

**COMICS: developing high throughput comet assays for DNA damage and DNA repair**



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on behalf of **COMICS** partners  
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...is an EC strategic targeted project (STREP) of the 6<sup>th</sup> framework, aimed at turning the comet assay into a high throughput assay suitable for use in screening chemicals for potential genotoxic and cytotoxic effects.

It relates to the EC policy on REACH and the philosophy of the 3R.



# The comet assay



1) Cells embedded in agarose on microscope slide



2) Lysis: 2.5 M NaCl, Triton X-100



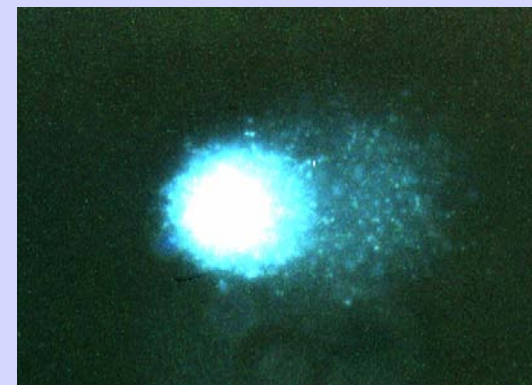
Nucleoid:  
supercoiled DNA

3) Alkaline incubation:  
0.3 M NaOH, 10 mM EDTA

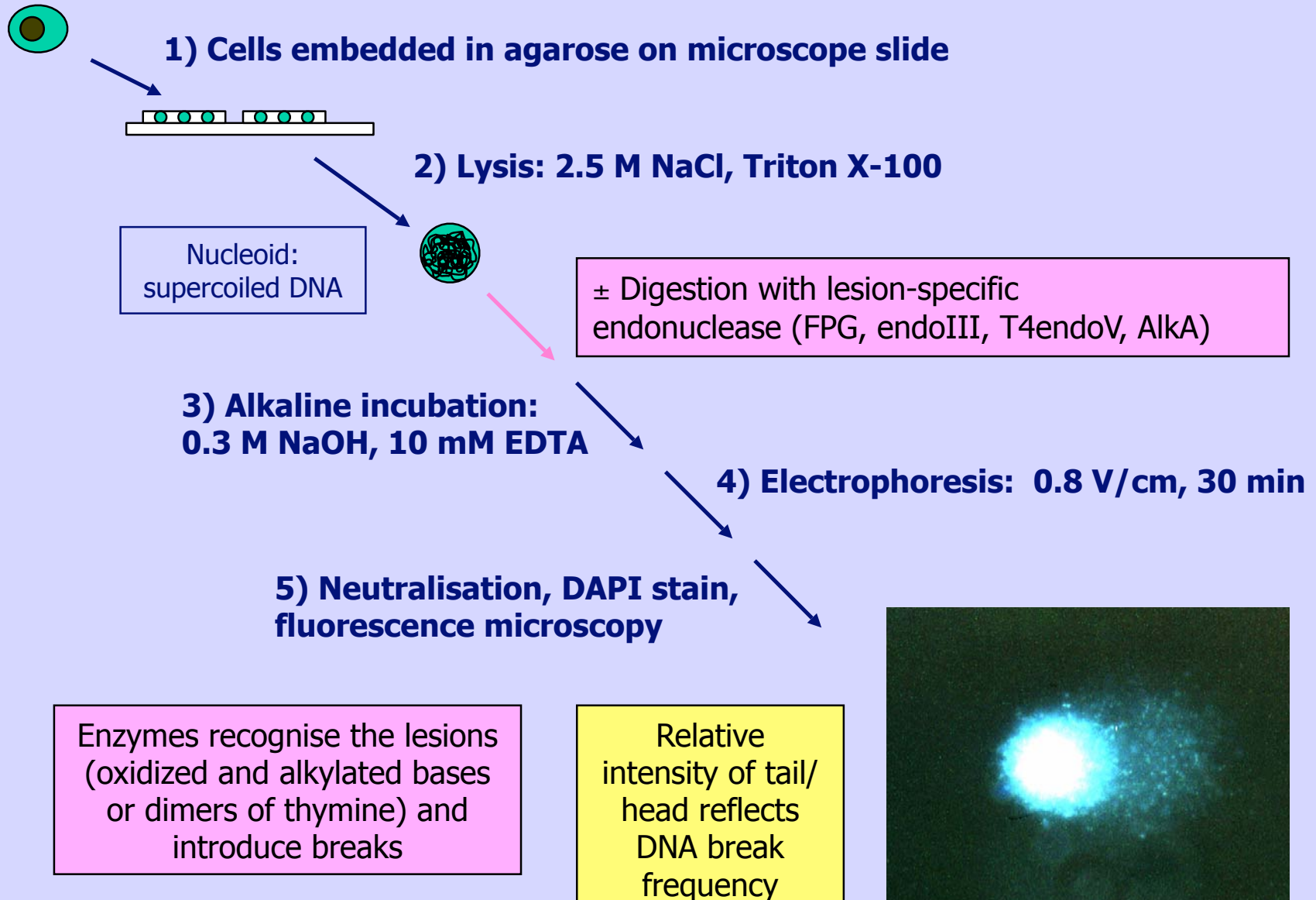
4) Electrophoresis: 0.8 V/cm, 30 min

5) Neutralisation, DAPI stain,  
fluorescence microscopy

Relative  
intensity of tail/  
head reflects  
DNA break  
frequency



# The comet assay modified with lesion-specific enzymes



- Basic research on mechanism of genotoxicity
- Biomonitoring
- Chemical testing (both in vitro and in vivo)

Fast and very versatile but:

- Labour-intensive
- Number of samples is limited by the size of the electrophoresis tanks
- Scoring is very laborious and very time-consuming



Medium- or high-throughput versions of the comet assay + an automated scoring system are necessary for monitoring damage in a large number of samples

## **Objectives of COMICS:**

- To increase the throughput of the comet assay, using multi-well format and 'cell arrays'.
- To develop further the cell array system as a parallel assay for cytotoxicity.
- To seek optimal cell types (e.g. metabolically active HepaRG).
- To develop a faster scoring method based on differential fluorescence of head and tail DNA
- To develop and compare methods for measuring DNA repair activity.
