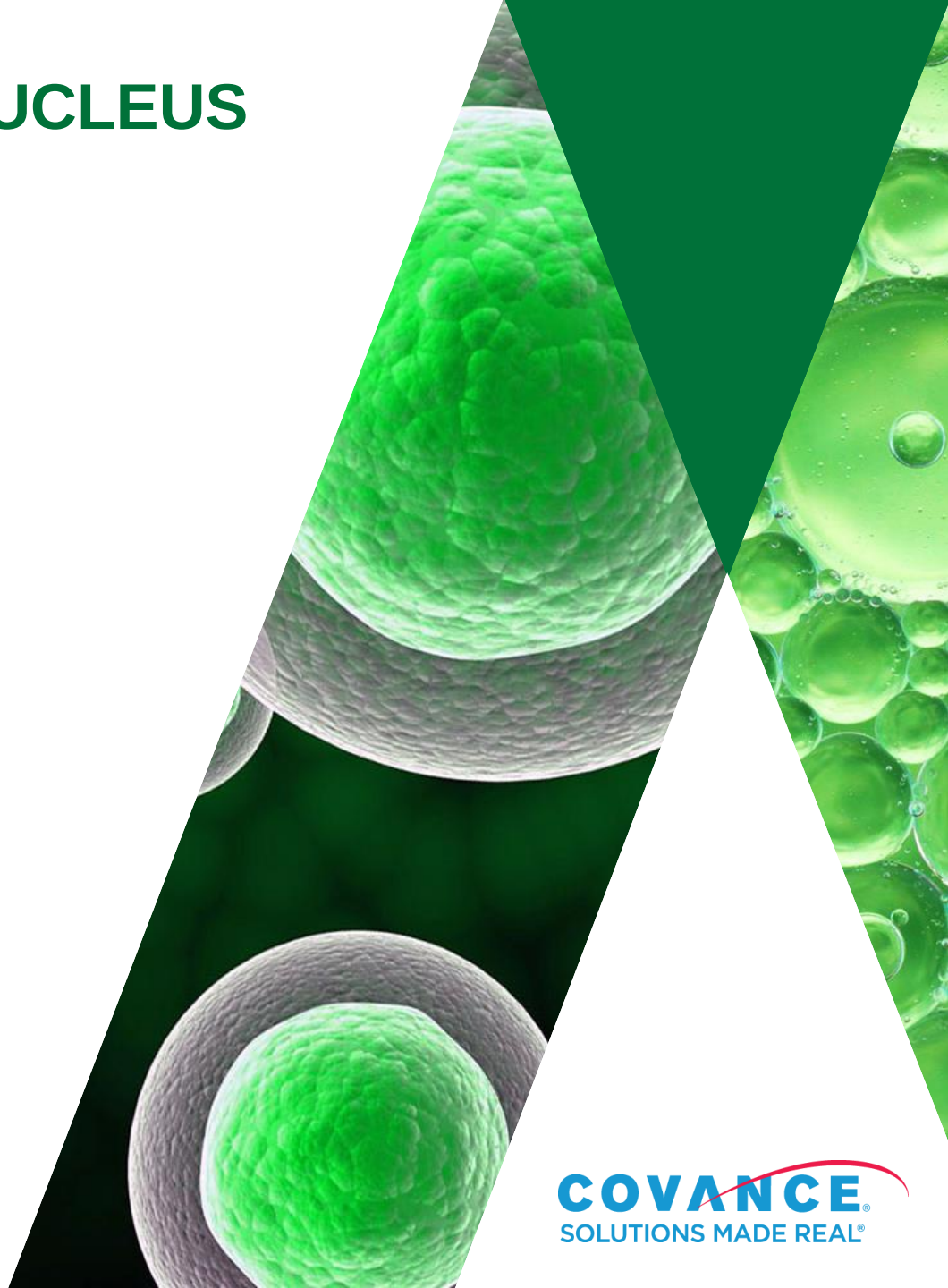
AUTOMATED MICRONUCLEUS ANALYSIS: IMAGE ANALYSIS AND FLOW CYTOMETRY

Jatin Verma, PhD



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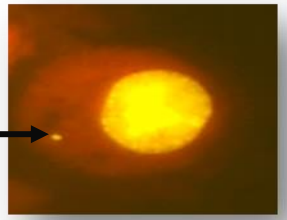
Introduction

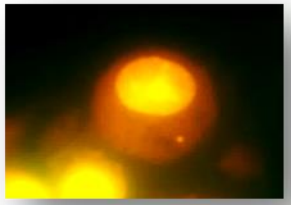
- The micronucleus (MN) assay is a vital cytogenetic tool that is routinely used to assess agents ability to cause structural or numerical chromosomal changes.
- In this assay, quantitative information on the induced DNA damage is obtained by scoring micronuclei (MN).

Micronucleus

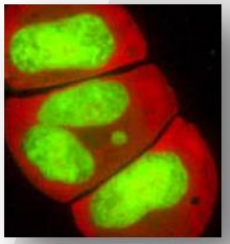


- The MN scoring can be carried out in the presence or absence of Cytochalasin-B (Cyto-B).

Mono-nucleated MN Assay



Cytokinesis Block MN (CBMN) Assay



- Both the in ***in vitro*** and the ***in vivo MN assay*** are incorporated in the regulatory toxicity testing guidelines (**ICHS2(R1) & OECD 487**).

