MetaSystems White Paper

Metafer Software in Genetic Toxicology Testing

WHITE PAPER

Automated image analysis is increasingly adopted in genetic toxicology to improve efficiency, standardization, and documentation in regulatory testing environments. The Metafer platform software has emerged as a widely implemented solution in this space.

This review of published genetic toxicology workflows demonstrates that Metafer is routinely used in GLP-compliant laboratories across pharmaceutical industry, contract research organizations (CROs), and public-sector institutions, indicating broad acceptance in high-standard regulatory environments.

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Genetic Toxicology in Preclinical Studies

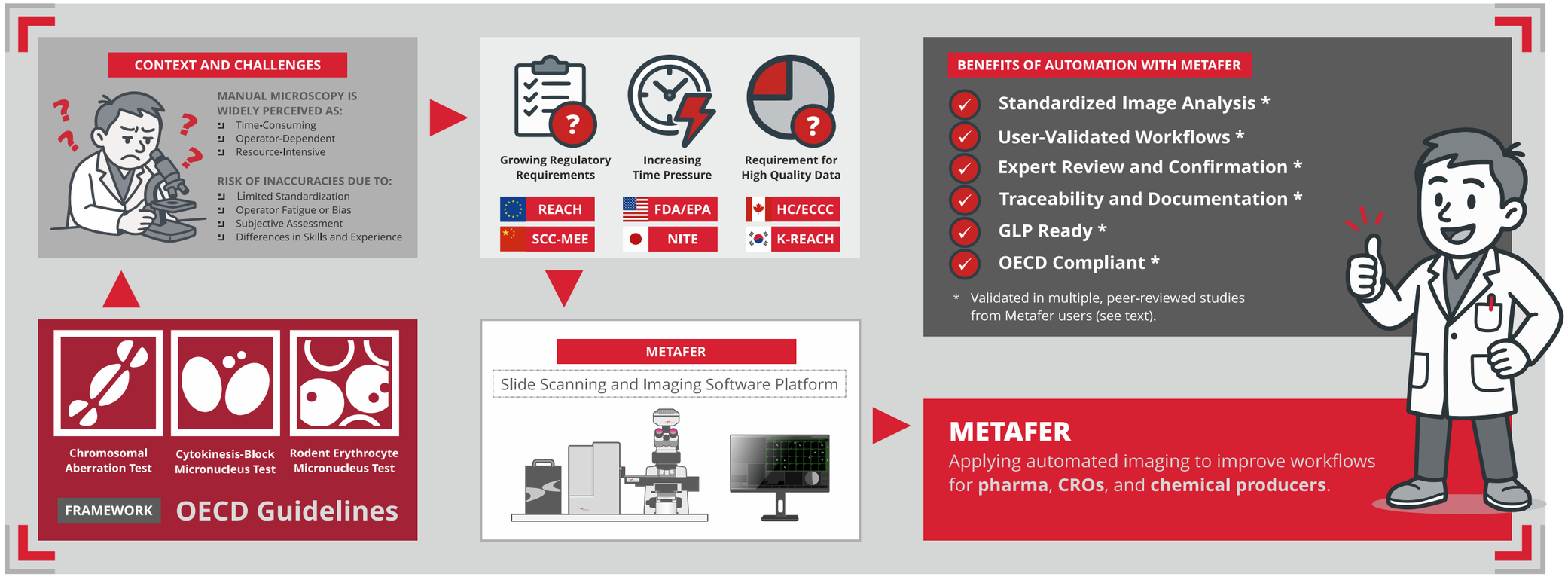
Genetic toxicology assays are designed to detect DNA and chromosomal damage that may lead to mutations, cancer, or heritable disorders. Regulatory guidelines (including REACH in Europe, FDA/EPA in the USA, SCC-MEE in China, K-REACH in Korea, and many more) mandate genotoxicity assessment using assays such as

- The chromosome aberration (CA) test,
- The cytokinesis-block micronucleus (MN) test, or
- The rodent erythrocyte MN test.

