Quantitative cytogenetics analysis and DNA damage evaluation in human biomonitoring by automated high-throughput high-content Image Cytometry:

Pathfinder™ system

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ABOUT IMSTAR

A visionary company: Innovative concepts in cellular imaging and development of cutting edge image cytometry technology for research and diagnostic

- IMSTAR is pioneer in the field of cellular analysis for Life Sciences research and *In Vitro* Diagnostic (IVD)

01

Strong corporate culture, with expertise in biophysics, instrumentation, modeling, complex morphology and phenotype characterization algorithms, cellular biology, medicine, and cytopathology

- Founded by Dr. Francoise Soussaline, PhD, an international expert in digital quantitative imaging

- Permanent consultant and advisory board including specialists in Toxicology, Cytopathology, Cytogenetics, Oncology

02

Invested over 35% of its revenues in R&D over the past 15 years. Strong Intellectual Property portfolio of 13 patents (US7092559), 98 copyrights and 15 trademarks to protect Pathfinder™ technology

- Strong links to key French and European networks in Life Sciences and Oncology personalized medicine

03

The Pathfinder™ range of products is compliant with the following regulations:

- EC Directive 98/79/EC IVD MD
- OECD TG 487

- Global customers base, , KOLs' Academic Labs and blue chip Companies: Pharmas, Nutrition, Tobacco, Cosmetic, Cytogenetic & cytopathology public & private labs

04

IMSTAR continues to innovate, particularly in the field of cellular tumor marker quantification for *In Vitro* Diagnostic

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ABOUT IMSTAR

Product: Pathfinder™ technology: First available fully automated Image Cytometry instrument in 2000. Continuous improvements in high performance Imaging scanning & analysis techniques, and development of new applications software

World-wide Market for Pathfinder™ systems: Installed base

- **Over 200** for Genetic Toxicology assays (MN, Comet, CAS); - **over 1000** for Cytogenetic (Karyo & FISH studies); - **over 150** for Cyto-Histo-Pathology; - **over 250** for advanced cellular characterization in LS Research, new Biomarkers Development and Drug Discovery
- **60% of the set-up in Asia** China first

Quality Policy integrated in products development, **CE (MD IVD) marked**

Network : International Academic Institutions, EU FP consortia, partnerships with Pharmaceutical, Agro-Food, Biotech & Cosmetic Industries

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THE CONTEXT

Health Market Challenges

- Promote **large volumes of genetic toxicology testing** for biomonitoring of population at risk as a result of industrial/environmental harmful exposure
- **Access in routine IVD** to numerous biomarkers tests, existing but not applied; currently expensive and labor intensive, yielding to low volumes of key data for marker assessment & diagnostic / therapeutic decision
- **Accelerate development of companion test** needed for drug prescription
- Accelerate the **discovery process of tumor genesis** as well as lower costs to speed up Academic research labs
- Develop new cost efficient technologies to address limited human expertise resources in fast growing health economies.

Our Answer

- **High-content, fully-automated & modular Image Cytometry System**, used for a variety of assays and diagnostic tests, with a focus on oncology.
- **Easy customization for new analytical** cellular imaging applications for third parties
- **Dramatic gain in workflow efficiency/cost** (over 10-fold), optimum use of human expertise, GLP archiving & traceability, and easier training.
- **An operator-independent 'smart'** data capture, processing and analysis assisting the expert for final interpretation
- **A comprehensive solution** to speed-up research, spread TCO* and facilitate technology integration in routine lab

* TCO tries to quantify the financial impact of deploying an information technology product over its life cycle. These technologies include software, hardware, support, training.

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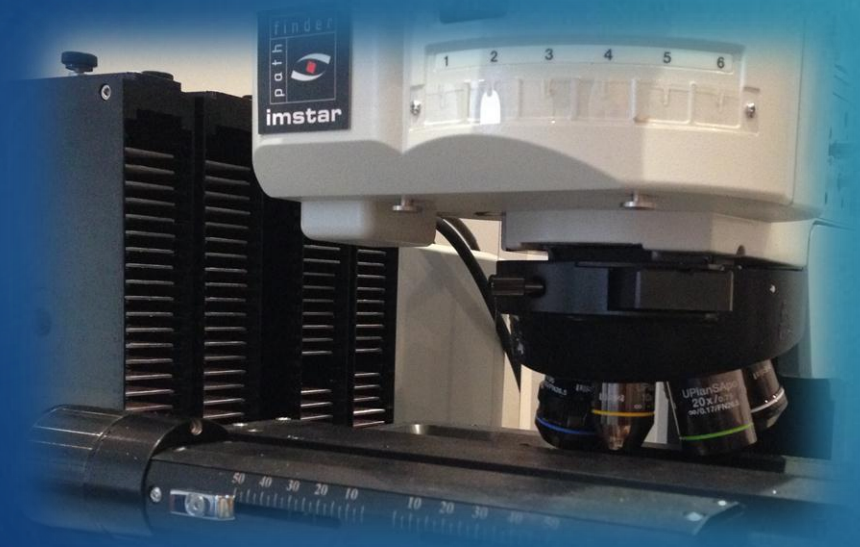
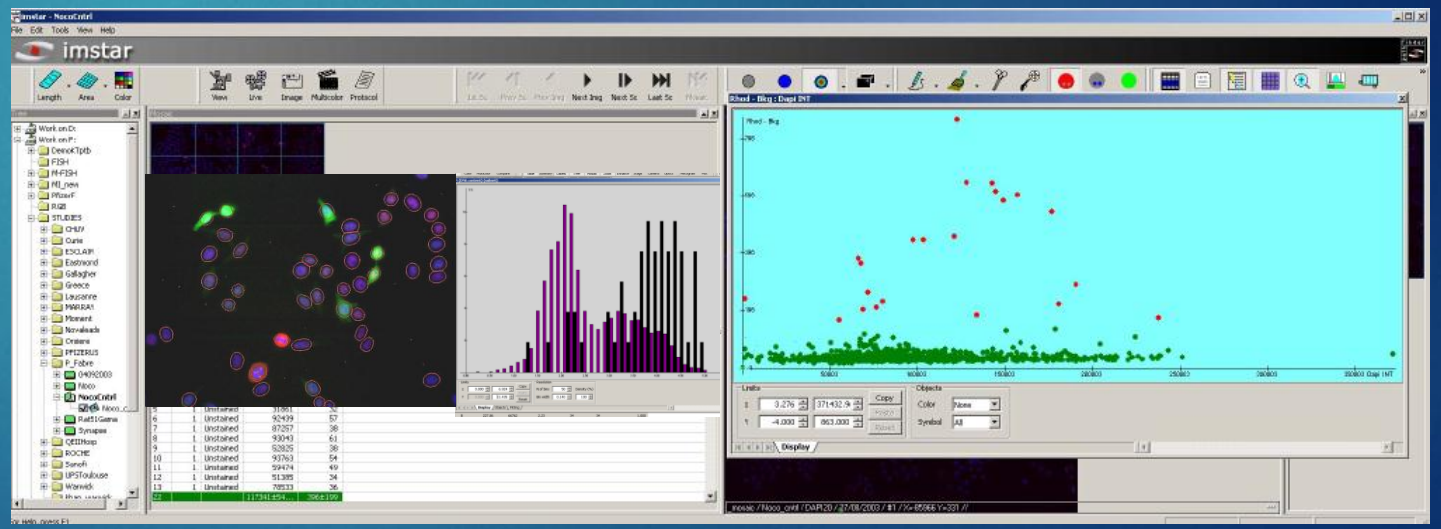
PATHFINDER™ ICS READER-ANALYZER (I)