All images are covance images

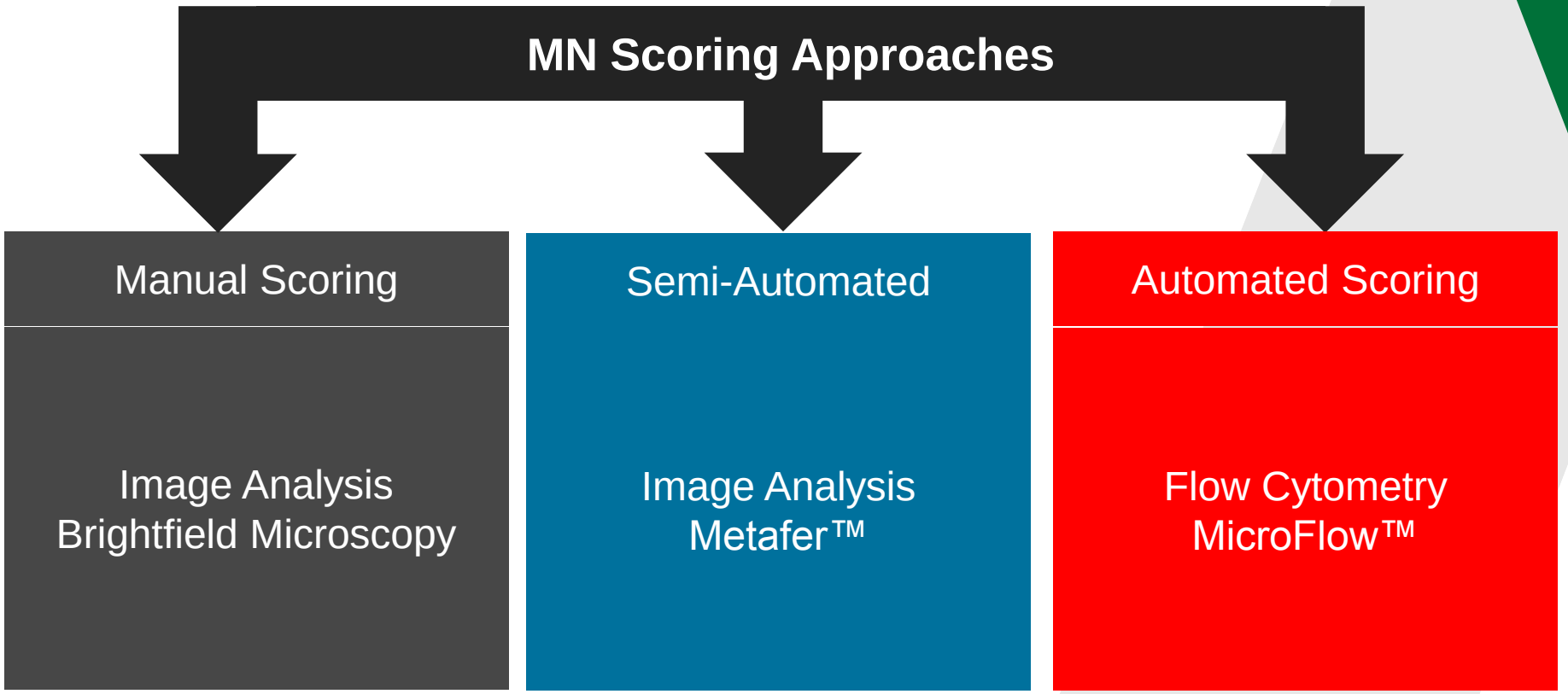
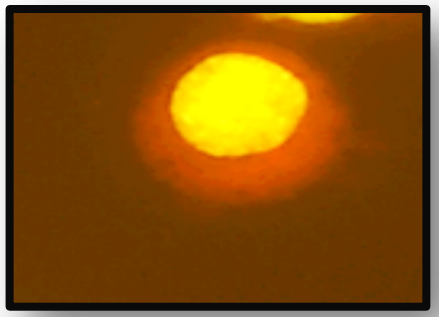
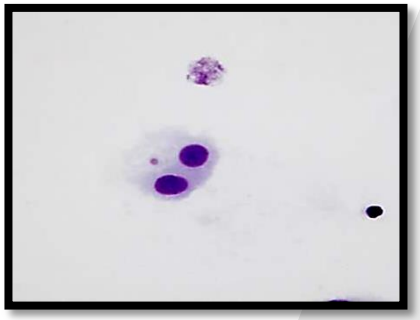


Image Analysis: Manual MN Scoring

- MN scoring is carried out manually by using either brightfield or fluorescence microscopy.
- The method is simple, economical and considered as a **“Gold Standard”** for MN assessment.
- Suitable for MN scoring both in the presence or absence of cytokinesis inhibitor (Cyto-B).
- However, manual scoring offers low-throughput, is time consuming (**30-40 minutes/slide**) and subjective.