Conventional manual microscopic scoring of these endpoints is widely acknowledged to be time-consuming, operator-dependent, and resource-intensive, particularly when large dose ranges and replicate studies are required. Furthermore, the risk of inaccuracies caused by a lack of standardization in data analysis, fatigue, operator bias, subjective interpretation, and variability in individual training and experience remains a significant concern when genetic toxicology tests are evaluated manually under the microscope.

Preclinical genetic toxicology testing is undergoing a structural transformation driven by increasing regulatory expectations, pressure on timelines, and the need for reproducible, high-quality data. The OECD genetic toxicology test guidelines emphasize robust study design, reproducibility, and clear documentation.

Validated in peer-reviewed studies and routinely used in GLP laboratories, the Metafer scanning platform software provides standardized and traceable image-based scoring that supports reproducible data generation within OECD-aligned genetic toxicology assays. As a result, it is positioned as a strategic asset for pharmaceutical companies, contract research organizations (CROs), and chemical safety laboratories.



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The Chromosomal Aberration Test

The in vitro chromosomal aberration (CA) assay is a standard regulatory test for detecting structural chromosomal damage in mammalian cells, as defined in OECD Test Guideline 473. It is widely used in pharmaceutical and chemical safety assessment to identify clastogenic effects induced by test substances.

A central methodological requirement of the assay is the identification and evaluation of metaphase spreads with sufficient quality and quantity to ensure statistical reliability. In conventional workflows, this process relies heavily on manual scanning and microscopic evaluation, which is time-consuming, labor-intensive, and subject to inter-operator variability. These constraints have driven the adoption of automated metaphase detection and image acquisition systems as supportive tools in modern genetic toxicology.

Metafer Validation Studies and Use in GLP Labs

Finot *et al.* (2011) from Covance Laboratories (France) reported an early validation study of the Metafer software, which they used to support the chromosomal aberration test on human lymphocytes and to determine the mitotic index in an automated manner. The authors emphasize the use of Metafer for image-based, on-screen aberration analysis, enabling cytogenetic evaluation from archived digital metaphase images rather than direct manual microscopy.

Subsequently, the same group and collaborators published further studies applying this semi-automated, image-based approach to chromosomal aberration analysis in the assessment of ethyl-paraben genotoxicity (Finot *et al.*, 2017).

In their book chapter on the In Vitro Chromosome Aberration Test, Registre and Proudlock (2016) confirm these findings and note that automated imaging systems substantially reduce slide scanning and metaphase search time in routine CA testing. They explicitly recognize automated metaphase finding and image capture as appropriate supportive tools within OECD-compliant assays, provided that final aberration identification and classification remain the responsibility of trained cytogeneticists.

In a practical application, Römer *et al.* (2015) demonstrate that Metafer can be integrated into an OECD-compliant in vitro chromosome aberration test as a semi-automated system for metaphase detection, localization, and retrieval. Expert human scoring of chromosomal aberrations was retained, while automation significantly reduced workload and data variability. This study provides clear evidence that Metafer-supported CA analysis is suitable for genetic toxicology studies conducted under OECD TG 473.

SOURCE CITATION

In Covance Laboratory SAS [...], the Metafer platform was fully validated [...] for chromosomal aberration assay to automatically score the mitotic index and to allow a manual seizure for chromosome aberrations through a user-interface.

Cited from: Finot *et al.*, 2011

CENTRAL CONCLUSION

Taken together, the publications cited on this page show that Metafer has become an established semi-automated platform for chromosomal aberration testing, reducing metaphase search burden and improving documentation while preserving expert led aberration classification.

The Cytokinesis-Block Micronucleus Test

The cytokinesis-block micronucleus test (also refered to as the in vitro micronucleus assay) is a core regulatory test for detecting both clastogenic and aneugenic mechanisms. As defined in OECD Test Guideline (TG) 487, the test quantifies micronuclei in interphase cells as an indicator of chromosomal damage or chromosomal malsegregation in mammalian cells. OECD TG 487 was originally adopted in 2010, revised in 2014, and most recently corrected in July 2023, and is widely accepted for regulatory submissions under frameworks such as REACH, FDA/EPA, and other safety guidelines.

