based on the principle of displacement of the green SYTO9 dye from cells with damaged membranes by the red dye, PI. In general, a range of stains can be combined to give multi-parametric data on aspects of membrane integrity, intracellular enzyme activity (cFDA), membrane potential, ($DiBAC_4$ (3)), intracellular pH (BCECF), and cell division (CFSE). It is beyond the scope of this paper to describe the attributes of the range of dyes available for food microbial applications but readers can refer to the chapters within the text of Wilkinson and other authors (2015) for a more detailed discussion on their merits. A key technical issue to remember when looking at dye combinations for differing microbial species/strains is that a preliminary check should be carried out to ensure the stains are not toxic to cells at dosages used in the particular application. Therefore a parallel experiment using the stains with cross checking of viability by plate counting should be carried out to establish correlation between methods and whether any bacterial strain or FCM-stain related toxicity is present.

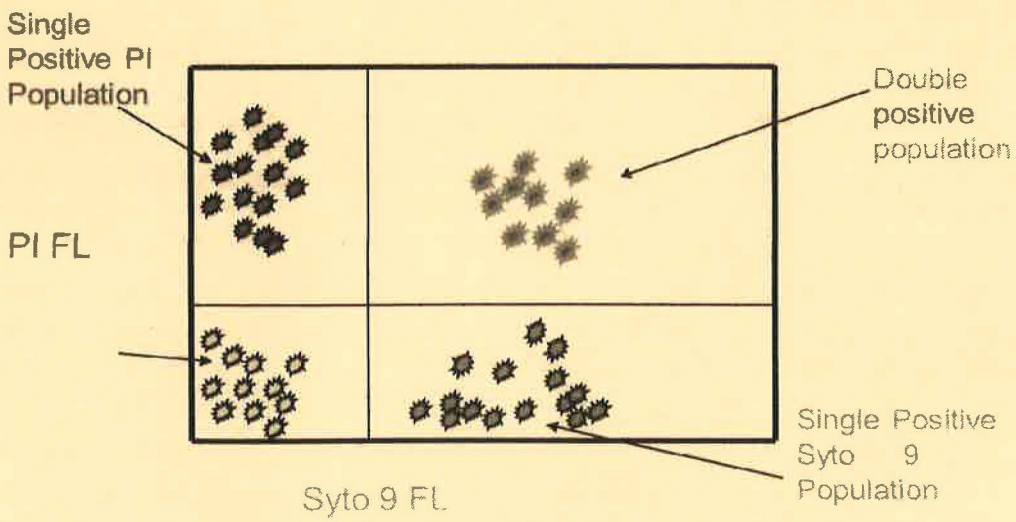

Fig 2. Flow Cytometry Profile arising from differential staining using SYTO9/PI combinations showing live, dead and damaged/permeabilised sub-populations.

Antibody Labelling and FCM analysis: progress, challenges and future opportunities

As mentioned earlier, the current available dye combinations are generally not indicative of the presence or absence of particular bacterial species within a mixed population. An avenue which is being developed is the use of antibodies to label specific bacteria and then either measure the fluorescence directly or amplify the primary antibody-bacterial cell complex using a secondary antibody which gives an enhanced signal suitable for collection by cytometry. One such application of this technology is Immunomagnetic Separation or IMS. This utilises