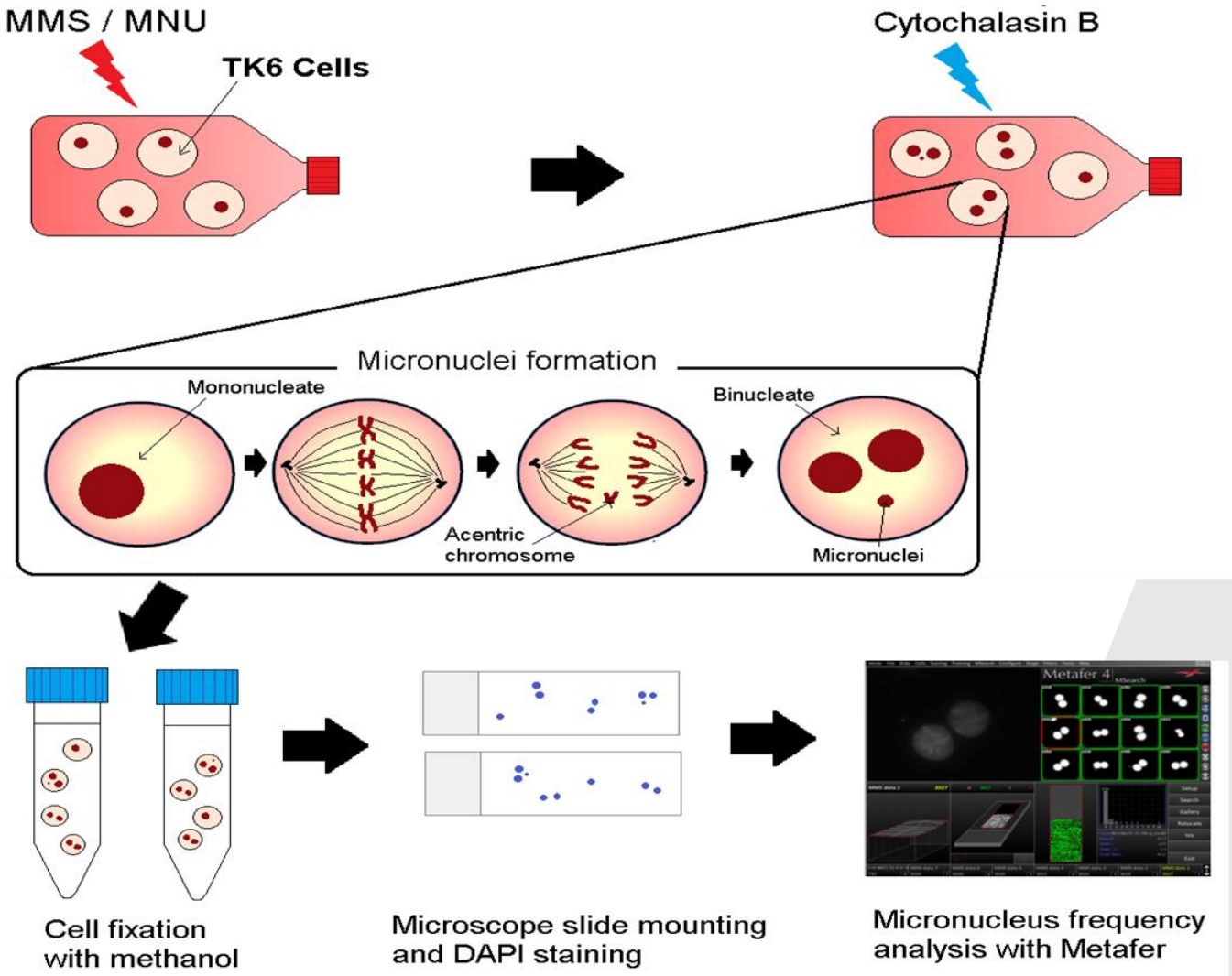
All images are covance images

Image analysis: Semi-Automated MN scoring

Image adopted from MetaSystems-international.com

- **Metafer 4™ (Carl Zeiss)** is a **semi-automated** image analysis platform.
- This semi-automated MN scoring platform is fitted with a fluorescent microscope (Zeiss axion), a motorised stage and a charge coupled device (CCD) camera for image capturing.
- Scanning and scoring of the cells/MN is carried out using the Metafer **MN search** software.
- Images of nuclei/MN are displayed in the gallery view and can be viewed for quality control.
- 2000 bi-nucleated cells and around 4000 mono-nucleated cells can be captured/assessed within **8-10 minutes**.

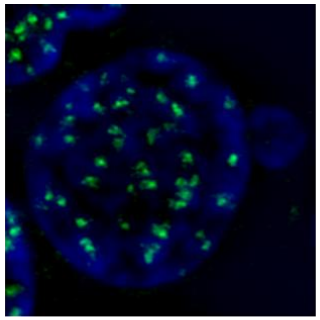
Image analysis: Semi-Automated MN scoring procedure



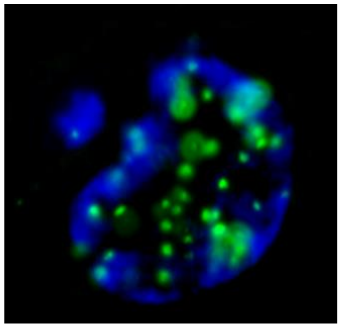
E. Wilde, 2017, A novel carcinogenicity tool to discriminate carcinogens from non-carcinogens

Image Analysis: Limitations of Semi-Automated MN Scoring Platform

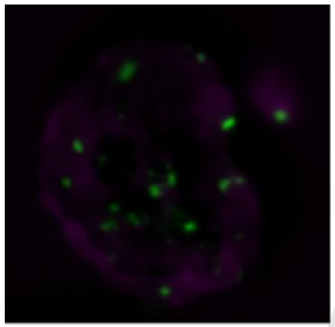
- Subjectivity in MN scoring due to human intervention (semi-automated approach).
- Quality of slide preparation.
- Optimisation of the classifier setting with various cell lines.
- Changes to cellular and MN size/morphology can cause underscoring.



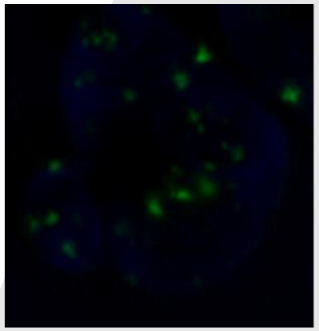
Circular MN cell



Crescent/Kidney bean shaped MN cells



Small size (**MN with 1 centromeric signal**)



Large (**MN 2 or more centromeric signals**)

Images belong to Jatin Verma, Evaluation of Chemicals in Binary Mixtures 2016

Flow Cytometry: MicroFlow™ MN Scoring Platform

- **Fully automated** MN scoring platform.
- Commercially available (MicroFlow™, Litron Laboratories, USA).
- Enables MN scoring in cell suspensions as compared to conventional slide base scoring with Image analysis.
- Offers **high-throughput** and **high-content** MN scoring
- **10,000 cells** can be scored within **1-2 minutes**.

