

THE PLURIPOTENT MICRONUCLEUS ASSAY (PMNvit): A NOVEL METHOD FOR GENOTOXIC POTENTIAL ASSESSMENT

Lerche, C. F.^a, Hölzel, B. N.^{a, b}, Allner, T.^a, Allner, B.^a, Lessing, F.^c, Stahlschmidt-Allner, P.^a

^a Gobio GmbH Institute for the Ecology of Waters and Applied Biology, Aarbergen, DE

^b Institute of Zoology, Johannes Gutenberg University Mainz, Mainz, DE

^c Fresenius University for Applied Sciences, Idstein, DE



Introduction

The micronucleus test is an assay for assessing the genotoxic potential of chemicals based on the formation of micronuclei (MN)

- 2 variants: *in vivo* (OECD TG 474) and *in vitro* (OECD 487, MNvit)
- 2 different modes of actions are known: aneugens impair the spindle apparatus and clastogens cause breakages of chromosomes
- MNvit usually performed with cultivated primary cells (e.g. lymphocytes) or immortalized mammalian cells (cancer cells)¹

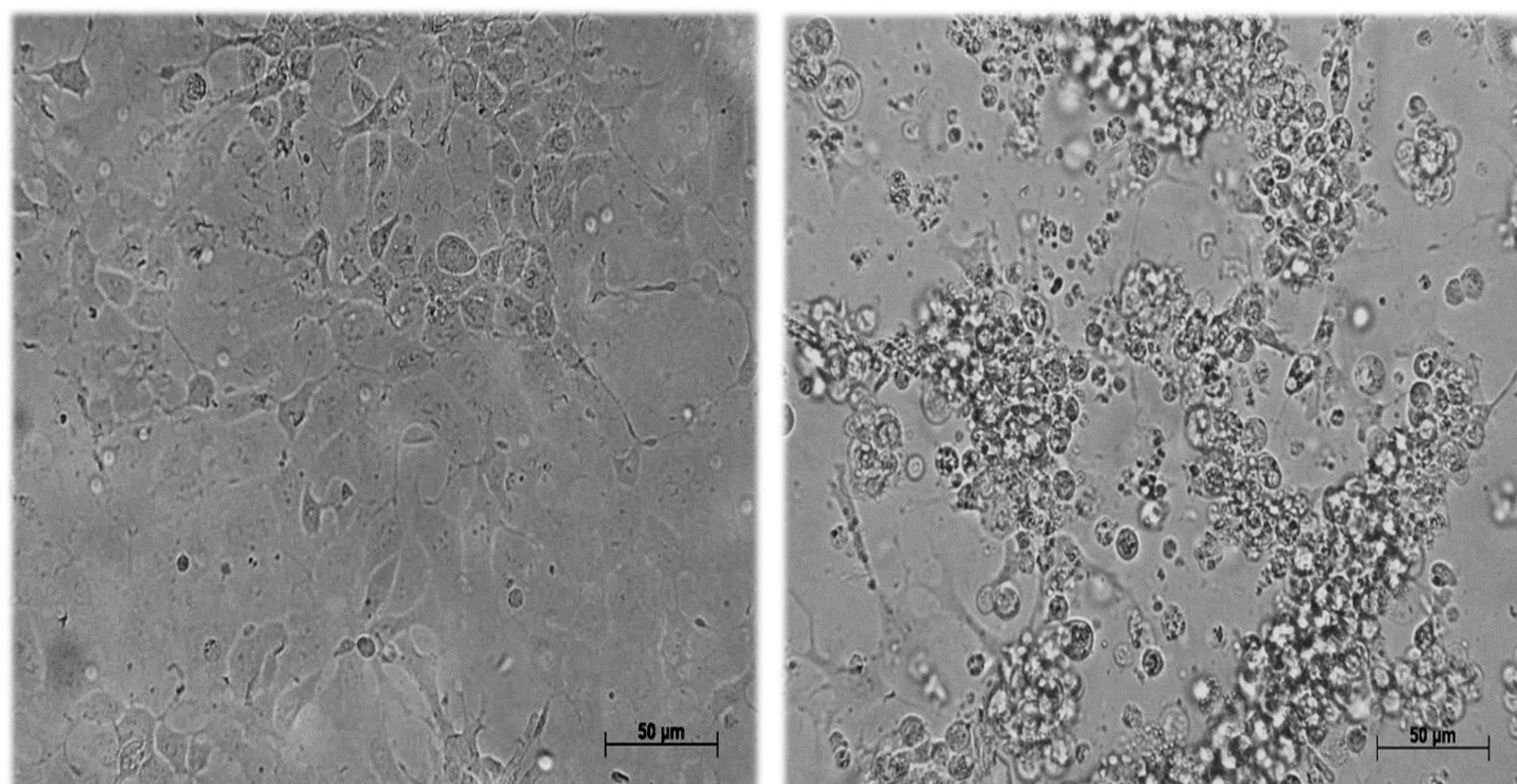
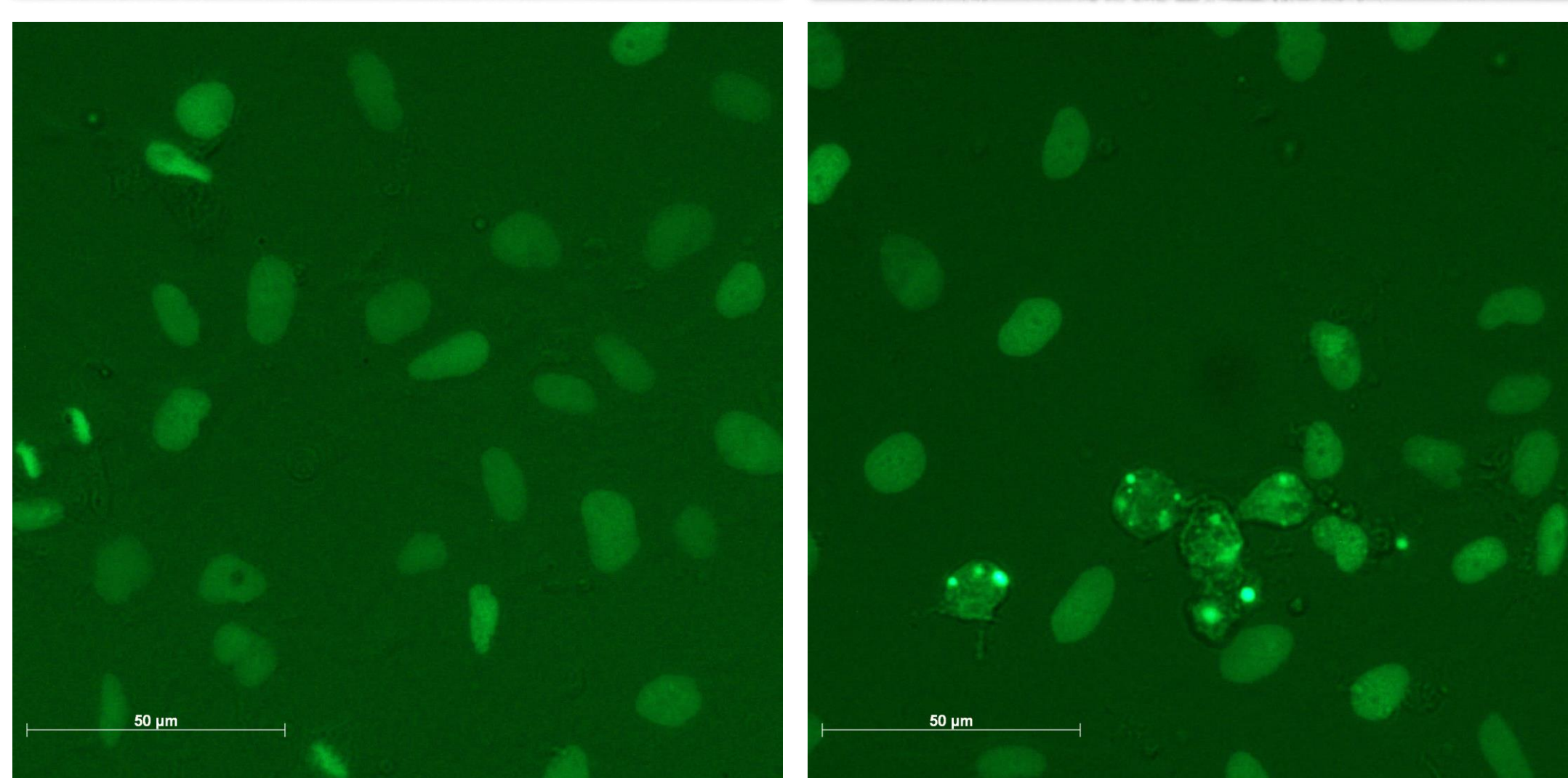
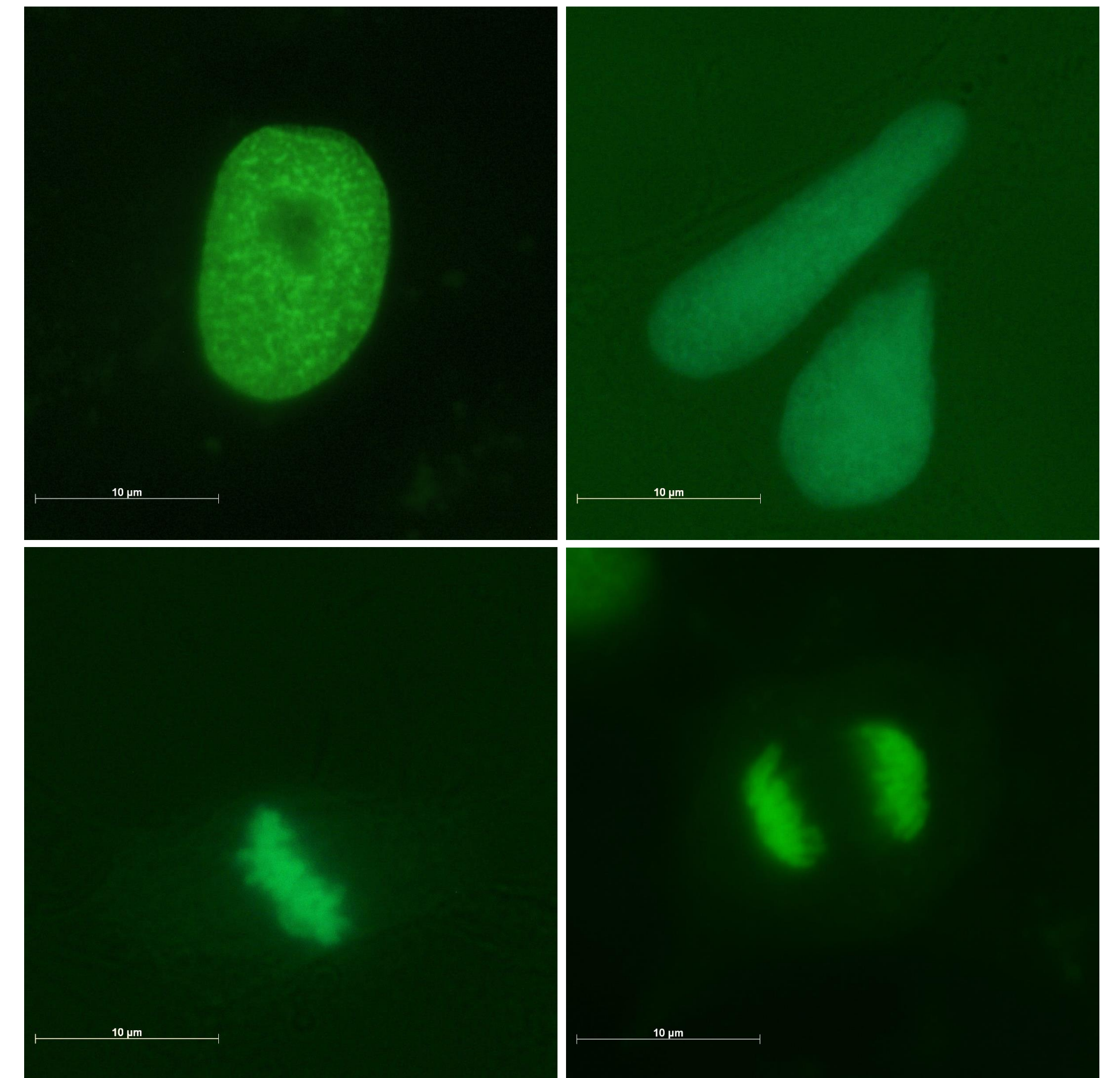