magnetic coated antibodies against target cells which are specifically labelled within a liquid sample and then retained or captured on application of a magnetic field, resulting in the concentration of target cells from a mixed culture. Thereafter, these cells may then be directly subjected to FCM analysis. Hibi et al., (2006) reported the separation of *Listeria monocytogenes* from mixed cultures by IMS followed by FCM analysis over range of 10^4-10^8 CFU/ml. In general, antibody capture of target cells such as *E. coli* 0157:H7 or *Listeria monocytogenes* requires further enrichment to allow cell numbers to reach detectable levels using FCM (from 10^4 cfu/ml and upwards). I would like to use a number of published studies to illustrate the challenges, progress and opportunities for immuno-FCM in food pathogen analysis. Wilkes et al., (2012) described the development of methodology for the rapid and sensitive detection of *E. coli* 0157:H7 in spinach. These workers used two proprietary reagents, A or B, containing either FITC-conjugated polyclonal antibodies against the pathogen or detergents conditioning chemicals for enhanced epitope presentation along with PI stain for dead cells, respectively. In terms of sample preparation, this product is quite challenging as regards particle interference, matrix colour interference and subsequent recovery and detection of pathogen. Spinach (25g) was spiked with 100µl of either 5 or 50 cells and followed by a 4 hour enrichment step. In terms of sample preparation, liquid media added to the spinach was decanted and used as the starting sample. Thereafter, the use of buoyant gradient centrifugation essentially allowed a concentration of cells which were then removed, washed and filtered (5 µm) prior to antibody labelling and cytometric analysis. The use of a specialised cytometer having a wide cross section flow cell with a 130nm resolution was reported by the authors as being superior for analysis of samples containing particulates likely to interfere with bacterial analysis. The approach used by these workers for FCM analysis was quite interesting and utilised a series of multi-dimensional gates beginning from the usual initial FSC and SSC plot, progressing thereafter to exclude PI positive dead cells and matrix particles to finally enable exclusive detection of live labelled *E. coli* 0157:H7. The performance of this qualitative or screening assay represented a Time to Result (TTR) of under 4 and a half hours, with a limit of detection of 1 viable cell in 25 g of sample. This excellent work represents tangible progress for immuno-FCM with the development of an assay suitable for generation of a "presence or absence" result for this pathogen well within a typical 8 hour production cycle. However, the inability to directly detect low pathogen numbers without an enrichment step along with the relatively detailed sample preparation procedure still falls short of a more desirable direct FCM based enumeration assay. Williams et al., (2015) outlined further progress on this assay by means of a Level 2 FDA approved validation process for detection of *E. coli* 0157:H7 in raw spinach. The validation procedure involved 20 spiked and 20 non-spiked samples for analysis with a comparison of FCM and an FDA approved q-PCR test. Levels of 1-4 viable cells per 100µl were inoculated into the test spinach samples with a 17.5 hour aging period before sampling. Subsequent preparation steps included the addition of a photobleaching agent, phloxine B, to reduce matrix colour interference and a 5 hour incubation to increase cell numbers to detectable levels. Using reagents A and B, detection was carried out as before. In this report another wide diameter flow cell cytometer was used but with a somewhat larger resolution of 170nm. Overall performance of the FCM method was very favourable when compared with the approved q-PCR method. Sensitivity was similar between methods at 2-4 cells per 100 µl, TTR for the FCM method was ~9h while that for the PCR was ~51h, this FCM method can potentially deliver data within a processing shift while its throughput was estimated at 20 samples during this interval. The number of false negatives for FCM was 4 out of 10 and for the PCR method was 5 out of ten. This study represents further progress towards a commercial and accredited qualitative FCM immuno-assay for food pathogen detection. Importantly, it provides solid data regarding the comparative performance of immuno-FCM with accredited assays such as q-PCR and therefore builds confidence for the adoption of FCM assays by the food industry. The work of Subires et al., (2014) also provides a very good insight into the complexities of FCM assay development for particular food groups such as prepared pasta salads which contain a range of ingredients any, or all, of which may be difficult to remove from the FCM analyte. These authors also report on a method to detect *E. coli* 0157:H7. The preparatory steps used to recover cells and reduce interference included pulsification in 63mm bags and centrifugal filtration. In this work, 10^3-10^7 cfu/g of live cells were inoculated into the unpasteurised salads which were stored at 4°C for 2 weeks. Detection of the target pathogen was carried out using a polyclonal