SOURCE CITATION

In conclusion, the semi-automated Metafer system provides a reliable, accurate and efficient method for the scoring of the in vitro CBMN assay in a range of human cell lines.

Cited from: Seager *et al.*, 2014

CENTRAL CONCLUSION

Taken together, the cited studies demonstrate that image-analysis–based micronucleus scoring using Metafer has progressed from validation to routine use, supporting regulatory submissions while enabling increased throughput and quantitative analysis.

Metafer Validation Studies and Use in GLP Labs

Finot *et al.* (2011) also used their Metafer-driven system for the analysis of mononucleated L5178Y cells and binucleated human lymphocytes. In their conclusions, the authors emphasized that automated analysis enabled the scoring of substantially larger cell numbers per experiment, resulting in increased statistical power and robustness of the generated data.

Later, Seager *et al.* (2014) from Swansea University (UK) evaluated and validated the Metafer software for in vitro mammalian cell micronucleus testing. The study demonstrates that, when supported by standardized slide preparation, optimized classifiers, and limited expert review, Metafer delivers reliable, reproducible, and biologically relevant results that are comparable to conventional manual microscopy—while substantially improving throughput, statistical power, and operational efficiency. The results revealed:

- Automated and manual scoring produced highly correlated dose–response relationships (R^2 up to 0.99, depending on compound).
- Benchmark dose (BMD) and lower confidence limit (BMDL) estimates were closely aligned across scoring methods.
- Scoring time per slide was reduced from ~45 minutes (manual) to ~15 minutes (semi-automated), representing a ~3-fold efficiency gain.

In a letter to the editor with reference to this study, Maertens and White (2015) from Health Canada added information on the automated proliferation index analysis (CBPI) with the same study setup, using Metafer as analysis automation tool.

Verma *et al.* (2017), also from Swansea, conducted a comprehensive evaluation of the Metafer image analysis platform against manual scoring and a flow cytometry-based method using human lymphoblastoid TK6 cells exposed to reference genotoxins. Key findings included:

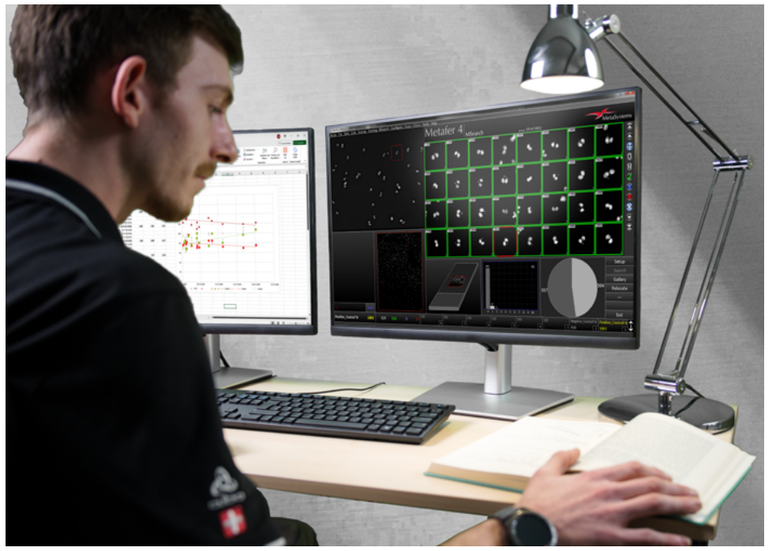
- Comparable dose–response relationships between Metafer and manual scoring,
- Reliable detection of clastogens (methyl methanesulfonate), aneugens (carbendazim), and weak genotoxic carcinogens (ochratoxin A),
- High reproducibility suitable for benchmark dose modelling.