MicroFlow™ MN Scoring Method

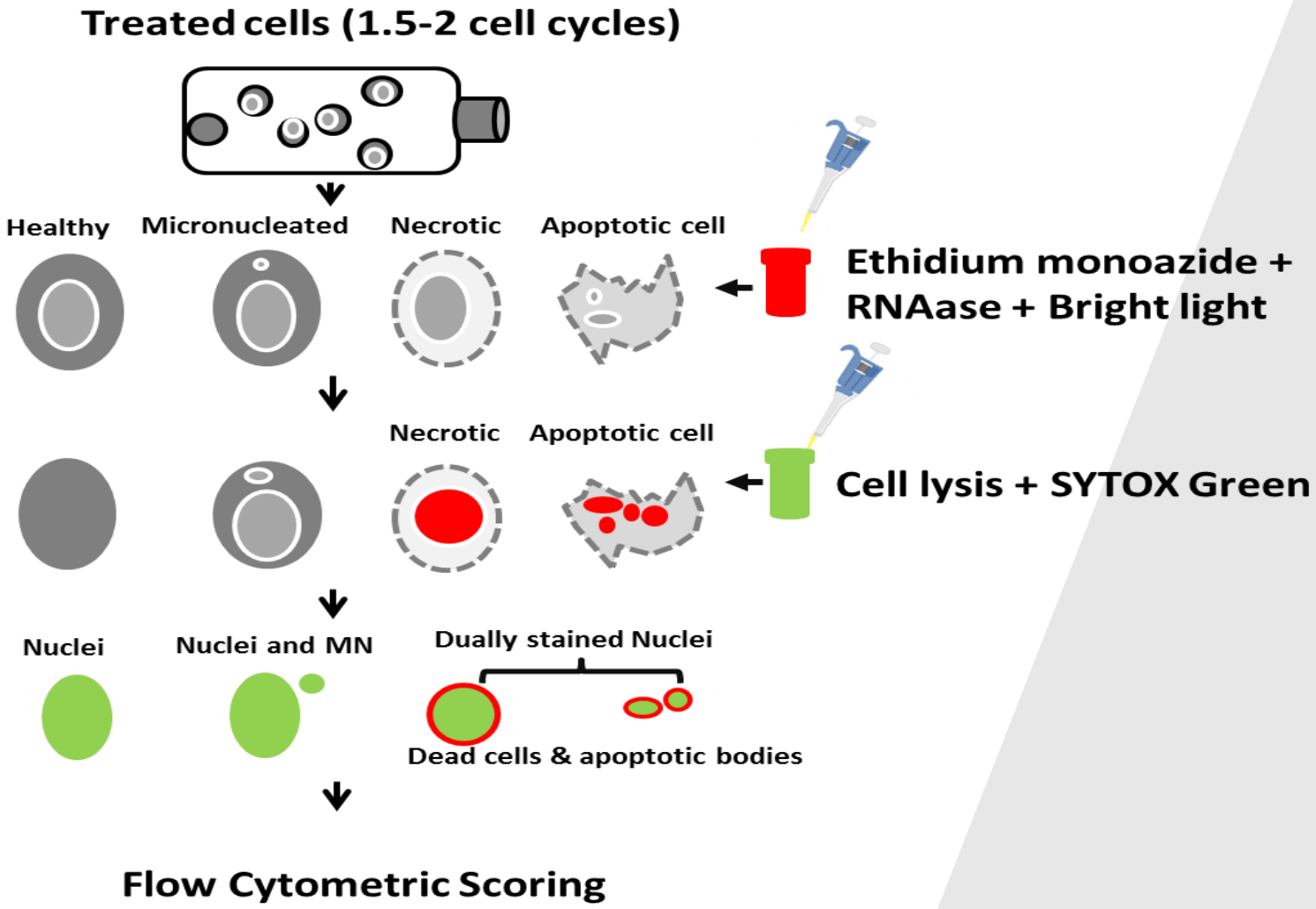
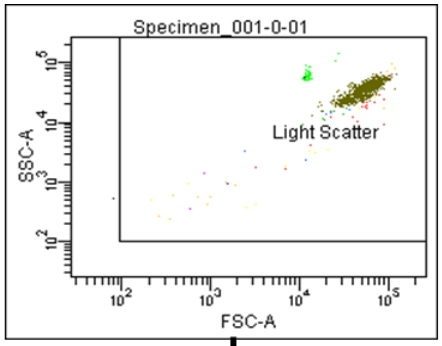
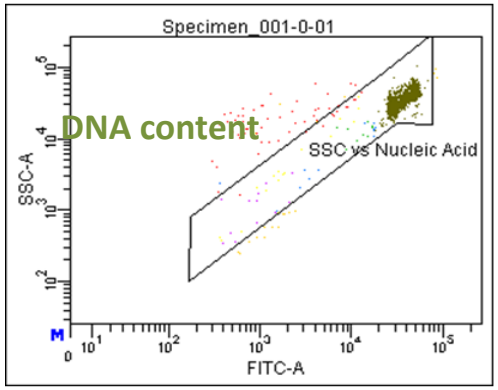
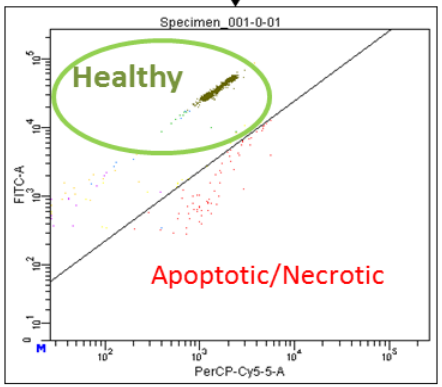


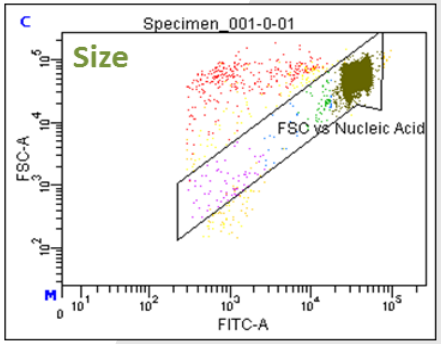
Image adopted/modified from MultiFlow™, Litron Labs USA.