antibody conjugated to R-phycoerythrin (R-PE, yellow-orange fluorescence), while live/dead staining was carried out on the labelled complex using SYBR GREEN I and PI. In this study, the authors did not use an enrichment step rather a direct labelling of a filtered and re-suspended cell pellet was undertaken. Correlations were also undertaken with conventional plate counting of samples. A careful selection of gates using a good range of controls allowed discrimination of cells from food particles and between live or dead/damaged cells. Data generated indicated an LOD of 10^5 cfu/g attributed to particle interference despite the preparatory steps undertaken. A good correlation was found between FCM and plate count data at a particular antibody concentration, showing the necessity for extensive assay optimisation for immuno-FCM to perform optimally. The additional information that multi-parametric FCM can provide was well illustrated from the SYBR Green I and PI combination which revealed that initially most cells had sustained membrane damage but appeared to have recovered by day 14. This physiological insight is beyond plate counting, and some other techniques, but may allow a greater understanding of the fate of stressed cells in foods and to estimate their potential for posing a latent threat to consumer safety from convenience foods. Using the above studies as a method to compare and contrast the ability of immuno-FCM to overcome the current issues with cell specific recovery and labelling it can be seen that good progress has been made towards the development of sensitive and rapid qualitative detection of particular pathogens in a limited range of foods. However, the direct species-specific enumeration of pathogens using sensitive quantitative immuno-FCM assays is still some way off and will rely on the development of novel methods to recover cells from foods in concentrations which can be detected by sensitive antibodies and using cytometers which are specially adapted for sensitivity at low event detection limits.

Spores: "the Final Frontier"

Spore forming bacteria are especially important to detect and quantify in the food industry, including *Bacillus cereus* in prepared consumer foods such as rice and cereal dishes. *Bacillus cereus* is ubiquitous in soil, and can enter the food chain at a very early stage such as at harvesting of cereals or into dairy products during milking. Generalised spore count monitoring in the food industry is often used to indicate the status of process hygiene and the likelihood of the associated presence of the highly dangerous pathogen *Clostridium botulinum*. Generally, testing for sporeformers in foods involves laborious methods initially in enrichment media, followed by growth on selective agars with confirmatory testing by molecular methods. Hence, FCM is being investigated as a potential method to detect and study spores and vegetative cells following outgrowth in foods. I will deal with progress made using FCM for the study of *Bacillus cereus* which was also reviewed by Cronin and Wilkinson (2009). In general, FCM has been successfully used to study a range of properties involving spores, their germination events, outgrowth of vegetative cells and survival of vegetative cells within model food systems. This in turn has formed the basis for cytometric and biochemical evaluations of the effects of various food processing treatments on spores/vegetative cells. Cronin and Wilkinson (2008) showed that a number of heterogenous sub-populations were generated following exposure of *B. cereus* endospores subjected to simulated cooking temperatures and time regimes using FCM together with SYTO 9 / PI and CFDA/ Hoechst 33342. In terms of the development of direct FCM based assays for spores and/or vegetative cells, there is still much work to be done on aspects such as cell recovery from foods, rapid differentiation of spores from vegetative cells and of course the provision of sensitive, low cost specific antibody probes with which to label cells for FCM analysis. The opportunities and challenges for FCM especially immuno-FCM for spore/vegetative cell detection in the food industry are worth re-iterating. Any new FCM method should be more rapid than growth-based methods, with data gained within hours rather than days currently required for plate counts. Confirmatory tests could be eliminated should specific antibodies become available while useful multi-parametric physiological data could also be generated by fluorescent staining of the same sample.

