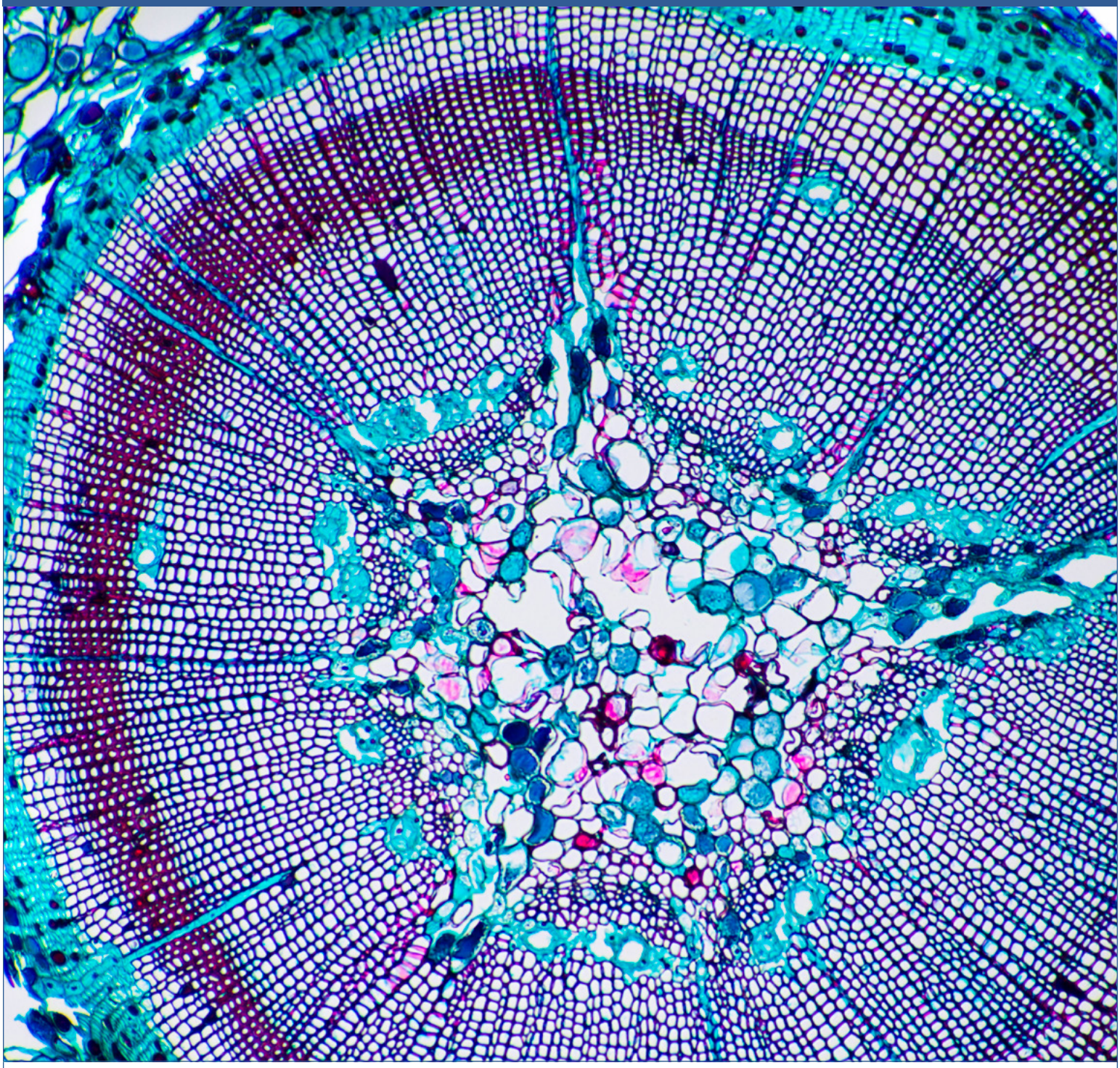
Flow Cytometry for Food Science and Agricultural Applications



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Flow Cytometry for Food Science and Agricultural Applications