Motorized Microscope-based Reader Analyzer for High-Content Screening (HCS) & Analysis in cells spread, cell' culture, cell-array and tissue sections

- Patented Quantitative Cell Imaging software engine for a range of applied solutions, integrated in modular hardware
- **HCS:** All information on individual cell, for thousands of cells at once, in **MULTIPLEX**, giving access to **DISTRIBUTIONS** of parameters
- Enables studies of **HETEROGENEOUS POPULATIONS** with all individual parameters for each (sub-)cellular "object"



Modular Pathfinder Master station



Pathfinder™ patented 'Review & Validate' by expert interface

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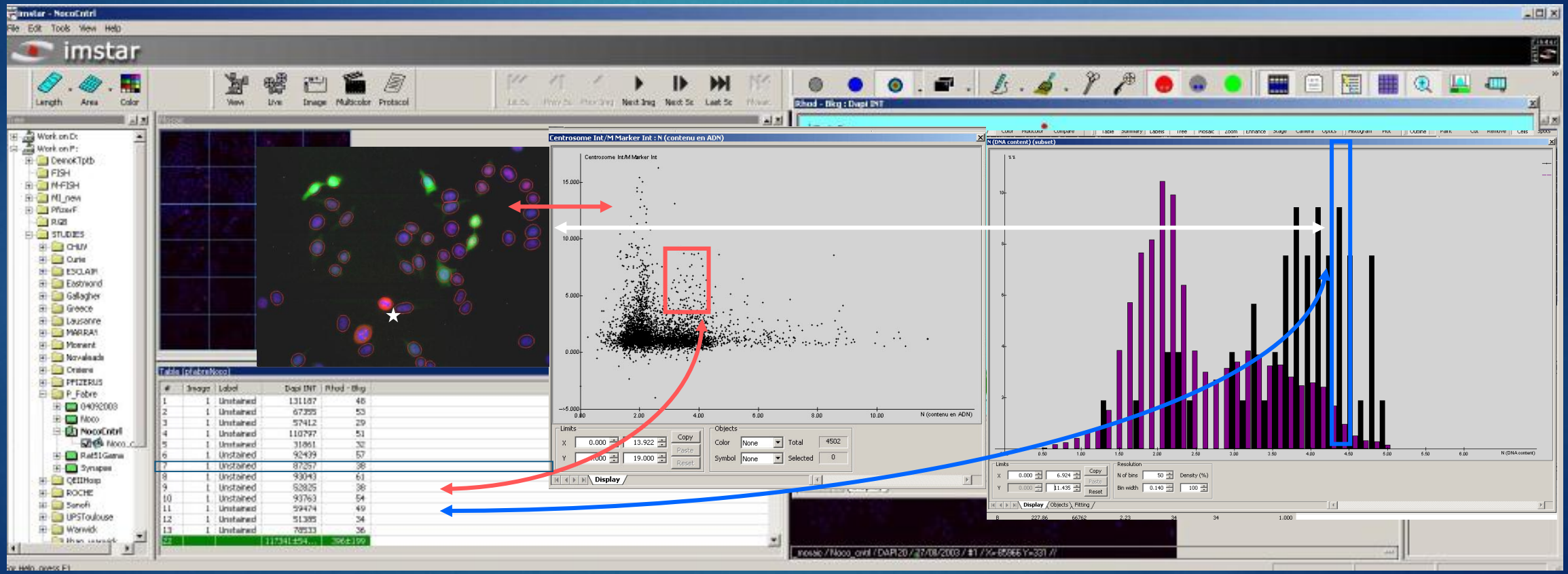
PATHFINDER™ ICS READER-ANALYZER (II)

- **Automated and interactive tools for study of markers in sub-populations per sample** (slide, pattern slide, cell on chip)
- **Unique interactivity** (patented) between detected objects and results
- Instant Histogram, Scatterplot, fitting curve **for fast assisted interpretation**



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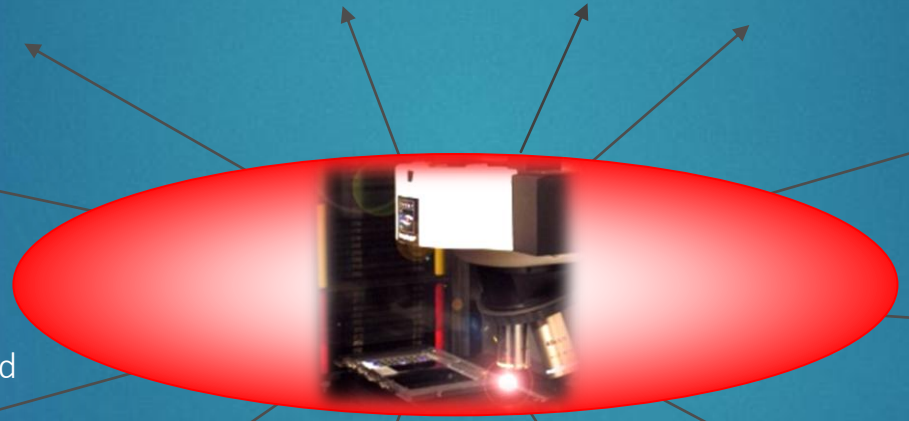
PATHFINDER™ ICS READER-ANALYZER (III)

Hardware: Instrument based on motorized photonic microscopy, designed for high content information and cell characterization on large number of samples



Fully automated capture & analysis with no human intervention

Up to 200 slides or 2880 wells in one batch

Fast Auto focus and Z staking

Bright-field or Fluorescence imaging

Adapted for slides, pattern slide, microchips, filters

Bar-code samples identification GLP compliant

2x to 100xoil for imaging magnification

Time-lapse from second to days scale

4x digital zoom with no-loss information

Multispectral multiplex capture (up to simultaneous12 labels)

Full control of capture conditions enabling optimal quantitative analysis

Optimal traceability and samples retrieval

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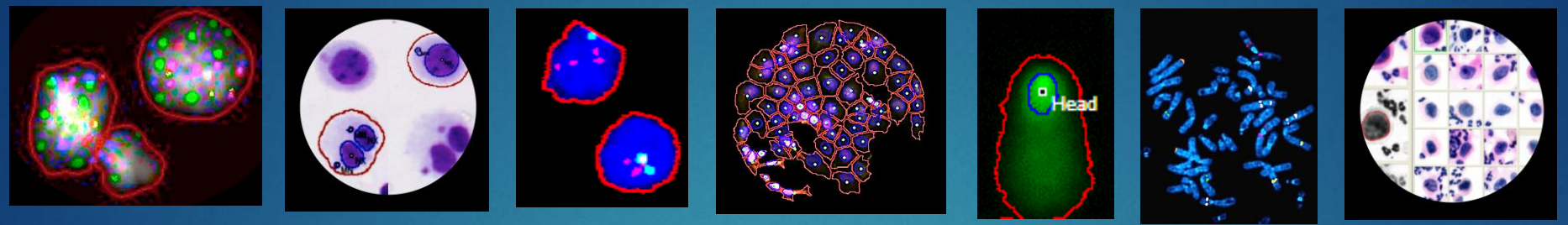
PATHFINDER™ ICS APPLICATIONS