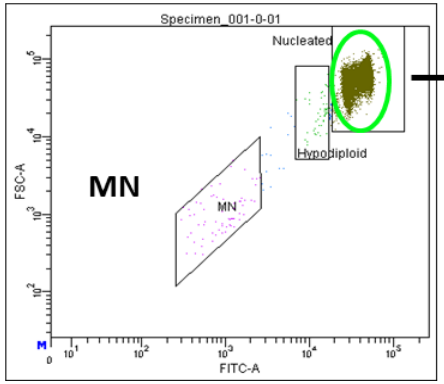
MicroFlow™ MN scoring platform

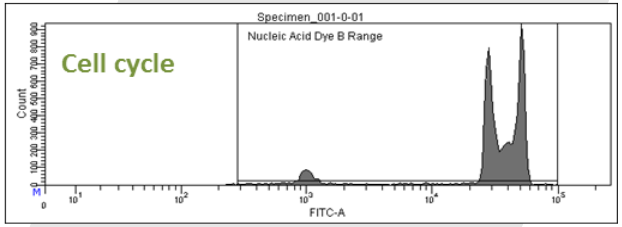












Unpublished data, Jatin Verma

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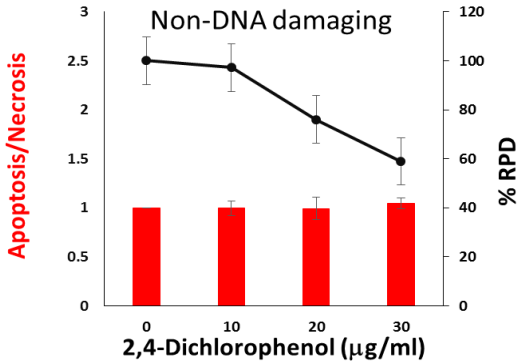
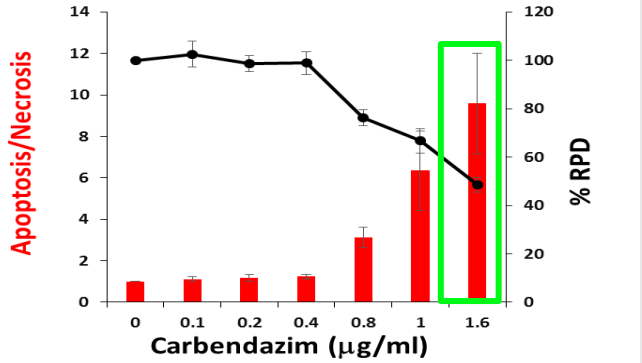
Advantages

EMA positive staining

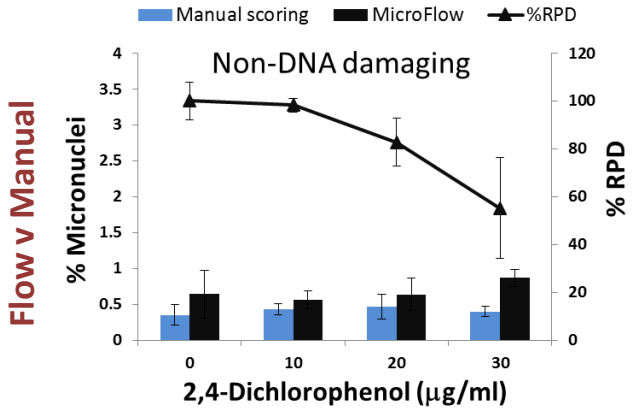
- Reduces the influence of apoptosis/necrosis on MN scoring and assists in top dose selection

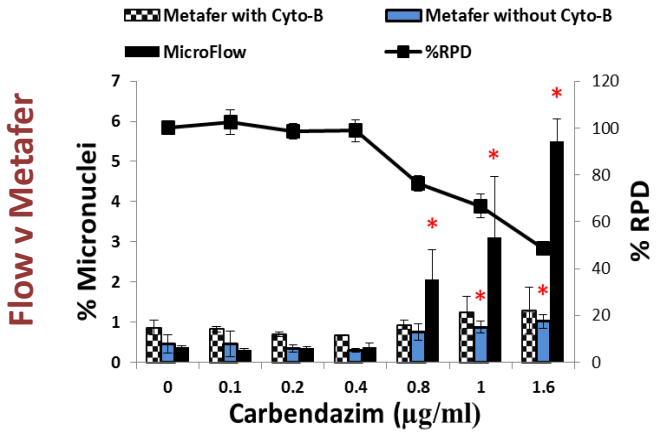




Suitability as an automated MN scoring platform for dose response analysis

- The results for MN responses with MicroFlow are comparable with Metafer and Manual MN scoring platforms