In a study with particular emphasis on the use, validation, and role of Metafer in in vitro micronucleus testing, Doherty *et al.* (2016) from Astra Zeneca (UK), reflecting an internal pharmaceutical end user perspective, present a comprehensive, regulator-oriented overview of the in vitro mammalian micronucleus (MN) assay, with detailed guidance on manual, semi-automated (image-analysis based), and flow cytometry–based scoring approaches. Within this framework, the book chapter provides a clear endorsement and practical validation of the Metafer image analysis platform software as a robust tool for semi-automated micronucleus scoring.

Routine Application in OECD-Compliant and GLP Laboratories

Across these independent validation efforts, a consistent pattern emerges: Metafer is repeatedly selected, evaluated, and retained in laboratories operating at the interface of regulatory compliance, high throughput, and quantitative risk assessment. The advantages of automated image analysis are particularly pronounced in studies requiring compliance with OECD guidelines and execution under Good Laboratory Practice (GLP) conditions. With the growing recognition of Metafer as a reliable tool for the standardization of micronucleus testing in genetic toxicology, the software has increasingly been applied beyond the preclinical testing phase of pharmaceutical development. Its use has expanded to include contract research organizations (CROs) and manufacturers of industrial and specialty chemicals, where robust, reproducible, and regulatorily acceptable genotoxicity data are required.

As a brief, non-exhaustive list of more recent papers, the studies by Reemtsma, Germany (Wieczorek *et al.*, 2020), Sciensano, Belgium (Sanders *et al.*, 2025), and Eurofins, Germany (Laux *et al.*, 2026) illustrate the routine, GLP compliant application of Metafer in regulatory and public sector genetic toxicology laboratories.



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The Rodent-Erythrocyte Micronucleus Assay

The in vivo rodent erythrocyte micronucleus assay is a cornerstone method in genetic toxicology for detecting chromosomal damage caused by clastogenic agents (chromosome breaks) or aneugenic agents (chromosome malsegregation) in living organisms. In this assay, rodents—typically rats or mice—are exposed to a test substance, after which immature erythrocytes (polychromatic erythrocytes; PCE) from bone marrow or peripheral blood are examined for micronuclei. Because erythrocytes expel their main nucleus during maturation, any retained micronucleus is readily observable, making the assay both sensitive and biologically relevant. The method provides in vivo confirmation of genotoxic effects observed in vitro and accounts for absorption, distribution, metabolism, and elimination. It is internationally standardized, routinely conducted under OECD Test Guideline 474, and constitutes a core component of pharmaceutical and chemical safety assessment frameworks and regulatory programs in the EU, Japan, the United States, and other countries.

Application of Automated Metafer-based Scoring in the in vivo Micronucleus Assay

In a 2016 study, Zeller *et al.* (Roche, Switzerland) reported a comprehensive multi-endpoint in vivo genotoxicity study in rats using methyl methanesulfonate (MMS), a well-characterised direct-acting alkylating agent. As part of this study, the bone marrow micronucleus assay was conducted using femoral bone marrow flushed, processed, and cytospun onto slides. After fixation, slides were stained with modified May–Grünwald–Giemsa. Scoring of micronuclei in PCEs was performed using the Metafer, enabling automated scoring of 4,000 PCEs per animal, consistent with regulatory expectations. In their study, the Metafer-scored bone marrow MN assay was a key contributor to both the biological conclusions (clastogenic dominance of MMS) and the methodological proposal (ratio-based critical effect sizes; CES).