- Cytogenetics in Genetic Toxicology & Genome Health Analysis
- Automated assays for DNA damage evaluation in Biomonitoring for environmental survey and population at risk: Micronucleus, Comet, H2AX and CAS assays
- Analytical cytogenetic
- Automated metaphases detection, capture and analysis, Multi FISH, subtle rearrangements
- In vitro Diagnostic and Theranostic on cytology and tissue section:
- Specific tests applied to quantitative cyto-pathology and rare events detection (CTC, q-FISH, Biomarker's morphometry & phenotype)
- Automated cytology Screening for assisted diagnostic
- Fast, reliable, cost effective solution to address needs of routine labs for tests (LB cytology Cervical cancer)
- Digital Cyto-Pathology
- Comprehensive modular solutions for routine & research applications; bio bank and virtual slides library

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PATHFINDER™ ICS for Biomonitoring (1)

- Human monitoring after exposure to environmental, occupational or lifestyle factors and potentially harmful compounds, in research and occupational medicine
- Genetic toxicology tests such as micronucleus and comet assay are providing crucial information and represent in sensitive and relatively easy methods to assess DNA damages
- Analysis of micronuclei (MN) is a standard approach for assessment of chromosomal damage in exposed populations and for in vitro genotoxicity testing. MN frequencies in cytokinesis-blocked lymphocytes (CBMN assay) is a reliable method as an early predictive biomarker for cancer risk
- Comet assay or single-cell gel electrophoresis is a well-established genotoxicity test and a simple method to detect a broad spectrum of DNA damage with high sensitivity, widely used in regulatory, mechanistic and biomonitoring studies

Main limitations

1. Number of cells scored due to time-consuming method,
2. Operator-dependent scoring
3. Limited number of parameters available without high-content quantification tools.

In conclusion: To increase the scoring accuracy and robustness, while saving scoring time by 10 -15x for several cell types screening (HL, L5178Y, V79, CHO, TK6, and HepG2), IMSTAR implemented an automated imaging module to be used in regulatory *in vitro* micronucleus studies compliant with GLP (OECD [C(97)186/Final]).

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PATHFINDER ™ ICS in Biomonitoring

Biomonitoring of Cancers risks or evaluation of treatment using Micro Nucleus assay

Contents lists available at SciVerse ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

HUMN project initiative and review of validation, quality control and prospects for further development of automated micronucleus assays using image cytometry systems

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[k] Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Rome, Italy

…"It is evident that automated scoring of micronuclei by automated image cytometry using different commercially available platforms [e.g. **Pathfinder™ (IMSTAR**), Metafer (MetaSystems), iCyte® (Compucyte)] is at a mature stage of development and that validation of these methods is progressing well."

The use of micronucleus (MN) assays in *in vitro* genetic toxicology testing, radiation bio-dosimetry and population bio-monitoring to study the genotoxic impacts of environment gene-interactions has increased steadily over the past two decades.

They are mainly performed by visual inspection in photon microscopy.

The **main limitations** of this method are:

1. **Number of cells scored** due to time-consuming method
2. **Operator-dependent** scoring
3. Limited number of parameters available **without high-content quantification** tools.

Automation of MN analysis allows

- **Faster, more reliable results** minimizing subjective MN identification. Improved accuracy, while reducing need for highly trained personnel'needs
- **Large scale analysis for multi-centers cohort studies**

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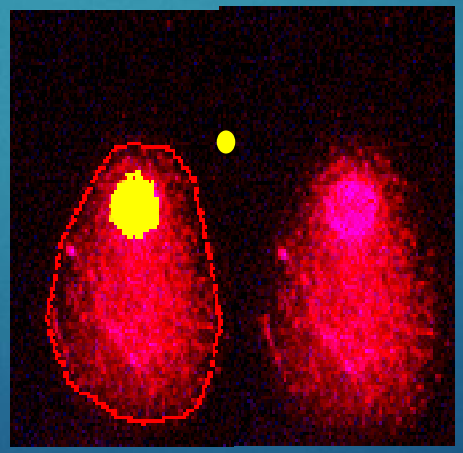
PATHFINDER™ ICS for CYTOGENETIC Tox

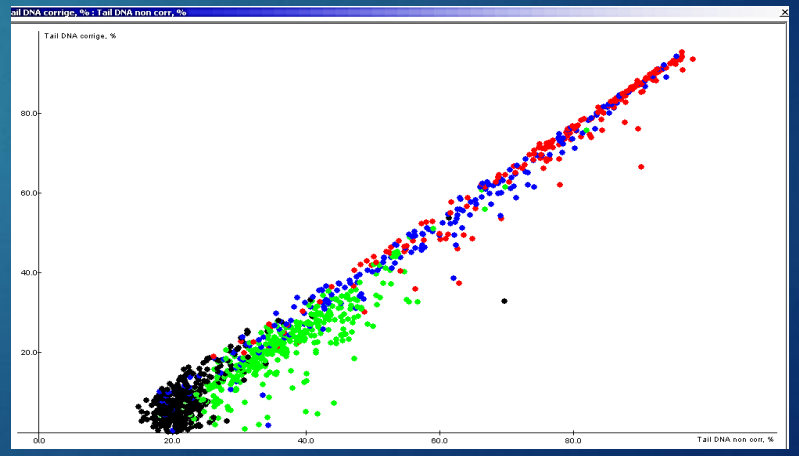
DNA damage assessment in Genetic Toxicology and Oncology treatment's follow up

High-content fully automated PATHFINDER™ ScreenTox™ platform can be used for 4 major assays' large scale analysis

- Comet assay
- Micronucleus test
- Chromosome Aberrations Scoring
- H2AX expression level

- Provides an accurate operator-independent evaluation of DNA damage and repair in high volume data
- Recognized as the reference system by blue chip customers and Centers of Disease Control





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PATHFINDER™ ICS embedded Know-How (I)

- **Modeling of the expert interpretation**
- Complex algorithms software modules embedded in Image Cytometry System
- Customized Scientific Protocol allowing high specificity and sensitivity

HUMN Scoring criteria (*Fenech M. et al., 2003, Mutat. Res., 534, 65-75*)

- MN diameter of less than 1/3rd but more than 1/4th of the nuclei diameter
- MN non-refractile; can be readily distinguished from artefacts such as staining particles
- MN are not linked or connected to the main nucleus
- MN may touch but not overlap the main nuclei and the micronucleus boundary should be distinguishable from the nuclear boundary
- MN usually have the same staining intensity that the nuclei; but occasionally staining may be weaker

Critical criteria for an automated Micro Nucleus cytome assay

- To detect cytoplasm as well as nuclei and micronuclei , with high efficiency
- To take into account variable or high cytotoxicity
- To take in account the limit of slide preparation for reproducible cell density/spacing, and homogeneous nuclei and cytoplasm staining (Standard Operating Procedure)
- Specific user interface for Digital review of detected micronucleated cells for final validation
- Possible relocation of detected objects for visual review under eye pieces
- To characterize MN morphology and position relative to the nuclei + FISH pancentromeric/centromeric probes for chromosome loss and non-disjunction
- Detection of other cytogenetic events: nucleoplasmic bridges, nuclear buds, necrotic, apoptotic cells, leading to automated « cytome » system