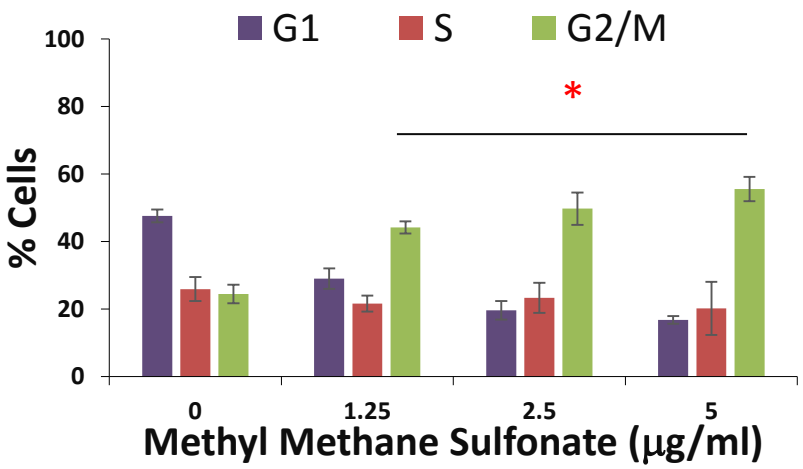


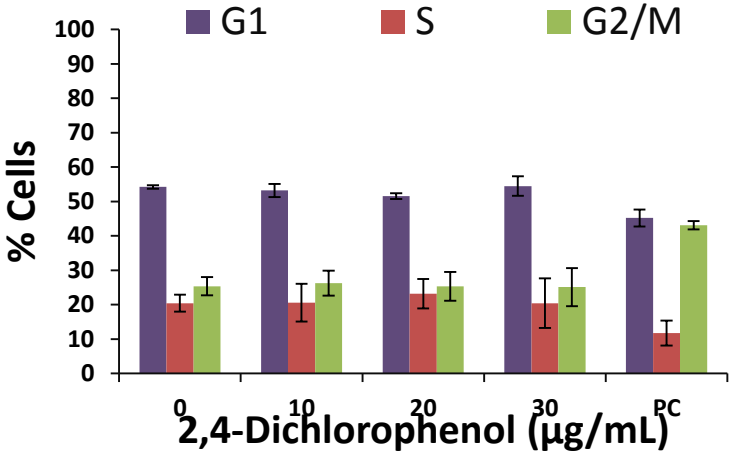


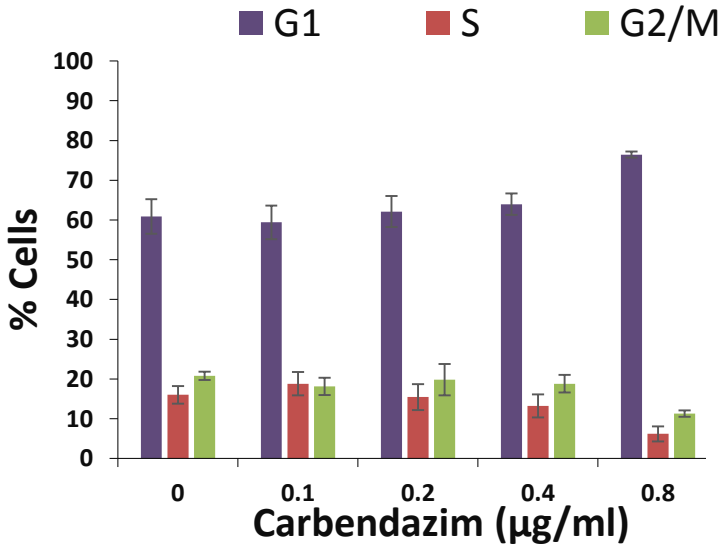
- Rapid scoring (10,000 analysed in (1-2) minutes) with reduced subjectivity (fully automated) and cell cycle analysis is an added advantage
- 30,000 nuclei means higher statistical power for dose response analysis

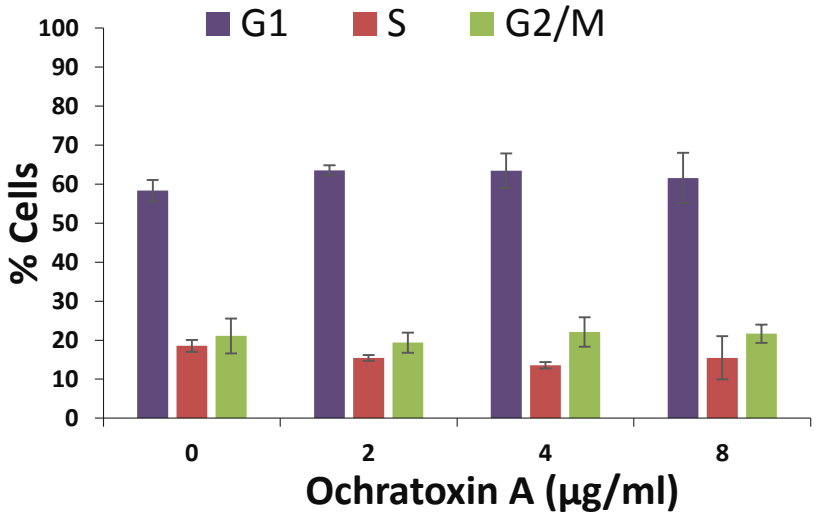
Verma, J., Rees, B.J., Wilde, E. et al. Arch Toxicol (2017) 91: 2689.
https://doi.org/10.1007/s00204-016-1903-8

Suitable to study cell cycle alterations





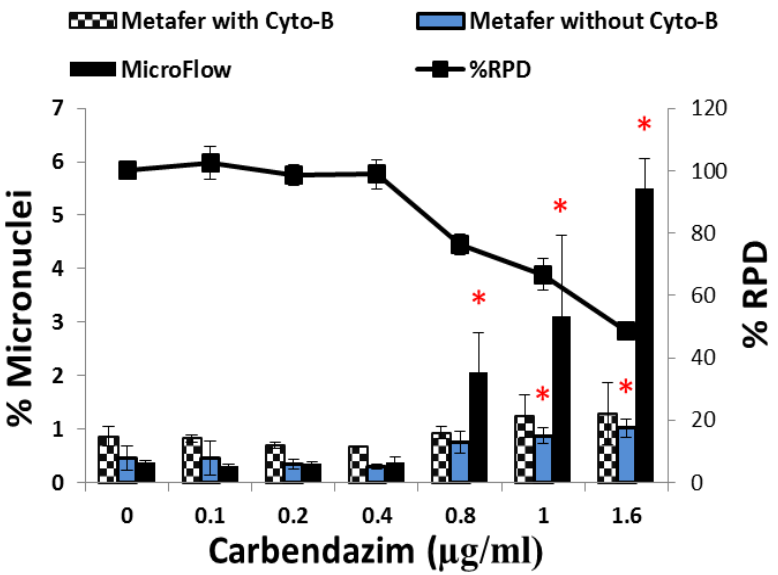




E. Wilde, 2017, A novel carcinogenicity tool to discriminate carcinogens from non-carcinogens

Limitations

Over estimation of MN induction



- MN within bi, tri and tetra nucleated cells
- Cells with multiple MN
- Early apoptotic cells with intact cytoplasm that resist EMA labelling

Cell lysis

MN cannot be co-related to the parent cell of origin due to cell lysis

Revalidation of the results

MN cannot be revalidated for quality control using human intervention

Verma, J., Rees, B.J., Wilde, E. et al. Arch Toxicol (2017) 91: 2689. https://doi.org/10.1007/s00204-016-1903-8

Summary: Image Analysis and Flow cytometry

Metafer™ (Image cytometry)	**MicroFlow™ (Flow cytometry)**
Semi-automated image analysis **(Subjective)**	Fully automated flow cytometric approach (Objective)
Can under estimate MN induction	Over estimate MN induction
4000 mono and 2000 bi-nucleates with MN can be scored in **10 minutes** (**Low through put**)	10,000 events (high-throughput) scored in **1-2minutes** (higher statistical power)
Suitable to study DNA damaging MoA	Cell cycle analysis is an added advantage (Multiplexing)
MN events can be re-validated to limit the effects of increased cytotoxicity/apoptosis on the MN scores	MN events cannot be re-validated/re-analysed

Verma, J., Rees, B.J., Wilde, E. et al. Arch Toxicol (2017) 91: 2689. https://doi.org/10.1007/s00204-016-1903-8

Future Automated MN Scoring Platform: Imaging Flow Cytometry

- Combines image analysis with flow cytometry to automate the MN assay.
- Suitable for MN scoring in human lymphoblastoid cell lines (TK6 and MCL5)
- No need for cell lysis, rapid (**20,000 cells <5 minutes**) and reliable (Adheres to the HUMN criteria for reliable MN scoring)
- Potential for multiplexing (H2AX, H3 and P53)
- Machine learning algorithms combined with automated imaging flow cytometry may help to fully automated MN assay.

