An automated scoring procedure for the micronucleus test by image analysis.

The micronucleus assay (MNT) in human lymphocytes is frequently used to assess chromosomal damage as a consequence of environmental mutagen exposure, to assess the effect of mutagens or to search for reduced DNA repair capacity after a mutagenic challenge. We have established an automated scoring procedure for the cytokinesis blocked MNT based on computerized image analysis (Metasystems Metafer 4 version 2.12). To evaluate the results we used the reproducibility of counts, established a dose-response curve for gamma-irradiation and used the ability of the system to differentiate between breast cancer patients and controls as a biological reference, a difference which we had observed before by visual counting. Blood cultures were irradiated with gamma-rays (2 Gy) at the beginning and treated with cytochalasin B during the last 24 h. The slides were stained with Giemsa for visual counting and with DAPI for automated analysis. Our test sample consisted of 73 persons (27 with breast cancer and 26 female and 20 male controls). A comparison between visual counting (controls, mean MN frequency 313) and automated counting (mean MN frequency 106) in slides from the same culture revealed a large drop for the automated counts. However, the automated counts were as reproducible as the visual counts [coefficient of variation (CV) on the sample approximately 20%; CV on repeated counts of the same slides...
approximately 5%] and both counts were highly correlated. Furthermore, the discrimination between cases and controls improved for automated counting of slides from the same cultures [visual odds ratio (OR) 16, P < 0.0001], with a strong dependence on the set of parameters used. This improvement was confirmed in a validation sample of an additional 21 controls and 20 cases (OR = 11, P = 0.0018) performed as a prospective or diagnostic test.