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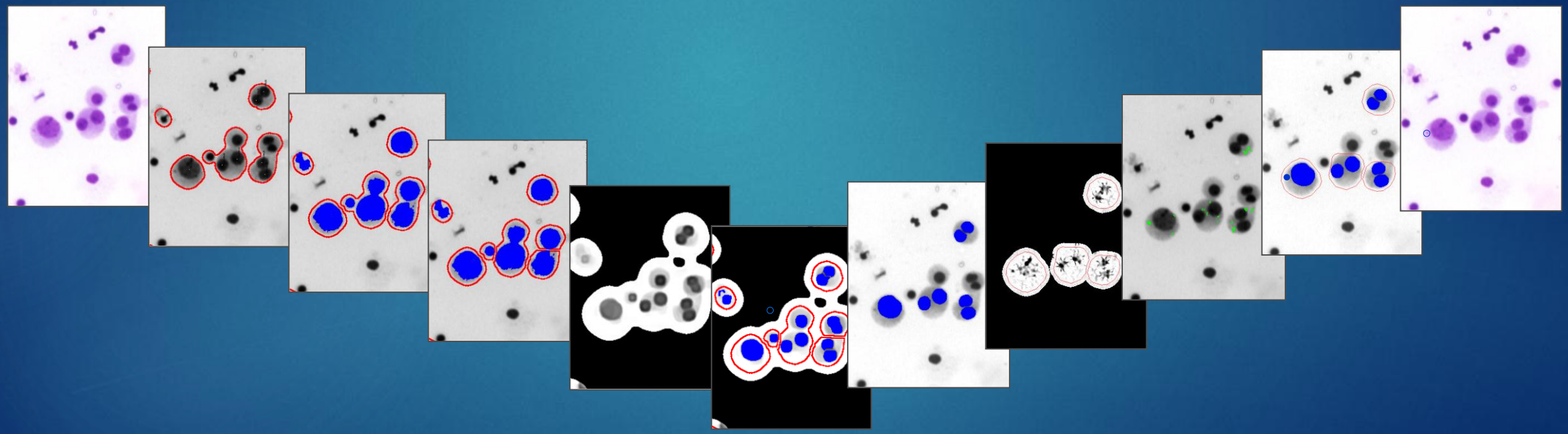
PATHFINDER™ ICS embedded Know-How (II)

- Modeling of the expert interpretation
- **Complex algorithm's software module (CSP) embedded in the ICS**
- Customized Scientific Protocol **(CSP)** allowing high specificity and sensitivity



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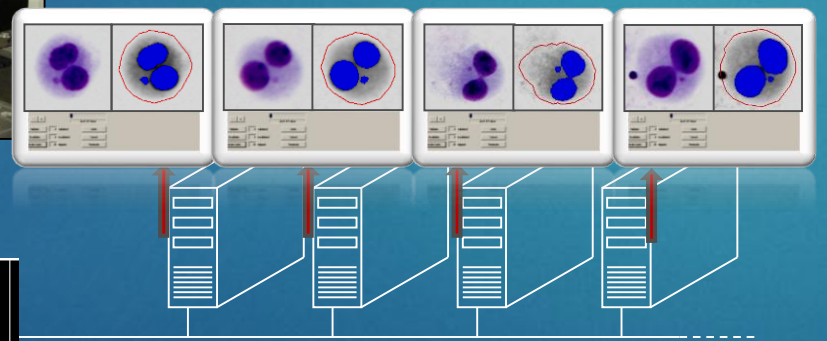
PATHFINDER™ ICS embedded Know-How (III)

- Modeling of the expert interpretation
- Complex algorithm's software module embedded in the ICS
- **Customized Scientific Protocol (CSP) allowing high specificity and sensitivity while reducing by over 10-folds expert time vs visual scoring**

Automated Pathfinder Auto MN Reader Analyzer Station

Nuclei /cell	% with MN	0 MN	1 MN	2 MN
Mono-	5.3%	2016	109	4
Bi-	8.1%	502	38	5
Tri-	0.0%	6	0	0



PATHFINDER\USER\AutoMN@PATHFINDER, 05.12.2008 16:05:46
Images\validation\

Automated

Nucl # / cell	% w/MN	No MN	1 MN	2 MN	3 MN	>3 MN
Mono-	1.00%	2399	23	1	0	0
Bi-	1.50%	2021	30	1	0	0
Tri-	4.90%	136	7	0	0	0
Tetra-	5.70%	50	3	0	0	0
Poly-	0.00%	0	0	0	0	0
Total:	1.40%	4606	63	2	0	0

Proliferation index: 1.52

Validated

Nucl # / cell	% w/MN	No MN	1 MN	2 MN	3 MN	>3 MN
Mono-	0.20%	2419	4	0	0	0
Bi-	0.40%	2044	8	0	0	0
Tri-	2.10%	140	3	0	0	0
Tetra-	5.70%	50	3	0	0	0
Poly-	0.00%	0	0	0	0	0
Total:	0.40%	4653	18	0	0	0