Figure 1: Unimpaired cytogenetic features

Figure 2: Monolayer morphology of treated and untreated cells

Materials & Methods

- *In vitro* MN assay with a pluripotent stem cell line from the Koi carp brain with GFP-labelled histones (H2B) for visualization of nuclear structures (KCB_{GFP}, DSM ACC3285 Budapest Treaty)
- Cells cultivated in 96 microtiter plates at 26 °C without CO₂, and exposed for 12-14 hours to model genotoxic compounds: colchicine (aneugen) and 4-NQO (clastogen)
- Determination of MN frequency in fluorescence microscopy photographs of living cells



Results & Discussions

- Increasing MN frequencies were determined in a dose-dependent manner for both compounds below cytotoxic ranges
- Background MN frequency was 0.65 % for the cell line (n > 18000 cells)
- Monolayer morphology can be used as indicators of cytotoxicity

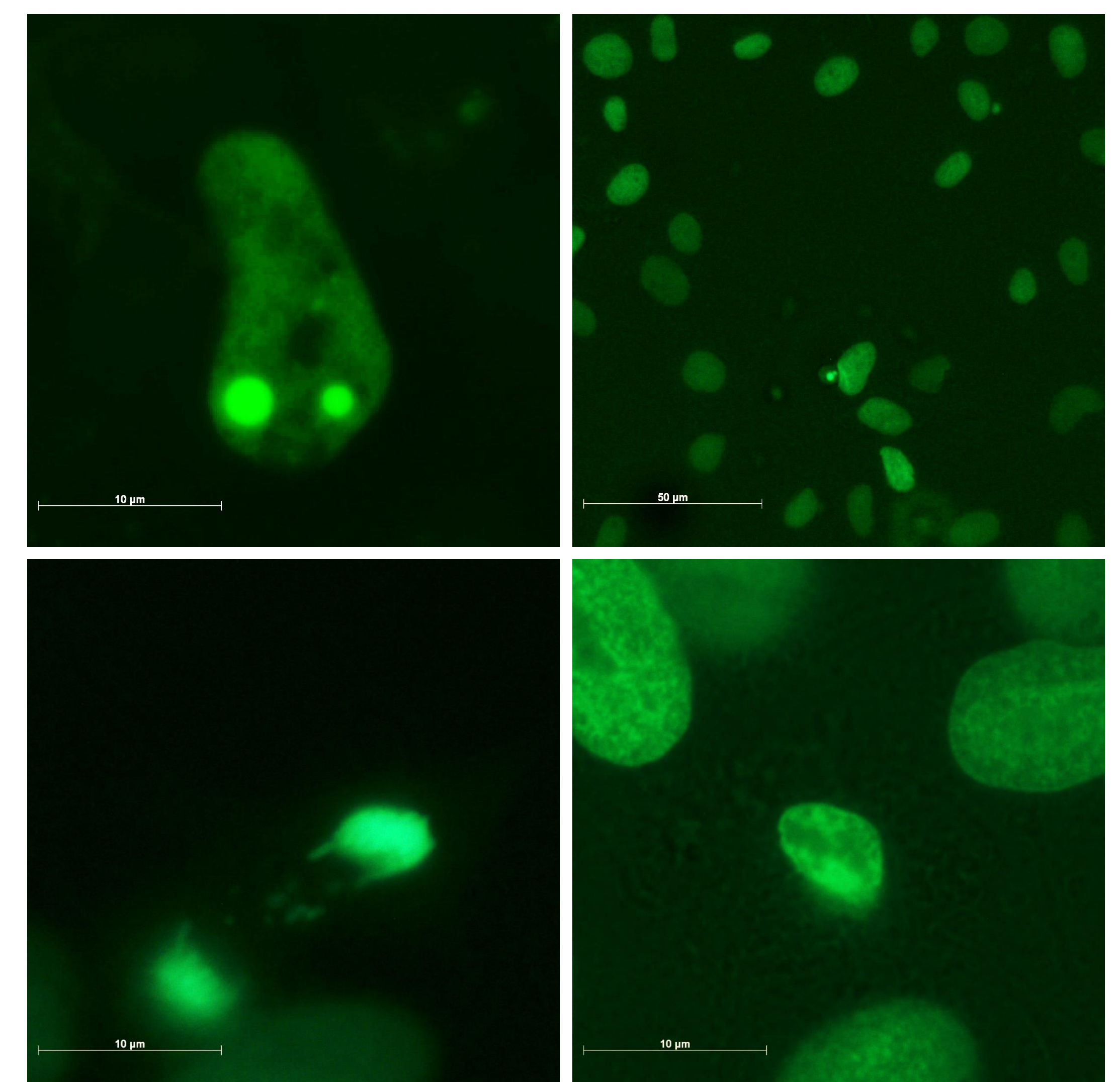


Figure 3: Genotoxic damage pattern

Conclusions

- Reliable assessment of MN frequencies with the PMNvit assay
- Clear improvement in terms of working time and expertise requirement compared with previously established methods
- After MN assessment survival of cells allows for the evaluation of long-term effects, enabling the inclusion of additional endpoints

Acknowledgment

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Reference

1) Fenech, M.; Chang, W. P.; Kirsch-Volders, M.; Holland, N.; Bonassi, S.; Zeiger, E. (2003): HUMN project: Detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. In: *Mutation research* 534 (1-2), S. 65–75.