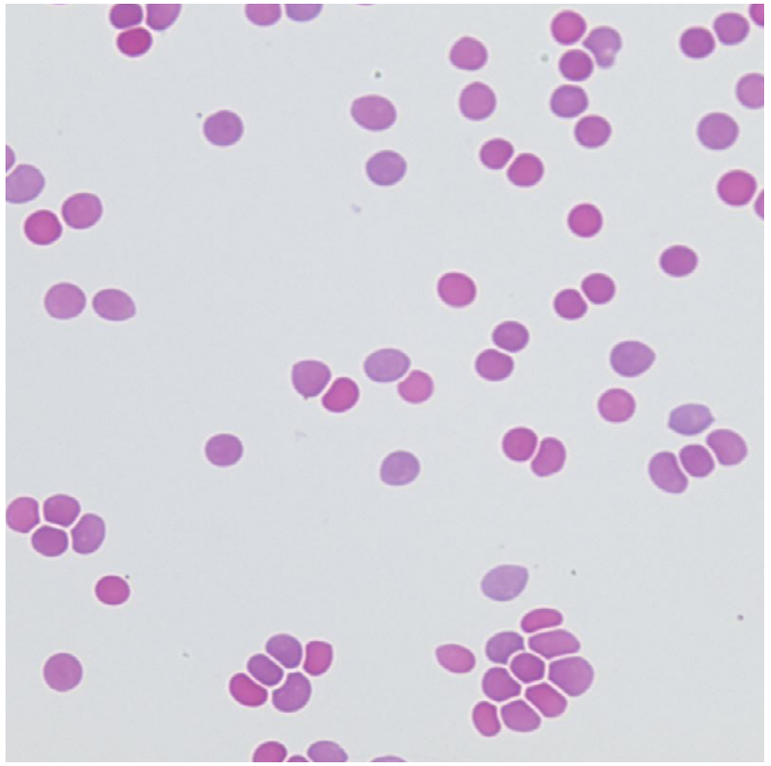
Extending this single-study validation toward an assessment of inter-laboratory reproducibility, Lovell *et al.* (2019) reported a large multi-laboratory analysis of historical negative control data for the rat in vivo micronucleus assay, based on contributions from experienced regulatory testing laboratories across Europe and North America. The dataset, assembled through an international genetic toxicology working group, included data generated in pharmaceutical industry, contract research, and academic laboratory settings, and provides a robust reference range for background micronucleus frequencies.

Within the multi-laboratory dataset, one participating laboratory study (referred to as “Lab G” in the publication) employed automated slide evaluation using the Metafer software following conventional slide preparation. The micronucleus frequencies generated using this automated approach fell within the overall historical negative control range observed across all contributing laboratories and were included without qualification in the pooled statistical analyses. No systematic differences attributable to the scoring method were identified, and results from the Metafer-based laboratory were comparable in magnitude and variability to data obtained using manual microscopy or flow-cytometric scoring, supporting the suitability of automated image analysis for in vivo micronucleus testing. Importantly, the inclusion of Metafer-generated data without qualification in a multinational historical control dataset represents a critical benchmark for regulatory confidence in automated scoring.

CENTRAL CONCLUSION

The studies evaluated here demonstrate that an automated image-analysis platform like Metafer can be fully compatible with OECD TG 474-compliant in vivo micronucleus testing, and is able to deliver results that are scientifically robust, reproducible, and regulatory relevant.

Across diverse laboratory settings, test substances, and exposure paradigms, Metafer-based scoring has been shown to reliably detect clastogenic effects, generate background frequencies consistent with historical control data, and support biologically meaningful interpretation. These findings underscore the value of automated microscopy as a mature and validated approach that enhances throughput and standardization while maintaining the sensitivity and integrity required for regulatory genotoxicity assessment.



In a Korea–Japan collaborative animal bioassay study, Kim *et al.* (2026) evaluated whether long-term exposure to mobile phone radiofrequency (RF) radiation is carcinogenic or genotoxic. The work was motivated by conflicting findings in the literature, particularly the U.S. National Toxicology Program (NTP) and Ramazzini Institute reports that suggested RF-related tumors in rodents.

For the genotoxicity component of the study, the authors used a Metafer-based automated scoring system implemented at the Korean Institute of Toxicology (KIT) in Daejon, Korea. They used Harlan Sprague–Dawley rats exposed to 900 MHz CDMA-modulated RF at a whole-body SAR of 4 W/kg, corresponding to a reference exposure level used in international human safety guidelines. In their study setup, Metafer was used to score a minimum of 4,000 PCEs and normochromatic erythrocytes (NCEs), to detect and count micronucleated PCEs (MNPCE), and to calculate the PCE/NCE ratio in randomly coded, standardized samples. Though they reported that RF-exposed animals showed no statistically significant increase in MNPCE frequency, the EMS positive-control group showed a clear, statistically significant increase in micronuclei, confirming that the Metafer-based workflow was sensitive to clastogenic effects.