Proliferation index: 1.52

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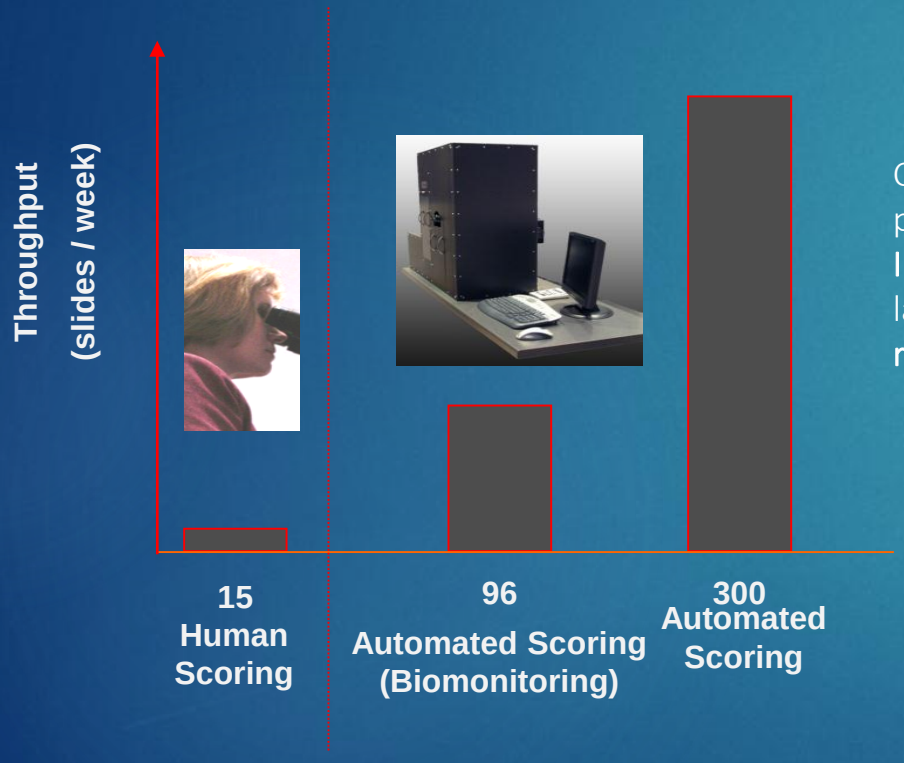
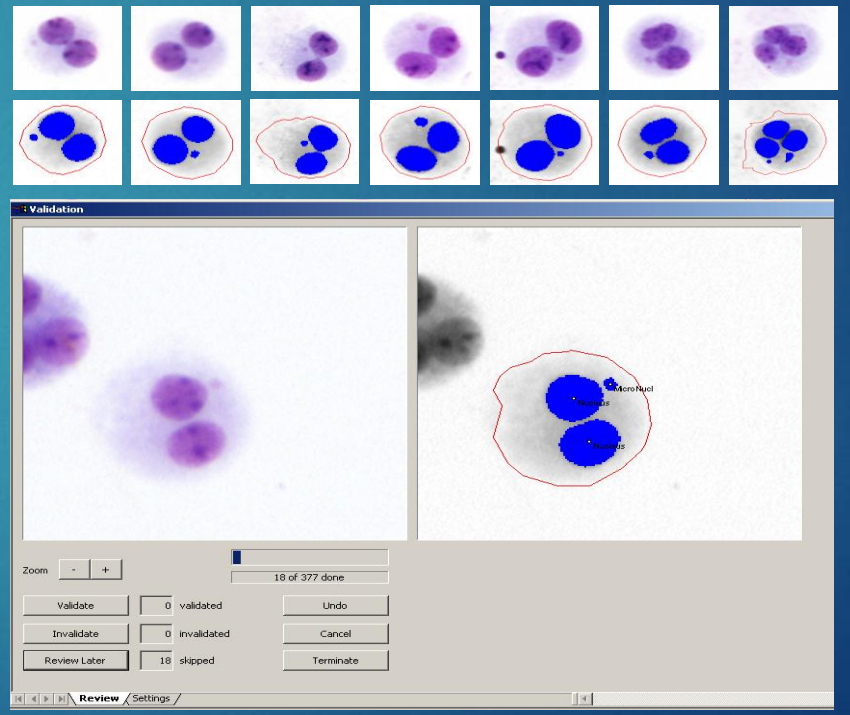
PATHFINDER™ ICS Fast validation process

- When necessary for any slide, visual validation by expert of every detected “event” such as MNs, but also BUD, BRIDGE, apoptosis, necrosis, mitosis… Comet, CTC…
- In absence of such capability, an imaging system should be validated using “Reference sample/slide”, itself analyzed by an alternative method



Candidate MN presented: **Validate**, **Invalidate** (=FP) or label it for **Future review**

Path Review and Validate™ specific user interface for fast validation



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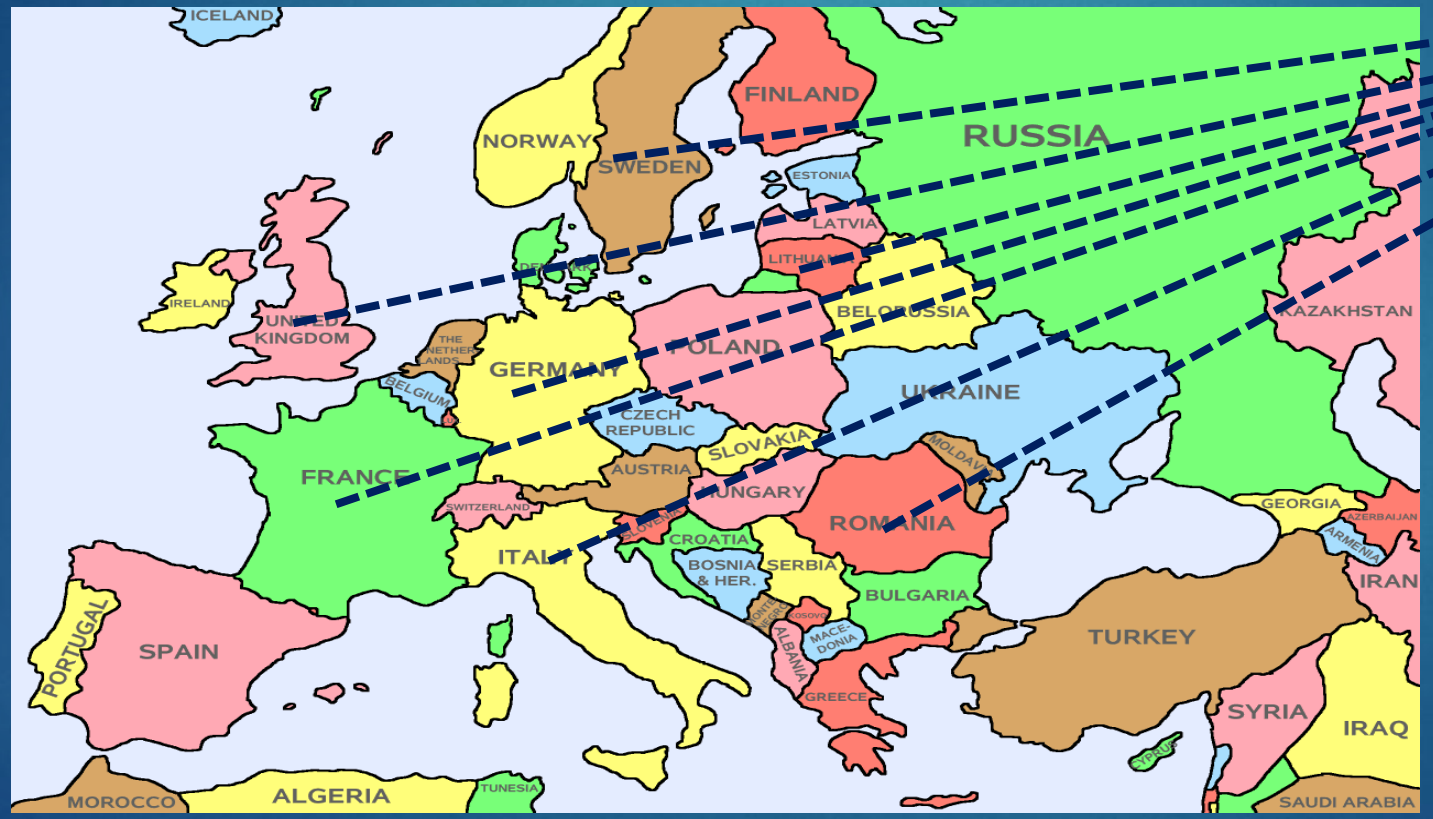
PATHFINDER™ ICS High throughput

Why High-throughput comet analysis ?