Flow Cytometry and Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria (LAB) are the main bacteria used as starter cultures in the fermentation of milk, cheeses, yogurts, wines, meats and vegetables. They are inoculated into food substrates such as cheese to produce lactic acid within a specific production time period. Thereafter, during ripening the LAB cells become non-viable and release their intracellular peptidase enzymes such as Pep X to ripen the cheese and generate the typical flavour for that variety. It has been known for many years that LAB strains especially *Lactococcus. lactis* subsp. *cremoris* or *Lc. lactis* subsp. *lactis* differ in respect to viability and that *Lc. cremoris* strains generally die off faster to autolyse and release intracellular enzymes e.g Pep X, thus ripening the cheese in a balanced fashion without off-flavours . However, the relationship between low viability, autolysis and enzyme release has not been fully answered and a question that has arisen over recent years is whether differences in cell permeability influences intracellular enzyme release into the food matrix. Traditionally, LAB viability has been measured by a decrease in population recovered on selective media such as L-M17. Quantification of strain-related autolytic properties is currently undertaken by measurement of the activity of released intracellular marker enzymes of which Pep X and Lactate Dehydrogenase (LDH) are notable. However, the issue of demonstrating and quantifying strain related permeability properties is not well suited to the previous methods and here is where FCM analysis has proven to be very helpful. The general area of FCM and LAB has been reviewed by Doolan et al., (2015). The application of FCM methodology for LAB was reported by Bunthof et al., (2001) for pure cultures grown in broth media who showed that for LAB and other species labelling with c FDA was useful for determination of the fate of live cells before and after heat treatment. However, these workers did not achieve good discrimination of live/dead cells for Lactococcal strains using c FDA but this was improved for Lactobacillus strains. Overall, TOTO-1 staining gave good discrimination of LAB in either live or dead states based on the generation of a highly intense fluorescence signal from dead cells. Bunthof and Abee (2002) successfully applied this FCM double-staining methodology for the determination of viability of LAB including probiotic bacteria in milk, starter preparations and in commercial probiotic drinks In terms of determination of strain-related cell permeabilisation properties in higher solids dairy products such as Cheddar cheese, Sheehan et al., (2005) used a combination of SYTO9 and PI to determine the percentage live/intact, permeabilised/damaged and dead sub-populations in cheese manufactured using two lactococcal strains and demonstrated the existence of differing percentages of permeabilised cells between these strains. Interestingly, one strain became non-viable, highly permeabilised and also released intracellular Pep X (*Lc. lactis* AM2). The other strain was less permeabilised (*Lc. lactis* HP) but these permeabilised cells did not appear to release intracellular Pep X to any substantial degree and typically the resultant cheese lacks optimum flavour and can be bitter. Overall, FCM and LAB including cheese starters and probiotics has benefitted significantly from FCM methodology which has allowed a unique insight into aspects of culture performance with economic and human health implications. It should be pointed out here that determination of LAB in fermentations in most cases is rendered less difficult than pathogen detection by virtue of the fact that sample populations from LAB fermentations are generally of the order of 10^6 cfu per ml or per gram and upwards. Hence, samples are often diluted (rather than concentrated for pathogen detection) to achieve correct cell concentrations for FCM analysis, for which those of us who work on FCM for LAB analysis are very grateful!

Sorting out the issues!

The use of fluorescent activated cell sorting (FACS) commonly known as "cell sorting" represents a higher grade of FCM-based analytical resolving power for use in food microbiology. Briefly, cell sorters are modified cytometers with the ability to gate and physically sample the cells from a sub-population of interest. This resolving power arises from the ability of the cell sorter to enclose a single cell of interest within a droplet which is then given either a positive or negative charge and deflected by passage through a high voltage electrical field (~5000 Volts) to be deposited onto an agar plate, test tube, or a microtitre plate well for further analysis (Muller and Nebe-von-Caron, 2010). Using this technology we can recover stained cells which have been exposed to various stressors and correlate their cytometric profile which may reflect their differing physiological states with their ability to recover and grow on various media. Therefore cell sorting can potentially simultaneously answer a number of

questions regarding FCM; (1) what equivalence has FCM with plate counting for particular bacterial species? (2) do VBNC cells exist within a population? and, (3) can a rapid sorting-based multiplexing assay simultaneously discriminate and confirm the presence of different species within a single sample? (4) can sorting evaluate the effects of stressors (heating, cooling, etc) on survival and recovery of cells. In terms of some of these questions, Kennedy et al., (2011) examined the responses of the food pathogens *E. coli*, *Listeria monocytogenes* and *Staphylococcus aureus* when subjected to the effects of various stressors encountered by pathogens during food processing. The strains were analysed by FCM for viability using SYTO9/PI or for the presence/absence of a functioning membrane potential by staining with $DiOC_2$ (3). Using FACS sorting of various sub-populations, these workers showed that extensively damaged cells (as per staining and FCM profiles) sorted onto various solid media were still capable of growth. Indeed, differing overall recovery rates were noted for the various pathogens on both selective and non-selective media and these recoveries were also affected by differing stressor treatments. This study clearly showed the potential for survival and outgrowth of damaged cells, it also demonstrated the heterogenous nature of the sub-populations generated within a culture following exposure to stressors and demonstrated the potential usefulness of cell sorting in gaining a novel profound insight into cell physiology and potential pathogenicity arising from persistence of damaged cells within foods. Cell sorting is still expensive and generally non-user friendly for applications within a routine food microbiology quality analysis laboratory however new cheaper sorters are becoming available and in the medium term could become lend themselves to specialised analysis of particular food pathogens.

