Confocal 3D DNA cytometry: Assessment of required coefficient of variation by computer simulation

To the Editor,

Ploeger et al. [4] presented an important work that provides accuracy criteria for assessing the DNA ploidy in thick histological sections. The work relates to image cytometry performed with a confocal microscope, a method that advances lately from research to clinical applications [1].

The success of the microscopy-based assessment is based on many parameters that have to be standardized and optimized to meet the required medical standards. These parameters can be divided into two different sets. The first set includes sample-related parameters such as the sample preparation method, the influence of the detergents and the variability of the tumor itself [4]. Other parameters are related to the measurement method and system. These include parameters such as the intrinsic noise acquainted with the detection system and the practical number of cells that can be monitored in a reasonable amount of time.

Ploeger et al. have showed that the accuracy level analyzed by the coefficient of variation (CV) of the histogram peak should be lower than 15%. It was also shown that the size of the population only marginally effect the final assessment accuracy (see Fig. 4). This result emphasizes the importance to gain the best possible signal with the smallest possible noise with the detection system.

The effect of the detection method and system was briefly mentioned by Ploeger et al. With this respect, I would like to highlight various developments that will most likely effect the applicability of imaging methods to applications such as DNA ploidy and others. These developments have the potential to significantly increase the throughput of cells detection so that many more cells can be detected in a given amount of time and to decrease the noise that is inserted by the detection system.

The first aspect relates to scanning methods. We lately witness breakthroughs in scanning methods that are based on either novel scanning techniques such as the array microscope [5] or methods that optimizes the detection by allowing a continues scan [3]. These methods significantly increase the throughput so that much larger cell-populations can be considered.

These methods were so far used mainly in transmission modes but it can be extended also to fluorescence microscopy. Even though the intensity levels in fluorescence are lower, these methods can still increase the throughput significantly. Currently these methods are not based on confocal setup, but emerging techniques have already been demonstrated that may extend these methods to confocal setting as well [2]. In addition, we lately see a growing number of applications that uses wide-field microscopy combined with deconvolution for extracting three-dimensional information [6]. The much-higher signal-to-noise ratio achieved by a CCD-based detector versus a confocal system that is based on a single-point detector compensates in many of these cases for the reduced resolution (mainly axial) and large depth of field. This can be relevant especially for applications such as DNA ploidy where spatial resolution may not be the most important issue.

To summarize, important advances in the development of clinical applications, such as for DNA ploidy detection descried by Ploeger et al., calls for improved and high throughput detection methods. I strongly believe that these methods are emerging and should be adopted, optimized and improved for actual clinical applications.

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