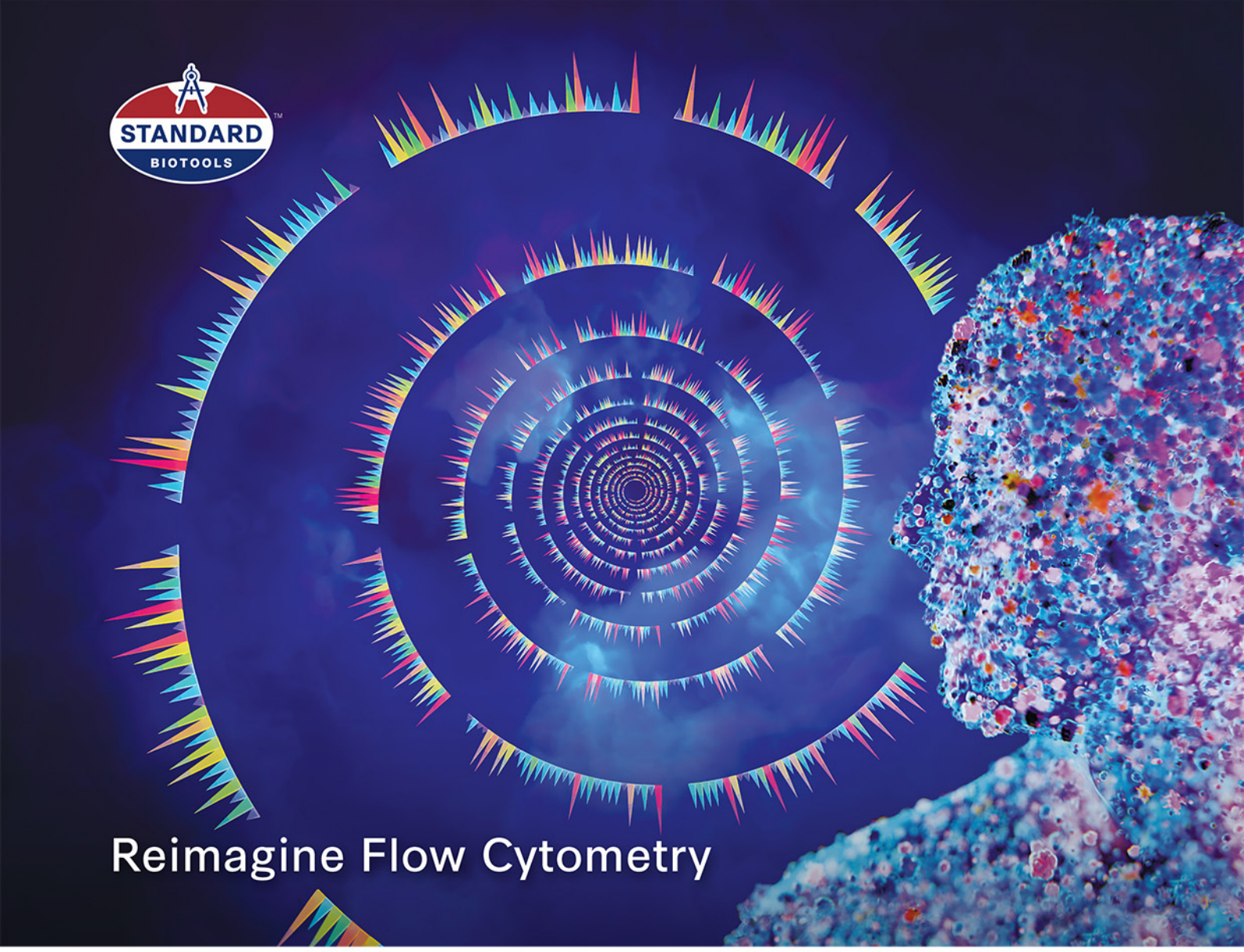

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EDITORIAL

ISAC CYTOMETRY Journal of Quantitative Cell Science PART A

Best practices in plant cytometry: The second tranche

1 | SETTING THE STAGE

Plant cytometry, the investigation and quantitative recording of the innate characteristics of plants at the cellular level, has rapidly matured over the last decades. This maturation has involved refining cytometric methods, from their first innovation, to accommodate increased levels of sophistication both in our knowledge of plant cellular systems, and in advances achieved in the design and performance of cytometric instruments. Together, this led to our prime purpose, the establishment of concepts of "Best Practices" in Plant Cytometry. This aims to thoroughly detail cytometric methods for rapid, accurate and reproducible application to questions in the basic and applied plant sciences. These Best Practices, being collectively published in Cytometry Part A, provide a resource of information that can be conveniently cross-referenced. It is hoped this will lead to improvements in the overall quality of published experiments involving plant cytometry, through identification and avoidance of common errors. It also allows us to track critical innovations at their points of introduction, thereby serving to decrease the incidence of publications that simply "rediscover the wheel". Through curating the appropriate reference sources, this collection also aims to serve as a nucleation site for discussion and additional expansion of plant cytometric techniques.