This study illustrates that Metafer-based scoring maintains assay sensitivity under challenging exposure conditions and correctly distinguishes negative findings from validated positive controls.

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Summary and Regulatory Context

Evidence Base Across Genetic Toxicology Assays

The literature reviewed here demonstrates that the Metafer automated imaging software platform has been extensively evaluated across the principal genetic toxicology assays used in regulatory safety assessment, including the in vitro chromosome aberration test, the in vitro micronucleus assay, and the in vivo mammalian erythrocyte micronucleus assay. Across these applications, Metafer-supported image analysis consistently yields endpoint frequencies, dose–response relationships, and statistical interpretations that are comparable to those obtained by conventional manual microscopy.

These findings have been reported for multiple cell types, species, test substances, and laboratory settings, indicating that performance is robust across diverse experimental contexts rather than dependent on individual study conditions.

Role of Automation Within Established Methodologies

Across assay types, Metafer is applied as an enabling tool within established test frameworks rather than as a fully autonomous analytical system. Slide scanning, image acquisition, automated object detection, and preliminary classification substantially reduce manual workload, while expert review remains responsible for biological interpretation and final endpoint confirmation.

This integration preserves methodological continuity with guideline assays and ensures that automation enhances efficiency and consistency without altering the underlying scientific principles of genotoxicity evaluation.

Data Quality, Reproducibility, and Traceability

A recurring advantage highlighted in the reviewed studies is the improvement in data standardization and traceability achieved through digital image archiving. Image-based documentation allows scoring decisions to be audited, revisited, and compared across studies, supporting transparency in regulated testing environments.

In addition, the ability to score larger numbers of cells within practical timeframes improves statistical power and enables quantitative analyses such as benchmark dose modeling. The inclusion of automated image-analysis data in multi-laboratory historical control datasets further supports the absence of systematic bias attributable to the scoring approach.

Positioning Within Contemporary Genetic Toxicology Practice

Collectively, the evidence positions Metafer as a mature image acquisition and analysis system that supports current genetic toxicology practice by improving efficiency, consistency, and documentation. Its use reflects broader trends toward digitalization and standardization in safety assessment while maintaining alignment with established assay designs and expert-driven evaluation.

Concluding Remarks

In conclusion, Metafer-based image analysis represents a technically sound extension of conventional genetic toxicology methodologies. When implemented within validated workflows and supported by appropriate expert oversight, it enables reproducible data generation, enhanced traceability, and efficient study execution without changing the scientific or interpretative foundations of guideline assays.

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About MetaSystems

For 40 years, MetaSystems has been developing innovative solutions for automated microscopy-based imaging for the healthcare and biotechnology sectors. Our headquarters are located in the southwest of Germany near Heidelberg.

We are a global company with an international team working in Germany and in our subsidiaries in North and South America, Europe, India, China and Japan. Our customers can be found in institutes, hospitals, and universities in over 100 countries around the world.

We continuously develop our products in close connection with our users, thus combining innovation with tradition. Our modern approaches include an advanced workflow management and the use of artificial intelligence. In many segments, this has enabled us to achieve an international top position.

MetaSystems software provides, among other functions, features to assist users with image processing. These include, but are not limited to, the use of machine and deep learning algorithms for pattern recognition. The output generated in this process should be regarded as preliminary suggestions and, in any case, mandatorily requires review and assessment by trained experts.

MetaSystems offers **Customization Packages** for application workflows that have been successfully implemented for customer labs using standard Metafer platform functionality. It is expected that they can be implemented for other customer labs using similar workflows and slide preparation procedures. If a Customization Package is purchased, MetaSystems product specialists will - based on their experience from other similar application cases - support the customer lab in adapting the Metafer software configuration to their needs. The performance of the solution will depend on the quality of the customer slides and the expertise of the users, MetaSystems cannot specify or guarantee any performance parameters. The validation of the solution for clinical use is the sole responsibility of the customer lab.

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