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Automation of the in vitro micronucleus assay for genetic toxicology testing on the Image Stream imaging flow cytometer

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The in vitro micronucleus (MN) assay may be a well-established method for evaluating genotoxicity. MN is made from whole chromosomes or chromosome fragments that lag behind during the metaphase-anaphase transition and are excluded from the most nuclei following karyokinesis. The presence of MN indicates chromosomal mutations and their quantification is used as an endpoint in genotoxicity testing. Manual microscopy is typically used to perform the MN assay but this is laborious, with low throughput and inter-scorer variability being of particular concern. Automated methods including slide-scanning microscopy and traditional flow cytometry are developed but these methods suffer from limitations including lack of cytoplasmic visualization (slidescanning microscopy) and therefore the inability to visually confirm the legitimacy of MN (flow cytometry). The ImageStream®X (ISX) imaging flow cytometer possesses the potential to beat these limitations because it combines the speed and rare event capture capability of conventional flow cytometry with the high-resolution fluorescent imagery obtained by microscopy. A method to perform the in vitro MN assay on the ISX has been developed using well-known aneugens and clastogens. High-resolution imagery of micronucleated binucleated cells are often captured and automatically identified and enumerated software that accompanies the ISX allowing the evaluation of geno- and cytotoxicity. Details describing the event of the ISX-based in vitro MN assay are going to be presented. The high throughput nature of the ISX overcomes many of the challenges in slide-based microscopy and conventional flow cytometry techniques. Significantly more cells can be collected and scored compared to microscope-based versions of the assay, improving the statistical robustness of the method. Additionally, all collected imagery are often stored in dose-specific data files. These results represent the primary step towards the event of a totally automated approach for performing the in vitro MN assay to assess cytotoxicity and genotoxicity using imaging flow cytometry.

A number of methods have been developed to perform the in vitro MN assay. Each of these has advantages and limitations concerning throughput, accuracy and visual confirmation of MN. The assay is most commonly performed through manual fluorescent microscopy which has the benefit of visual confirmation of MN; however, this method is time consuming and prone to scorer subjectivity thanks to both interscorer variability and fatigue when many samples must be scored 18 Automated microscopy methods are developed using software algorithms that are ready to identify and capture images of fluorescently-labeled nuclei and MN 19, 20, 21, 22. While these automated methods remove the tedious nature of manual slide-scoring, they are doing not typically allow visualization of the cytoplasm and thus it are often difficult to associate one (or multiple) MN to a particular cell. Also, many of these methods currently do not support automatic scoring of polynucleated (POLY) cells, a required parameter for cytotoxicity calculations when the assay is performed with Cyt-B 12. Furthermore, some inherent slide-making concerns, such as ensuring high slide quality and optimal cell density, can be difficult to optimize 23. Laser scanning cytometry (LSC) methods were introduced in the early 2000s and have been recently improved with the advent of new generation instrumentation. LSC systems leave the identification and automatic enumeration of MN also as mononucleated (MONO), BN, and POLY cells. While LSC can differentiate MN from other DNA positive objects through the use of multiple stains, many systems are still hindered by the need to create high quality microscope slides with adequate cell separation 24, 25, 26. Finally, conventional flow cytometry methods have been developed to perform the in vitro MN assay. One of the original publications by Nüsse and Marx described a method based on ethidium bromide fluorescence and forward and side scatter intensities to differentiate between debris, nuclei, and MN 27. Recently, an imaging flow cytometry method to perform the CBMN assay specifically for use in triage radiation biodosimetry was developed 40, 41, 42, 43, 44, 45. Results from this method demonstrated that cellular images of both the cytoplasm and DNA content might be captured at higher throughput than other automated methods and stored during a data file, eliminating the need to create microscope slides 40. Candidate BN cells with and without MN were automatically identified in IDEAS through the use of mathematical algorithms to implement the scoring criteria developed by Fenech et al. 9, 46. The ISX-based version of the CBMN assay was shown to automatically score more BN cells in a fraction of the time compared with manual microscopy, therefore enhancing the statistical robustness and speed of the assay. A dose response calibration curve to guage the frequency of MN per BN cell was created from 0 to 4 Gy and was similar in magnitude to other calibration curves published in the literature 43. Finally, through the use of blinded samples it was verified that the method could generate dose estimates to within ±0.5 Gy of the true dose, sufficient for triage radiation biodosimetry 44.

All of these proposed future research directions would increase the capacity of the ISX to provide more robust and comprehensive results from the in vitro MN assay than are currently available from all other methods.

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