- To use lesion-specific enzymes for different kinds of DNA damage – oxidised and alkylated bases, UV-induced damage and bulky adducts.

- To develop fluorescent in situ hybridisation on comets, to study gene-specific DNA repair.
- To validate the comet assay in its various forms, assessing reproducibility and robustness, comparing results in different laboratories.
- To develop reference and internal standards for use in the comet assay.
- To make the various innovative products available for use by companies and researchers investigating DNA damage and repair.

- University of Oslo, Oslo, Norway (CO-ORDINATOR: Andrew Collins)
- Commissariat à l'Energie Atomique (CEA), Grenoble, France
- Institut National de la Santé et de la Recherche Médicale (INSERM), Rennes, France
- Slovak Medical University, Bratislava, Slovakia
- Uppsala University, Uppsala, Sweden
- Institute of Experimental Botany, Prague, Czech Republic
- IMSTAR S.A., Paris, France
- Chiron AS, Trondheim, Norway
- TATAA Biocenter AB, Göteborg, Sweden
- Astra Zeneca, Macclesfield, United Kingdom
- Thistle Scientific Limited, Glasgow, Scotland
- Severn Biotech LTD, Kidderminster, United Kingdom
- BIOPREDIC INTERNATIONAL, Rennes, France
- Norwegian Institute of Public Health (NIPH), Oslo, Norway
- JOINT RESEARCH CENTRE (JRC-ECVAM), Ispra, Italy

## **University of Oslo, Norway**

- To develop and calibrate a cell array for a medium-throughput assay.
- To collaborate in the development of automated analysis.
- To develop an in vitro repair assay based on the comet assay to detect different pathways, and to compare this method with DNA repair chips.
- To develop stains and staining procedures for two-colour fluorescence
- To develop FISH combined with cell array to make scoring easier and quicker
- To apply padlock probes to detect gene-specific DNA repair in comets

