RAPID EVALUATION OF DNA DAMAGE WITH COMET ASSAY IN COMBINATION OF HIGH THROUGHPUT MICROSCOPY

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Introduction: Comet assay is well known method for DNA damage evaluation. However, it also possesses some limitations related to the problem with manual entry of each comet for analysis that decreases the performance and measurement quality. Here we suggest the way for modification of this method based both on advances in microscopy and digital processing.

Methods: Peripheral blood mononuclear cells (PBMC) of different origin were used for experiments. DNA damage was determined by Comet assay method (according protocol of 4250-050-K; Amsbio), based on acrydine orange fluorescent staining. PBMC were placed in thin film on slide. After transfer and staining, slides were analyzed under fluorescent microscope Carl Zeiss Cell Observer SD with magnification x 20. «Tile» setup of ZEN software was used to create a scan area of ~2x1 cm of ROI at slide to collect sufficient number of singe comets. FIJI/ImageJ(NIH) software was used to process images converted in advance to 8-bit grayscale TIFF. It was done in the following order: 1) first high filtering (DoG) was applied to remove speckles and illumination defects; 2) subtracted images were converted to binary mask by threshold adjustment. Original image was used as a reference for binary mask, and this part was carried out by experienced researcher; 3) «Analyze particles» tool of FIJI/ImageJ(NIH) was used to detect nuclei in automated mode. Size limitation for particles was set to avoid false recognition of a range of debris and overlapping nuclei.

Results: There ~500-2000 objects have been recognized in differently scaled samples with time interval ~30 min/sample). Damage was evaluated in simplified mode as a ratio of diameters of nuclei and comet (circularity). The following results for circularity were obtained: control=0,595 (0,003 SEM), n=696, peroxide=0,219 (0,004 SEM), n=881.

Conclusion: Despite some limitations related to manual entry/handling of samples and biased selection of the samples, single comet assay is most precise method of analysis of DNA damage. The proposed method is less precise and requires the improvements in filtering and recognition algorithm. However, it may serve as a tool for fast and unbiased measurement of large sets of samples.