BIOMONITORING

Blood Collection from Mothers & Children



Comet Analysis

NATIONAL RESEARCH CENTRE FOR THE WORKING ENVIRONMENT

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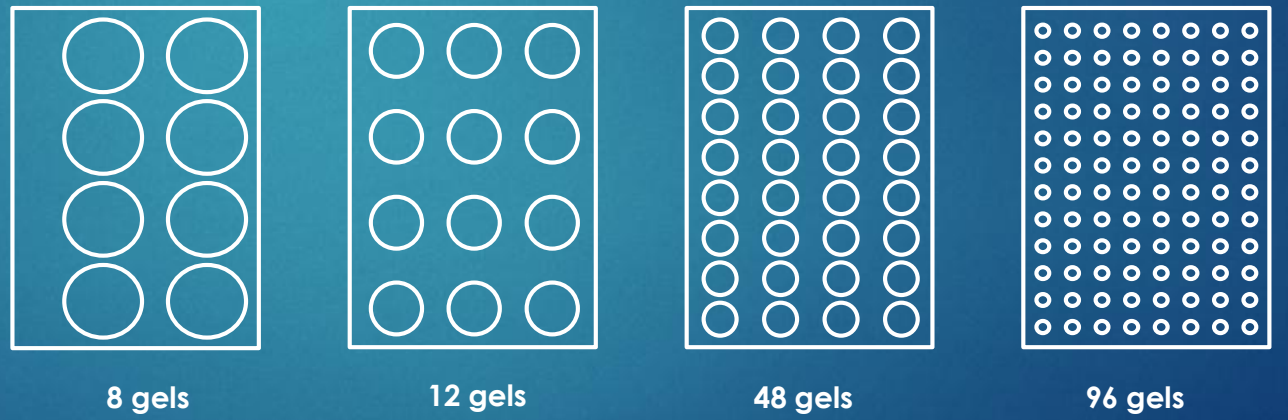
PATHFINDER™ ICS High throughput

High-throughput comet protocol Encapsulation

Petra Jackson, National Research Centre for the Working Environment, Copenhagen Denmark

Standard glass slides
Trevigen CometSlides™
Cell Biolabs, Inc.
NORGENOTECH

GelBond®film



Mutagenesis, 2012; 28, 333-340
High-throughput comet assay using 96 minigels.
Gutzkow,K.B., Langleite,T.M., Meier,S., Graupner,A., Collins,A.R., Brunborg,G

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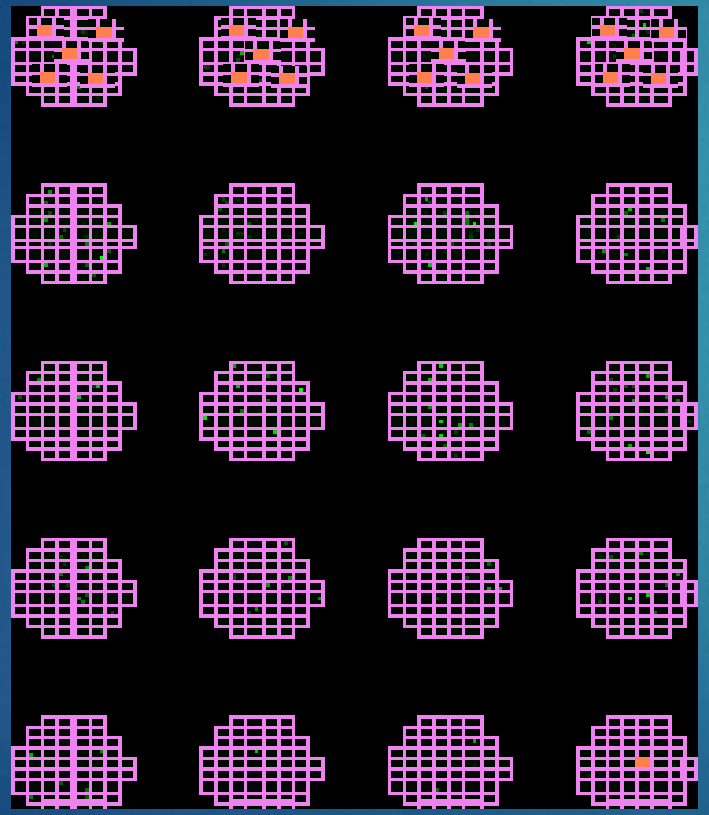
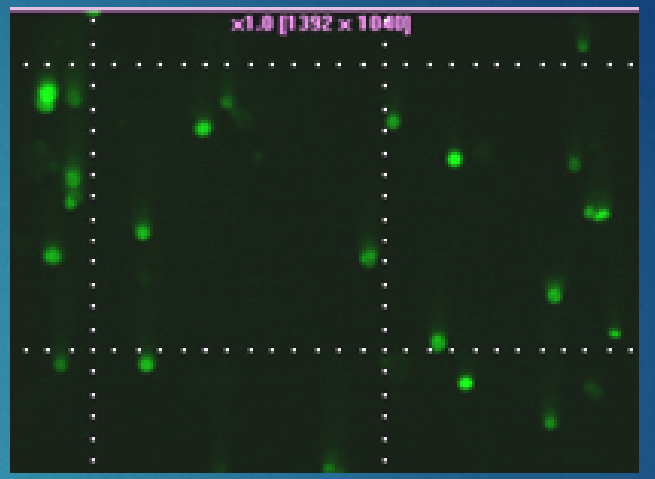
PATHFINDER™ ICS High throughput

High-throughput comet protocol
Fullyautomated scoring

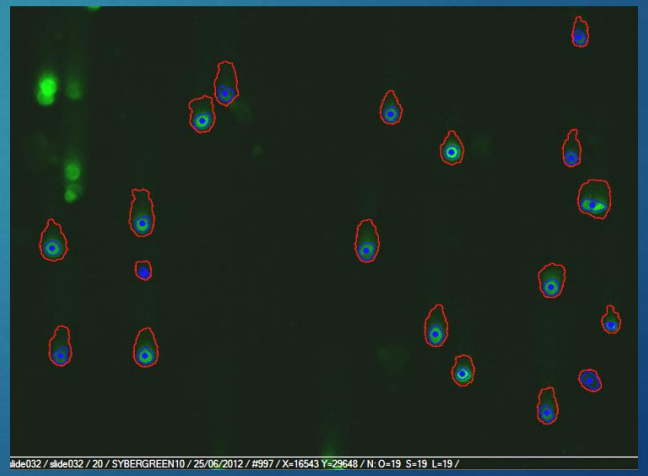
1) Landmarks:



2) Image capture:



3) Analysis:



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PATHFINDER™ ICS High throughput

Fully-automated scoring High-throughput comet protocol

Mutagenesis, 2011; 26, 393-399
The influence of scoring method on variability in results obtained with the comet assay.
Azqueta,A., Meier,S., Priestley,C., Gutzkow,K.B., Brunborg,G., Sallette,J., Soussaline,F., Collins,A

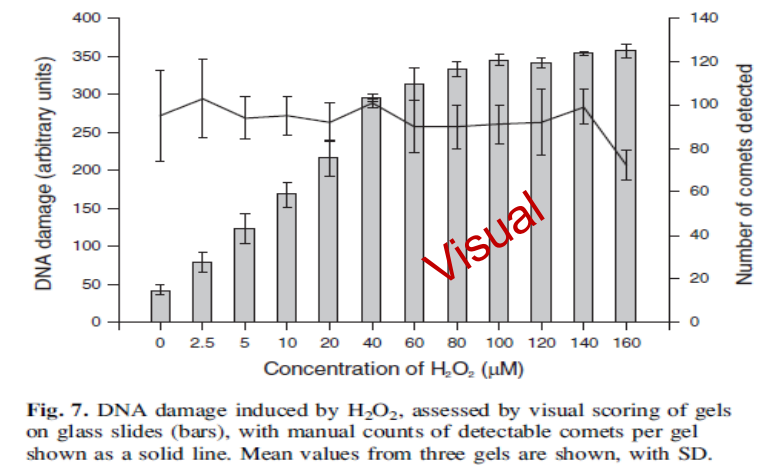

Fig. 7. DNA damage induced by H_2O_2, assessed by visual scoring of gels on glass slides (bars), with manual counts of detectable comets per gel shown as a solid line. Mean values from three gels are shown, with SD.

