Cytometry PART A
Journal of the International Society for Advancement of Cytometry

ISAC
International Society for Advancement of Cytometry

Computational Analysis of Cell Images

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CYTOMETRY as a field is ever evolving, both in aspects related to methods and technology (including hardware and software) as well as applications in health sciences. Powerful modern instruments have become more accessible, and enabled us to probe the intricate structure and function of cells with unprecedented accuracy and specificity. With high-throughput instruments such as automated microscopes and imaging flow cytometers already commonly available software for extracting biological information from larger, and more complex datasets, has become increasingly the focus of significant research efforts (1). The powerful combination of modern instruments and software has enabled researchers to start asking increasingly complex questions, obtaining more accurate predictions, and extending the range of applications. In this *Cytometry Part A* special section, we have collected four papers that exemplify some of these trends.

Siddiqui et al. [this issue, page 296], for example, utilized laser-scanning cytometry to study the phosphorylation of the core histone protein H2AX (γH2AX) in response to ionizing radiation (IR). They report a significant increase in nuclear γH2AX foci 30 min after IR exposure, amongst other findings. Nikonenko and Bozhok [this issue, page 309] describe a new, efficient, computational technique for quantifying nuclear shape symmetry and show they can be predictive of abnormality in thyroid cells. Tosun et al. [this issue, page 326] describe a new computational technique for quantifying nuclear chromatin content and report it can be highly effective in providing diagnostic information of malignant mesothelioma directly from cytology specimens. Nielsen et al. [this issue, page 315] report that nuclear texture features have high prognostic value in a population of 254 uterine sarcomas. Dialing back even just 10 years ago, such studies would have been harder to conduct.

As our ability to measure and study the relevance of morphology and other cellular properties increases, important issues related to reproducibility also apply. To put it succinctly, how do we make sure we are not "fooling ourselves" with data? It is a well known fact that, for a fixed dataset, as the number of measurements (features) made per cell increases, so do the chances that specific linear (or nonlinear) combinations will be significant in the statistical sense. Hence, when hypothesis regarding differences between states or conditions (e.g., healthy vs. diseased) are being characterized and estimated directly from image cytometry data, it is crucial to enforce the concept of separating training data, or data used to discover the effect (claim or hypothesis), from testing data, or data used for assessing the validity or the claim [this issue, Tosun et al. page 326; Nielsen et al., page 315].

Looking toward the future what should we be hoping from automated software for cell image analysis? Beyond enhancing automation and robustness of certain operations (e.g., cell or nuclear segmentation), it will be important to develop cytometry methods to transcend findings between different experimental datasets, so that findings reported from these kinds of studies [this issue, Siddiqui et al., page 296; Nikonenko and Bozhok, page 309; Tosun et al., page 326; Nielsen et al., page 315] can be validated in different laboratories. As we are all too familiar with, the value of certain numerical features currently used for quantifying populations of individual cells can vary greatly and unpredictably as a function of image quality, resolution, cell culture preparation, etc. The development of image cytometry calibration standards (2) is likely to play an important role in this process, as

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Published online in Wiley Online Library (wileyonlinelibrary.com)
DOI: 10.1002/cyto.a.22653

will the software that is able to extract more physiological information from such data. Enhancing our ability to characterize biophysically interpretable information, such as number of cytoskeleton filaments, amount of protein in different subcellular compartments, etc., will significantly enhance the ability to understand changes between cell populations, or changes of the same population over time.

Literature Cited

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