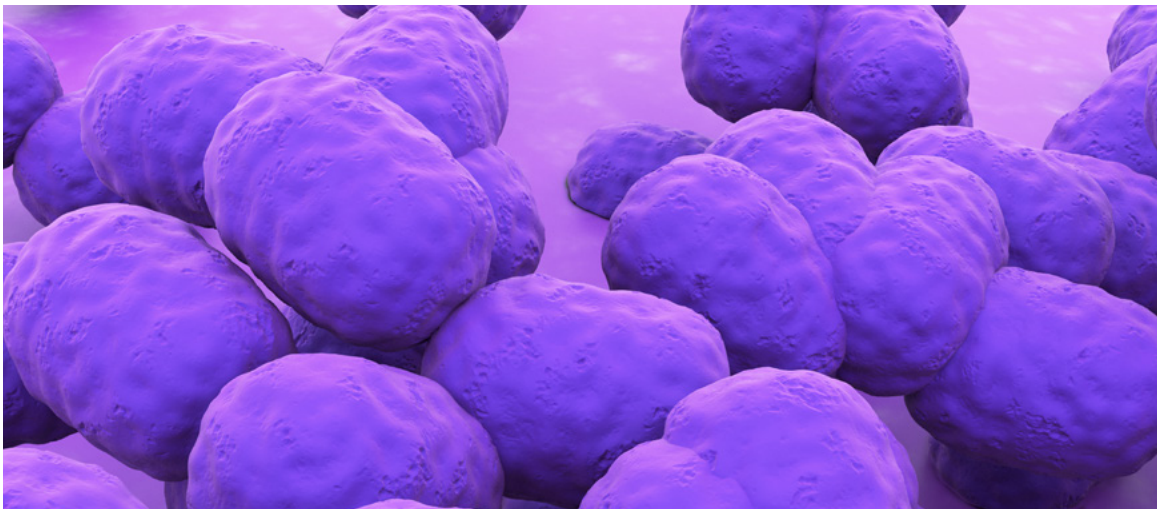
Flow cytometry has been a mainstay in biomedical research, particularly for studying cells of the immune system. But as flow cytometers and flow cytometry reagents have become more sophisticated and customizable, scientists have developed protocols for measuring a wide variety of cell types, including plant cells and bacteria. Flow cytometry protocols are being used more frequently for food science and agricultural applications, and have been developed into validated assays that can meet the needs of industry scientists.

This white paper features several alternative flow cytometry applications in food science and agriculture that can meet the demand for rapid, precise, and reproducible results in fields highly reliant on accurate cell counts and downstream single cell analysis techniques.

Probiotic Assessment Using Flow Cytometry

The human gastrointestinal tract is filled with bacteria that are critical to maintaining the integrity and function of the digestive system, protecting against gut-associated pathogens and aiding in digestion . Infections, antibiotic treatment, and chronic conditions like irritable bowel syndrome have been associated with imbalances in beneficial gut bacteria. Our scientific understanding that this imbalance can be restored by ingesting fermented foods goes back more than a century to the work of Nobel Laureate Elie Metchnikoff. Probiotic bacteria are found in fermented food products like yogurt, sauerkraut and tempeh, and work over the last several decades has identified the key bacterial strains that have probiotic properties. In 2001, a formal definition of probiotics was proposed by the Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.”