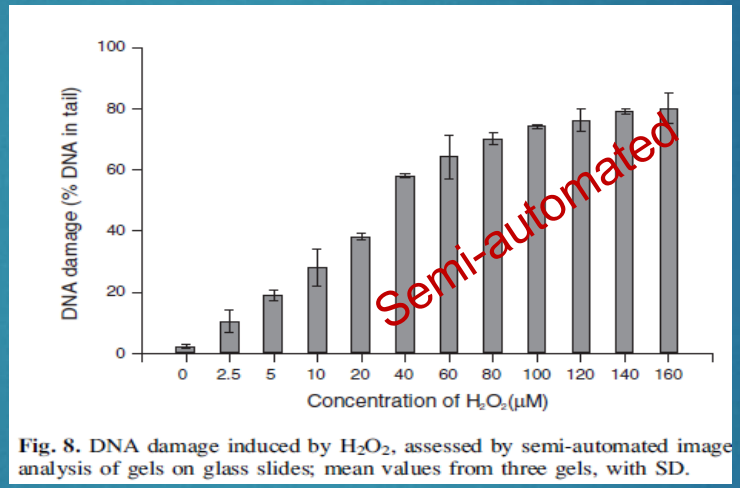

Fig. 8. DNA damage induced by H_2O_2, assessed by semi-automated image analysis of gels on glass slides; mean values from three gels, with SD.

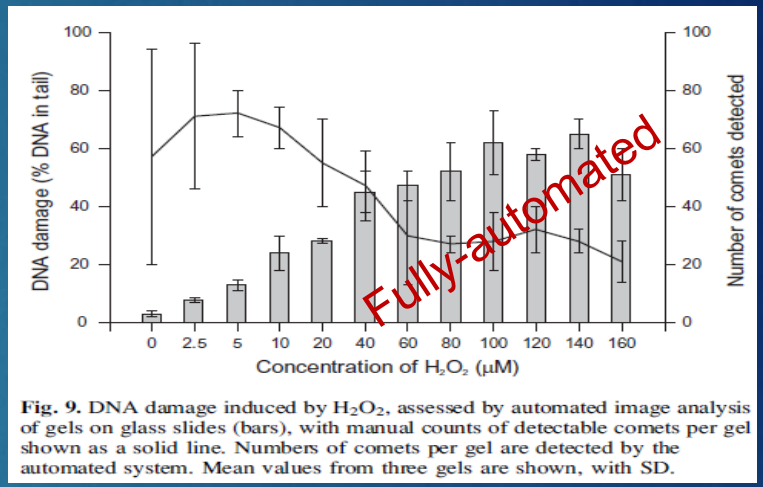

Fig. 9. DNA damage induced by H_2O_2, assessed by automated image analysis of gels on glass slides (bars), with manual counts of detectable comets per gel shown as a solid line. Numbers of comets per gel are detected by the automated system. Mean values from three gels are shown, with SD.

Mutat Res, 2012; 749 (1-2), 70-75
The influence of the number of cells scored on the sensitivity in the comet assay.
Sharma,A.K., Soussaline,F., Sallette,J., Dybdahl,M

Low levels of DNA strand breaks:
Optimal number of cells scored 300-600

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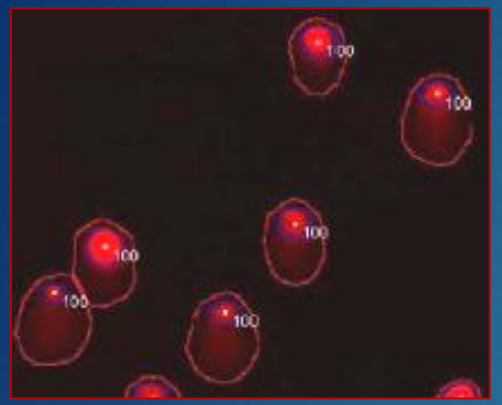
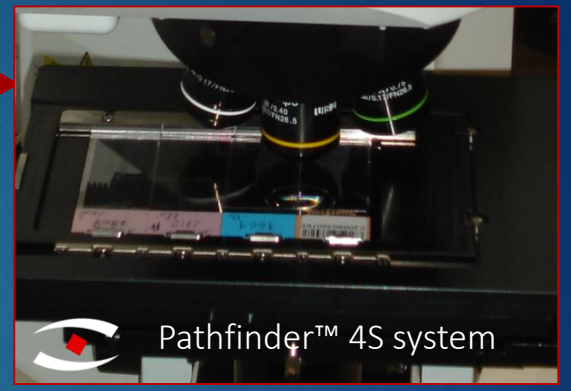
PATHFINDER™ ICS High throughput

The influence of the number of cells scored on the sensitivity

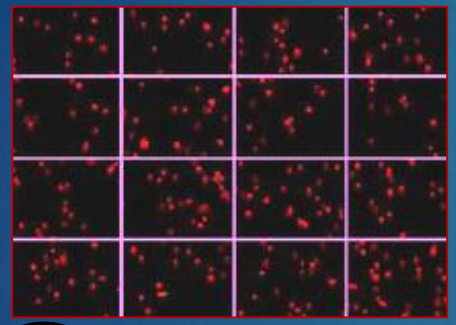


1

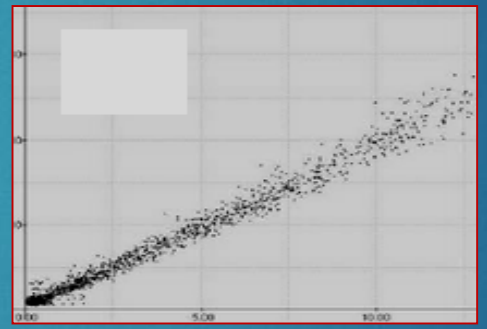
110 slides are scanned by Pathfinder™ ScreenTox Auto-COMET, in one day;
Unattended processing for comets detection and scoring overnight by the Fully Automated (FA) system

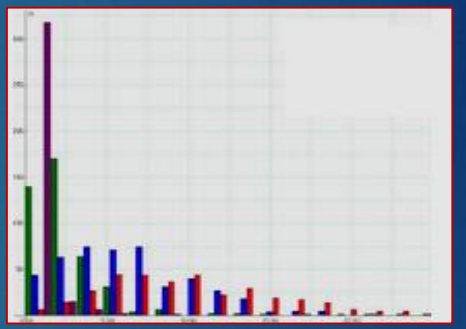


Automated comets detection and % tail DNA evaluation of 100 - 700 randomly selected cells/slide



o Head	Tail DNA non corr, %	Tail % DNA corrected
2597	66.6	6.3
2272	66.6	7.7
9530	59.0	10.0
4611	64.7	0.0
4582	61.4	4.1
3524	59.2	0.0
3896	67.1	3.3
5268	64.3	8.4
5930	62.9	3.6
6035	62.1	10.1
	51.5±10.2	10.7±13.0





2

Differences in % tail DNA of 100 - 700 cells scored by fully automated system analysed by one-way ANOVA analysis of variance with Dunnet's test to compare 3 concentrations vs corresponding control group. The influence of the concentrations and the number of cells scored on the CVs was investigated by two-way ANOVA analysis.

