MetaSystems White Paper

Obtaining CBPI in Micronucleus Tests

Introduction

The cytokinesis-block micronucleus (CBMN) assay, a version of the in vitro micronucleus assay (MNvit), is widely used to test substances for their ability to cause DNA damage. Typically MNvit tests are used in the frame of biological dosimetry, toxicology, and environmental studies. Recommendations for the design of MNvit tests are published by the OECD in the respective guideline no. 487 (2010).

In brief, the CBMN assay utilizes chemical blocking of cell division (with the actin polymerisation inhibitor cytochalasin B) after treatment of cells with the potentially mutagenic agent in culture. Since blocking only affects division of cells (cytokinesis), but not mitosis, the resulting cell population consists of a mixture of cells with one (mononucleates), two (binucleates), or three or more nuclei (multinucleates). To ensure that cells have undergone exactly one mitosis after treatment, micronuclei are usually only scored in binucleates. The micronucleus rate is taken as an indicator for the DNA damaging capacity of the tested substance.

The OECD recommends to also quantify the effect of cytotoxicity, i.e. the impact of the tested substance on cell proliferation. In the CBMN assay cytotoxicity is expressed by the Cytokinesis-Block Proliferation Index (CBPI), which is actually a ratio between the three sub-populations of cells with different numbers of nuclei (for details on the calculation please refer to the OECD guideline 487).

Micronuclei in binucleates are automatically scored with **Metafer MNScore** (preferrably using DAPI as nucleus dye). Automated calculation of CBPI is possible with **Metafer MetaCyte**, if cytoplasm is stained separately with Propidium iodide.

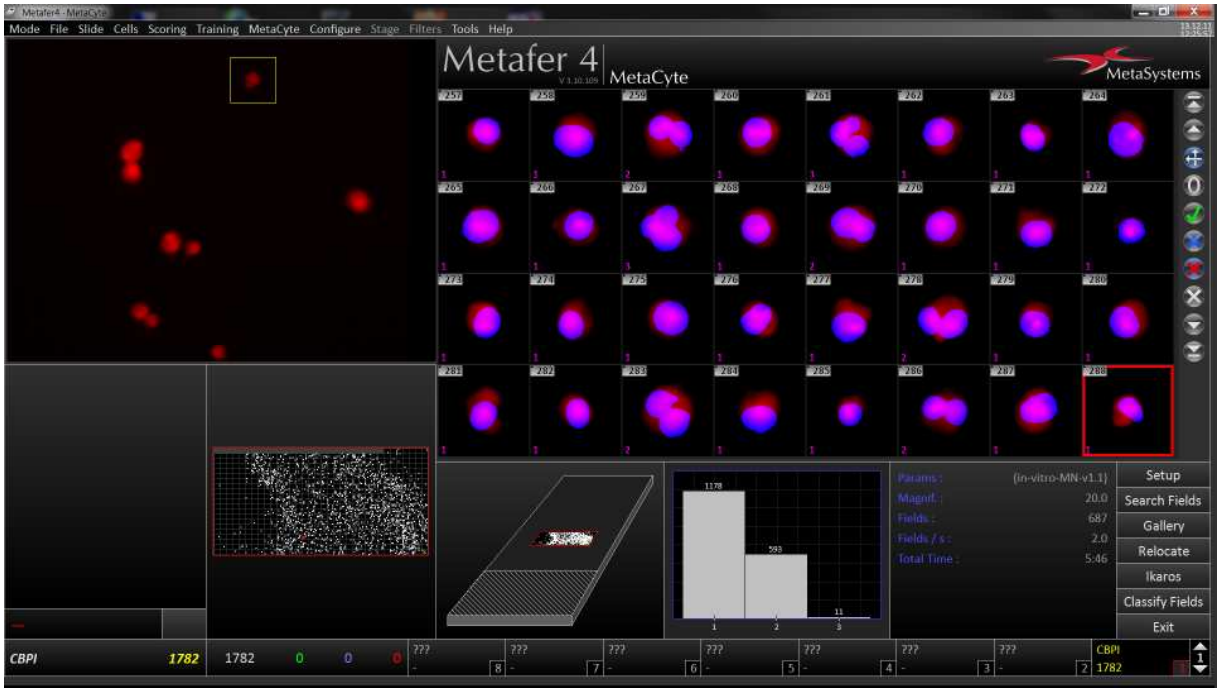

Fig. 1: Metafer MetaCyte gallery with results of the CBPI scoring. Numbers in the corners of gallery images show the nucleus statuses (1: mononucleates; 2: binucleates; 3: multinucleates). The histogram in the lower part of the screen summarizes data for all cells.

WPC-CBPI-2011-11-01-X.pdf

MetaSystems White Paper

Obtaining CBPI in Micronucleus Tests

Functionality

Automated scoring of micronuclei in cells prepared for the MNvit assay is done with **Metafer MNScore**. Recommended staining for automation is DAPI (fluorescence mode), as this dye is more specifically staining nucleic material than for example Giemsa. However, evaluation of nucleus number per cell requires additional cytoplasmic staining. Good results could be obtained with a low concentrated Propidium iodide staining, which enables the system to detect cell boundaries in the red color channel.

Samples prepared accordingly can be automatically scored with **MNScore** to obtain the number of micronuclei in binucleates. A linked **MetaCyte** search will afterwards scan the same slide region to detect the number of nuclei in each cell. Detection is based on algorithms to evaluate the morphology of the nucleus clusters within each cell after binarizing the nucleus images.

1. Automated Detection of Micronuclei in Binucleates
   Binucleates are detected based on nucleus morphology. Micronuclei are detected within the vicinity of detected binucleates and automatically counted. Micronuclei scoring results are displayed in the gallery image corners of the respective binucleate. Data are also displayed as a histogram, allowing for fast selection of sub-populations.
2. Linking of Scans
   The scan for micronucleus detection can be automatically linked to the CBPI scan; no user interaction is required.
3. Automated Determination of Nucleus Counts
   Cell boundaries are identified based on the Propidium iodide signal. Images of nuclei within each cell are binarized and automatically analyzed for their morphology. Mononucleates, binucleates, and multinucleates are identified, and a number for each sub-group is shown in the gallery image corners of the respective cell. A histogram shows the count of cells and allows for selection of sub-populations.

Contact MetaSystems Worldwide

Headquarters:	MetaSystems Germany	tel +49 6205 39610	info@metasystems.de
Asia and Australia:	MetaSystems Asia	tel +852 2587 8333	info@metasystems-asia.com
USA and Canada:	MetaSystems Group	tel +1 617 924 9950	info@metasystems.org
Web:	www.metasystems-international.com		