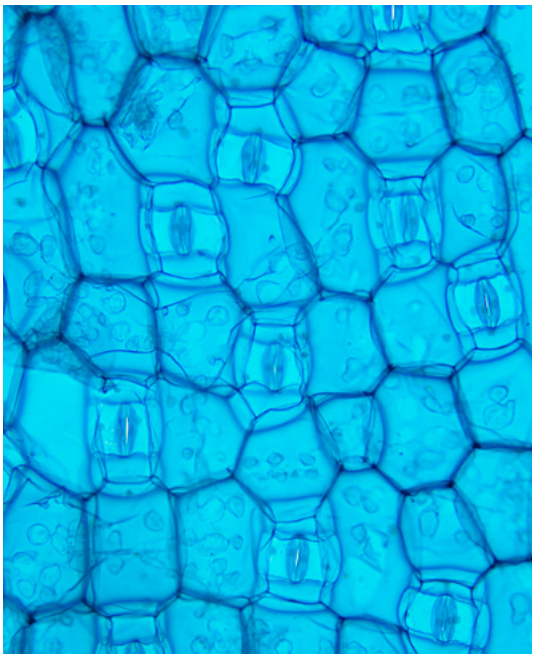
Ingestion of probiotic bacteria has been shown to help treat or manage several intestinal diseases, including infectious diarrhea, antibiotic-associated diarrhea, and inflammatory bowel disease. Probiotic intake has also been linked to beneficial effects on metabolic syndrome , and probiotics, particularly members of the Lactobacillus species, are now considered the third most popular dietary supplement behind vitamins and minerals . This growing demand for probiotic products, which typically must include information about the number of viable probiotic bacteria present in a product has created a demand for accurate and reliable methods for measuring the viability. In the United States probiotics may be regulated as food, dietary supplement or drugs, depending on its intended use and formulation . In these scenarios, the quantity of live probiotic bacteria must typically be included on the label.



Standard bacterial culture techniques have been used to estimate bacteria counts, but these methods take several days to perform and can only estimate the number of replicating bacteria. This can result in an underestimate of probiotic bacteria existing in the viable but non-culturable state (VBNC). Flow cytometry is not culture-dependent and uses fluorescent probes to measure viability of bacteria with multiple parameters including membrane integrity, metabolic activity, DNA replication and intracellular signaling. Flow cytometry-based viability assays can be done on bacteria immobilized in complex matrices such as fermented dairy products. In 2015, the International Organization for Standardization (ISO) published a standardized method (ISO 19344) to quantify probiotic bacteria in starter cultures, probiotics and fermented products, which has been seen as a major advance in quantification that meets the probiotic industry's burgeoning demands. Standardized flow cytometry assays have several advantages, including greater consistency, rapid turnaround, high throughput capabilities and the ability to measure viability with multiple parameters.

Probiotic manufacturers and fermented food producers are seeking expert help in flow cytometry-based quantification assays, as this technique is considered more technically specialized compared with classical probiotic quantification protocols. Contract research organizations have taken notice of the demand for probiotic bacteria quantification and are establishing specialized operations in both the United States and Europe to meet these needs. This will remain an area of potential growth as the health benefits of probiotic bacteria are studied in greater depth at both the basic research and clinical levels.

Using Flow Cytometry for Ploidy Analysis and Plant Breeding



Plant science has used flow cytometry for decades for estimating DNA content and ploidy levels in plant specimens. Ploidy is the number of sets of chromosomes in a cell, and living organisms can vary widely in ploidy levels depending on species characteristics or stage of the life cycle. Humans are diploid organisms and cells carry two sets of chromosomes, excluding gametes, which are haploid. In contrast, many plants are polyploid species, and some argue this larger amount of genomic material may confer evolutionary benefits.

Nuclear DNA content (C-value) in plant cells and ploidy can be quantified by flow cytometry using fluorochromes that stain DNA . This method is considered superior to alternative methods for accurately estimating genome size, replication cycle stage and level of ploidy. Flow cytometry can be performed on nuclei isolated from plant cells, plant cultures, callus or seeds, and this approach has been integral to determining C-values for numerous plant species in the field and in agricultural settings. Ploidy analysis can be carried out with C-value data and information about known ploidy levels from the same species. These protocols have been optimized and standardized to assure that DNA-staining dyes are not biased toward binding to specific base pairs, and that flow cytometers can measure C-values over a broad dynamic range. Beyond basic research, plant breeders use flow cytometry to characterize parent plants, evaluate ploidy of progeny, and screen seeds after interploidy crosses.

Plant breeders do not typically have flow cytometers on site, and often seek out contract research organizations that have expertise in plant cell analysis. These evaluations can be done rapidly and at high-throughput levels to meet the need of large scale plant breeding operations.

Conclusion

Flow cytometry is being recognized as a valuable tool to scientists in the food and agricultural fields. The rapid, precise, reproducible and scaleable nature of flow cytometry protocols lends itself well to use by industrial partners in probiotic supplement, food industries and plant breeding. New and refined methods will continue to make flow cytometry an indispensable method to these industries and beyond.

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