3

Caco-2 cells (EMS) and HepG2 cells B[a]P were also scored by semi-automatic (SA) system Kinetic Imaging 5.5, UK or Perceptive IV Image Analysis System (scoring of 50 cells /gel)

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PATHFINDER™ ICS High throughput

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The influence of the number of cells scored on the sensitivity in the comet assay

Anoop Kumar Sharma[a,*], Françoise Soussaline[b], Jerome Sallette[b], Marianne Dybdahl[a]

Summary

- For the comet assay of *in vitro* samples, using a **Pathfinder ScreenTox Comet fully-automated system allows the scoring of several-folds higher number of cells.**
- **For low levels of DNA damage, < 9% tail DNA, scoring of 600 cells, and for levels of DNA damage between 9-16% tail DNA, scoring of 300 cells increased the sensitivity, compared to 100 cells scored.**
- **These findings could be particularly important for bio-monitoring studies**
- **The human time needed to perform the study is reduced by 20-folds** using the Fully Automated system. It could be reduced by over 30-folds with the last generation of automated Pathfinder systems with 50x12-gels automated slide-loader.

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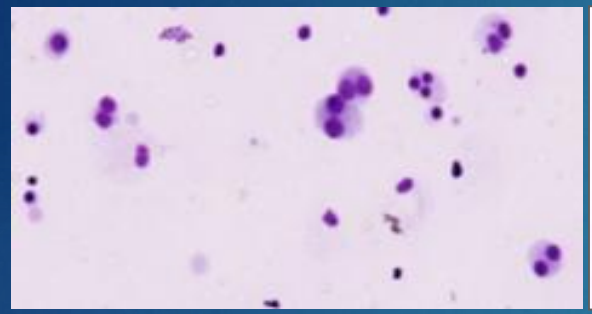
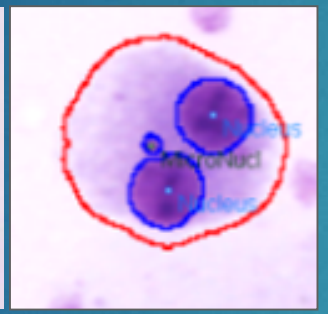
PATHFINDER™ ICS in Cancer Prevention

- DNA damages in biomonitoring testing
- Early cervical cancer detection
- Early detection of cancer recurrency

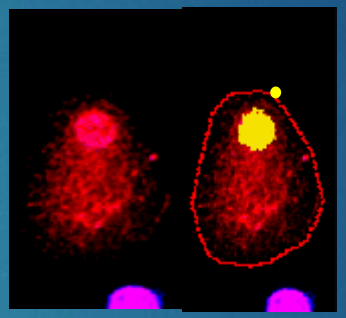


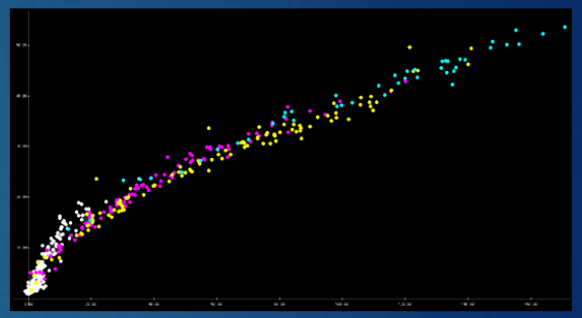


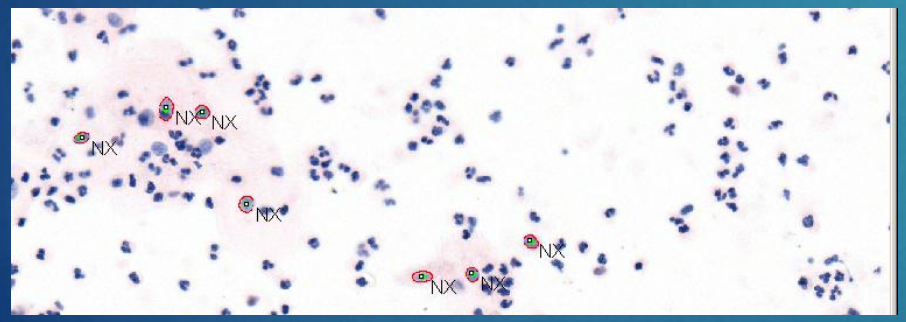
User validated

Nucl/cell	%w/MN	0 MN	1 MN	2 MN
Mono-	0.9%	228	2	0
Bi-	0.4%	257	1	0
Tri-	0.1%	8	0	0
Total:	0.6%	494	3	0

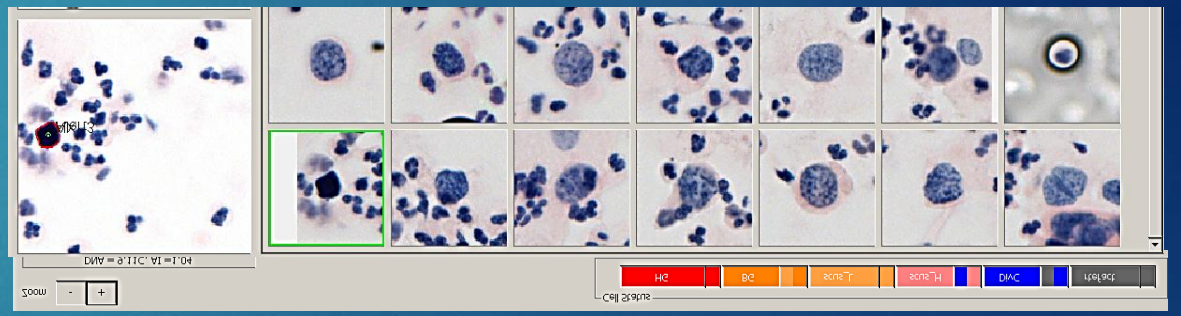
Proliferation index: 1.56







Sorted Alert slide
Review & Validate:
HSIL HPV & biopsy required



Automated detection of all suspicious CTC in lung cancer: After Review & Validate confirmed presence of CTC > 50 cells: **Highly positive sample.** Gain in expert time: **6folds**

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IMSTAR a COMPANY with a VISION

- Innovation and cross knowledge are the starting points for new analytical tool design
- Change in paradigm proved to be the starting point for many breakthroughs in medical research and routine practice
- Know-how, vision, passion and perseverance of a few people can change, sometimes drastically, the natural history of a disease

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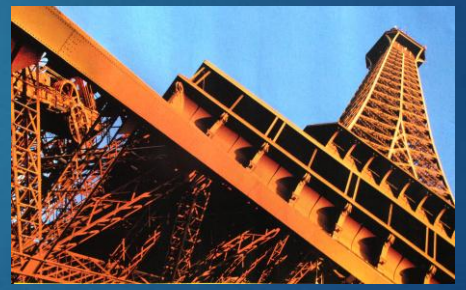

THANK YOU FOR YOUR ATTENTION!

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