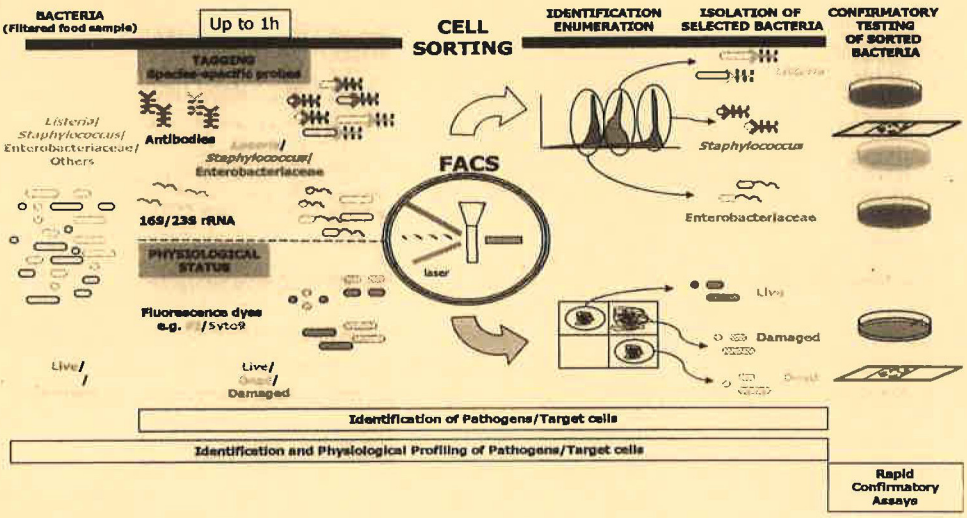

Fig 2. Potential applications of Cell Sorting to Pathogen/Target Cell detection in Food Microbiology (Source: Cronin, Alonso-Gomez and Wilkinson, 2016)

Let it Flow! How the water quality sector is showing the path towards acceptance and accreditation of FCM methodologies for the Food Industry

In order to see what possibilities for FCM and Food Microbiology there may be, it is necessary to compare and contrast with an industry/sector in which FCM is becoming a standard quality and production monitoring technique. The provision of a simple reliable and rapid microbiological test for water quality has long been a goal of workers in this field and traditionally it involved an agar based heterotrophic plate count (HPC) which was of limited usefulness. Egli and Kotzsch (2015) describe in detail the development and widespread acceptance (including by national regulatory bodies) of an FCM based assay to determine total cell count (TCC) and the ratio of High Nucleic Acid to Low Nucleic Acid content of cells in the population of a drinking water sample. The clearly demonstrated unrivalled ability of FCM to rapidly generate a meaningful data set from water samples was the result of work done by the drinking water research group at Eawag in Switzerland and led initially to the uptake

by laboratory practitioners and thereafter by the regulatory authorities such that validation and accreditation of FCM methods for water quality are now in place in Switzerland and work is now nearing completion for the use of specially developed cytometers for on/in line quality control which will provide continuous quality control data and enable immediate remedial action to be undertaken in response to problems. This, in my view, is the vision we can have for flow cytometry in the food industry, however many technical and regulatory challenges remain before we can reach this goal. Overall, much progress has been made and indeed a few FCM based analytical quality control systems have been commercialised so perhaps the "cytometry based future" may not be that far distant!

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