Amnis EMD Millipore

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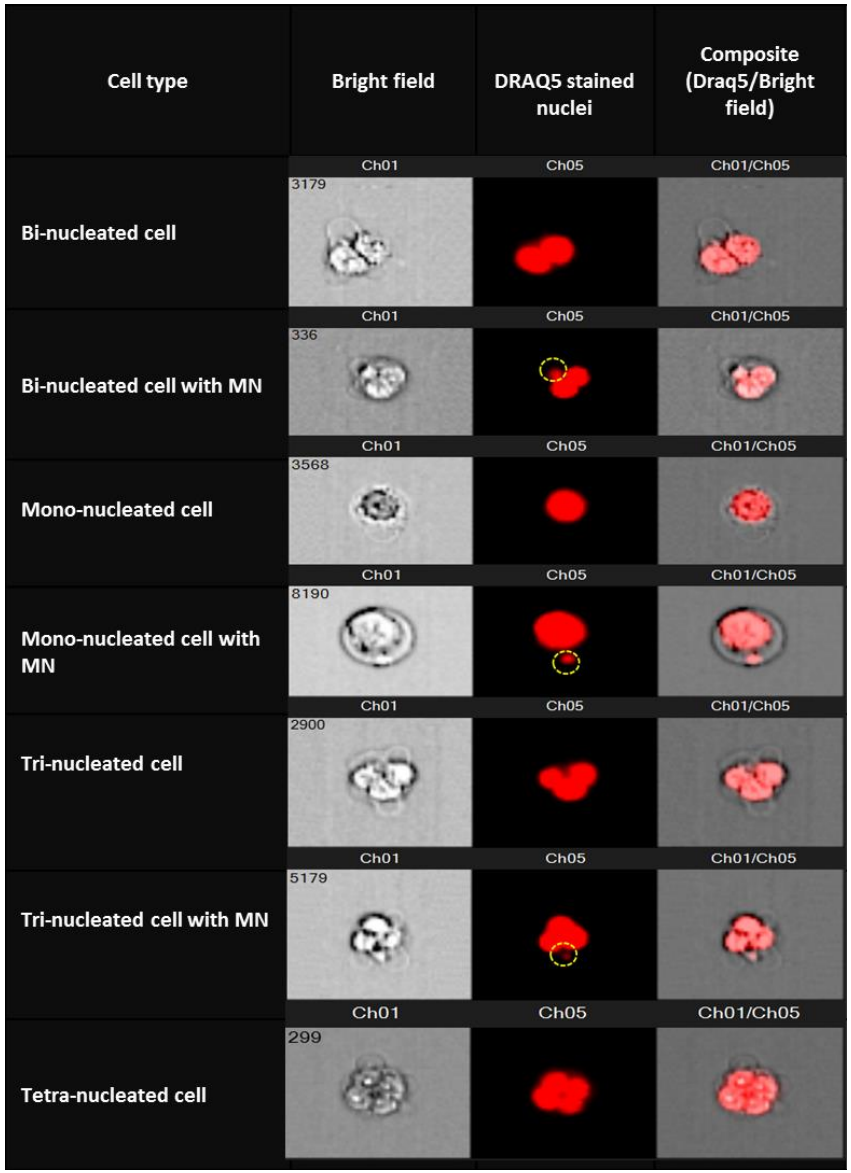
Imaging Flow Cytometry



IDEAS based classification of cells as Mono-nucleated, bi-nucleated, tri-nucleated, tetra-nucleated cells with and without MN. Left panel denotes cell types, Bright field panel (channel 1), Nuclear stain panel (DRAQ5 in Channel 5) and Composite panel (DRAQ5/Bright field overlay). MN are highlighted using yellow circles

Investigating FlowSight® imaging flow cytometry as a platform to assess chemically induced micronuclei using human lymphoblastoid cells in vitro (Mutagenesis https://doi.org/10.1093/mutage/gey021) Verma 2018