In 2021, we edited an initial tranche of Best Practice chapters, which subsequently were published on-line and then formally in Cytometry Part A. The first part of the Best Practices collection included Galbraith et al. [1] that provided an editorial introduction and overview of the first volume, Čertnerová and Galbraith [2] that dealt with flow cytometry (FCM) of algae, Kron et al. [3] that focused on cytometry of pollen and spores, Loureiro et al. [4] that dealt with preparation and cytometric analysis of plant nuclei for estimation of nuclear DNA content, Talhinhas et al. [5], that focused on FCM for fungal nuclear DNA quantification, and Doležel et al. [6] that presented best practices on chromosome analysis and sorting.

2 | MOVING FORWARD

In the second tranche of articles of the Best Practices collection, Čertnerová [7] comments on the challenges of analyzing small genomes using flow cytometry, from instrument requirements to changes in lysis buffers, lack of appropriate FCM standards, missing ploidy level data, and reduced genome size estimates to support studies aiming towards correlations between this trait and other functional traits.

Galbraith [8] describes the somewhat unexpected power of crowd-sourcing plant cytometric methods for measurement of plant genome sizes. Based on the establishment of a freely searchable, curated database hosted by the Royal Botanical Gardens at Kew [9], it was possible to extract "Gold Standard" nuclear DNA content values across plant species. These values are derived from worldwide submissions and used a variety of instruments and controls. Comparison of these to values obtained via our current best practices and using latest generation cytometers revealed remarkable correlations across genome sizes with the Gold Standard values identified by Kew. This strongly implicates convergence of methods and instruments to provide estimates of the true sizes of plant genomes.

Čertner et al. [10] presents guidelines for selecting the optimal plant tissue for FCM analysis, considering their mitotic activity, the possible occurrence of endopolyploidy, and how secondary metabolites interfere with FCM measurements. Best practices regarding sampling material, and material preservation strategies and storage, are also presented.

Since FCM measures relative fluorescence intensities of nuclei stained by a DNA fluorochrome, for ploidy determination, and for the estimation of the nuclear DNA content in absolute units, comparisons are required to a reference standard of nuclei of known DNA content.

Temsch et al. [11] present the advantages and disadvantages of standardization procedures. The authors comprehensively define the requirements of a reference standard, and the best fitting standards according with a plant genome size value. Recommendations are also given for establishing new standards. All of these will be valuable particularly when integrated into available data repositories such as that at RBG Kew [9].

In a detailed effort covering the most common applications of plant FCM, Sliwinska et al. [12] discuss the advantages and limitations of establishing plant ploidy, genome size, DNA base composition, cell cycle activity, and level of endoreduplication, while comprehensively providing advice on how to obtain accurate and reliable data, as well as how to manage troubleshooting during sample preparation, cytometric measurements, and data handling.

Antoniadi et al. [13] describe the applications of flow sorting to plant cell systems, focusing on single cells devoid of cell walls (protoplasts) produced by enzymatic solubilization of the plant cell walls under hypertonic conditions. The use of endogenous and transgenic sources of fluorescence is described, and the importance of measurement of cellular integrity and viability is emphasized. Examples of downstream measurements employing sorted protoplasts are provided.

Dunker et al. [14] describes the potential of multispectral imaging flow cytometry for improving our knowledge about environmental

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monitoring, with increasing accuracy, in a variety of research fields (such as plant–pollinator interactions, fossil samples, air, water, or food quality) and focal organisms that currently mostly depend on manual microscopic methods. In addition, best practice recommendations are provided.

3 | WHERE NEXT?

In these two special issues, a total of 13 articles concerning Best Practices in plant cytometry have been published. One article, regarding a further, very important topic, Best Practices for instrument settings and raw data analysis in plant flow cytometry, is in process. Taken together, these special issues constitute the most comprehensive guidelines for the correct application of FCM and cytometric sorting to plants. Such key rules for the reliable application of FCM and FCS to plants will benefit researchers, facility managers, journal editors, and reviewers. For some of the articles, guidelines that need additional empirical or theoretical work have also been clearly identified. When new data is obtained, or when critical comments, new ideas, and/or practical suggestions are provided, we intend to update the current versions of the manuscripts. In addition, as new applications emerge, the virtual issue can continue to be updated with new articles. Comments from interested persons are always welcome.

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