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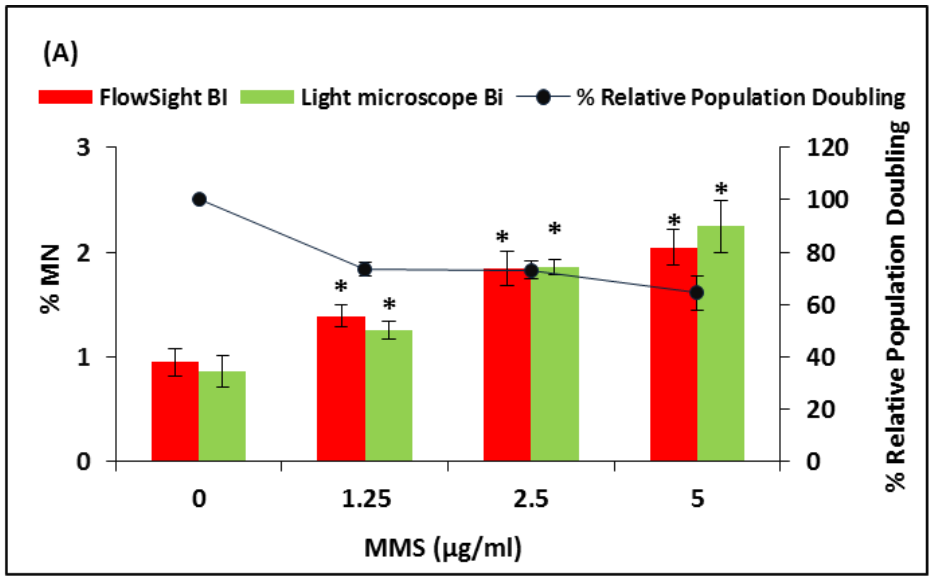
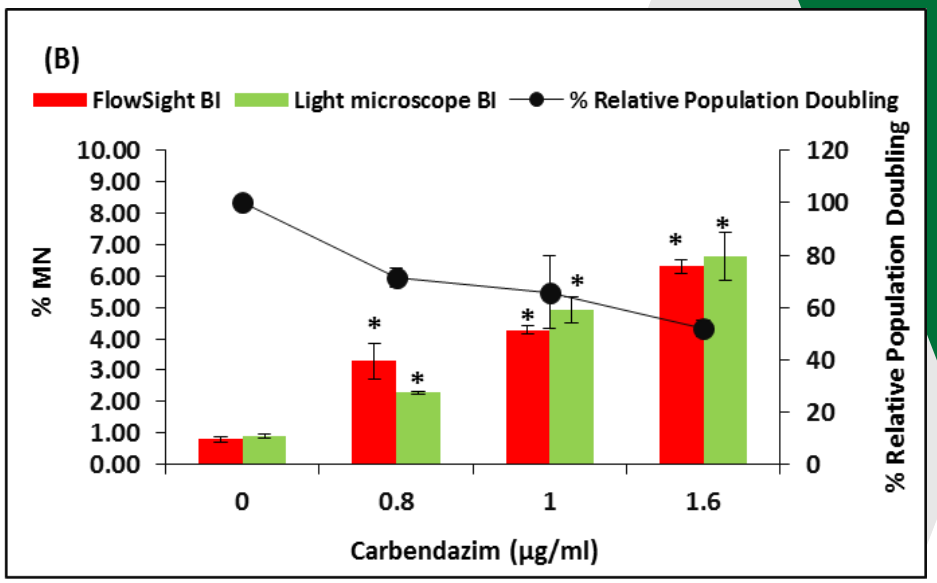
Imaging Flow cytometry Vs Manual Scoring





Comparison of the MN responses derived by using FlowSight (red bars) and manual scoring (green) in TK6 cells treated with MMS (A) and Carbendazim (B) for 30 hrs. * indicates a significant increase in the MN formation over the control ($p<0.05$). Error bars represent mean $\pm$ SD (n=3).

Verma et al 2018: Investigating FlowSight® imaging flow cytometry as a platform to assess chemically induced micronuclei using human lymphoblastoid cells in vitro (Mutagenesis https://doi.org/10.1093/mutage/gey021)

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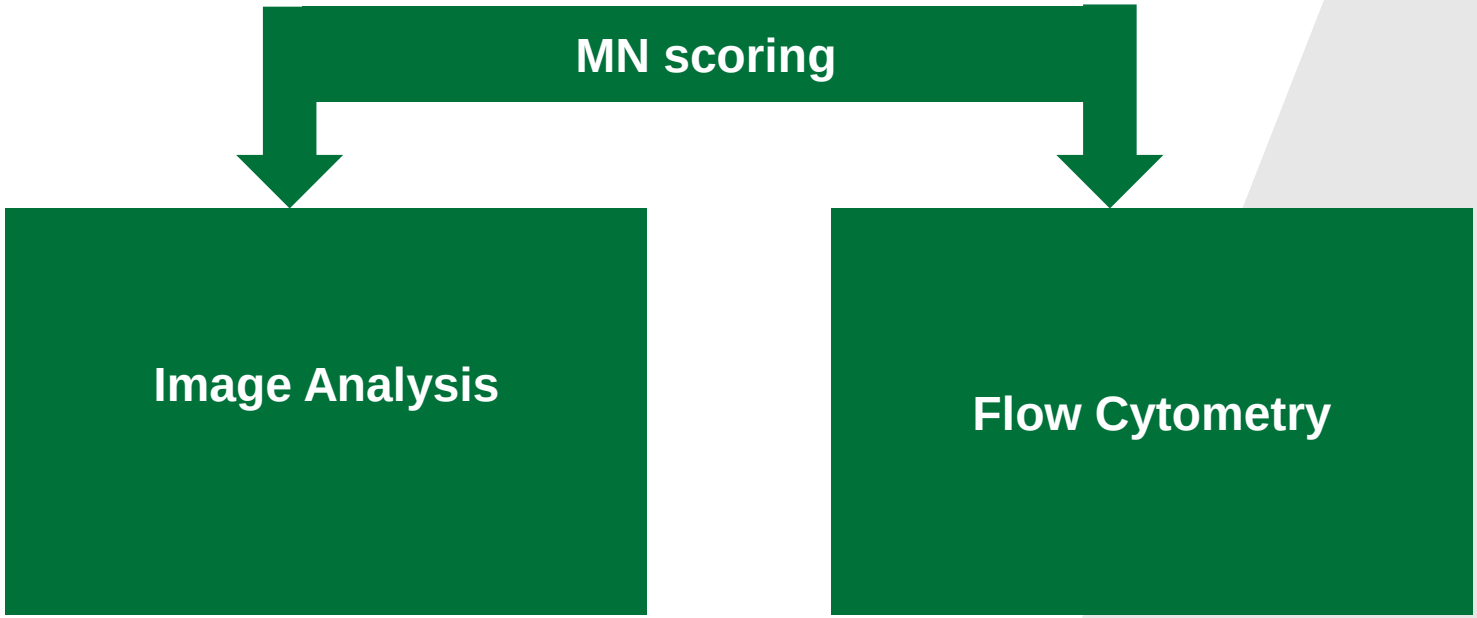
Specificity or Speed?



Thank You

Robert Smith (Study Director)
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Dr Julie Clements (VP Global Lead Genetic Toxicology)

Dr George Johnson
Dr John Wills
Dr Paul Rees
Dr Huw Summers

Dr Steven Bryce
Dr Stephen Dertinger

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