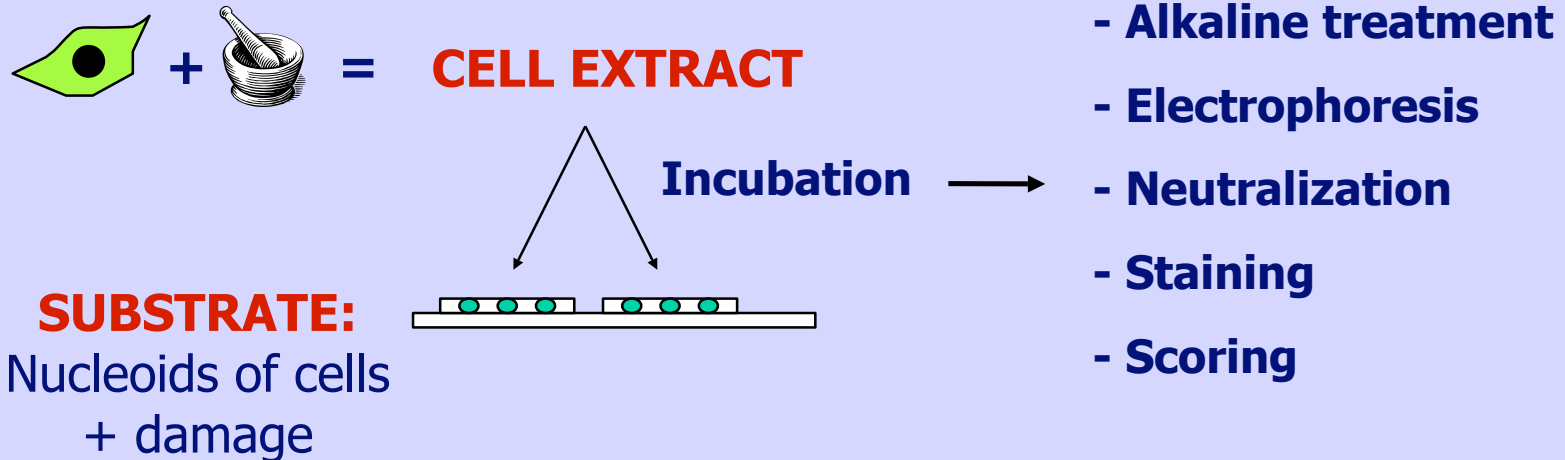
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IN VITRO REPAIR ASSAY: estimates the repair activity of cell extracts by measuring their incision activity



Nucleoids containing 8-oxoguanine ⇒ BER

Nucleoids with cyclobutane pyrimidine dimers ⇒ NER

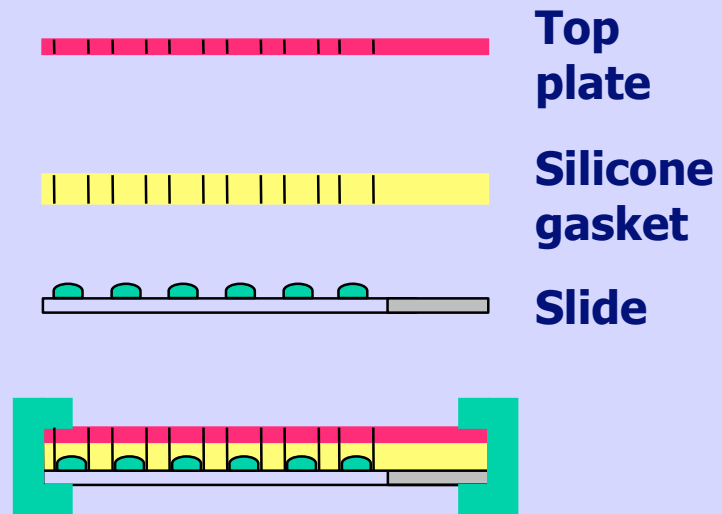
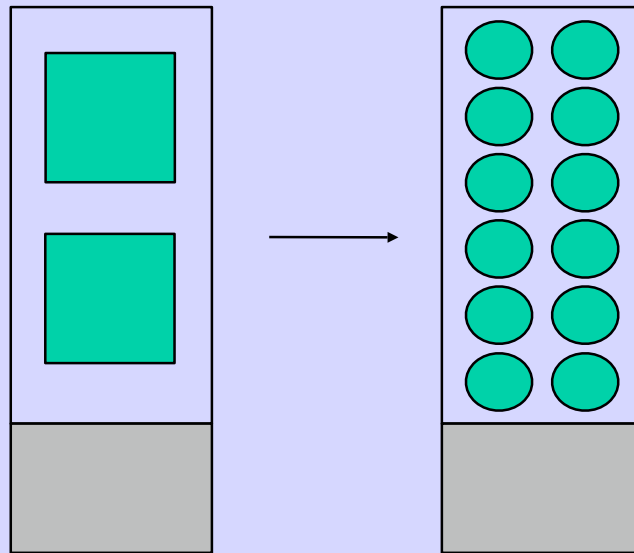
\* Comparison of in vitro repair assay with DNA repair chips

\* Development of an assay to detect the capacity for repair of alkylation damage

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## MEDIUM THROUGHPUT ASSAY

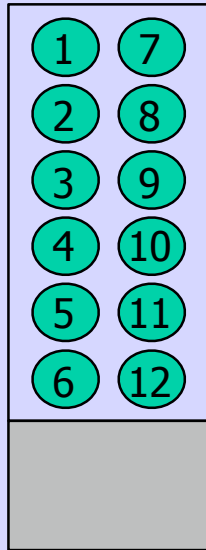


12 gels/slide

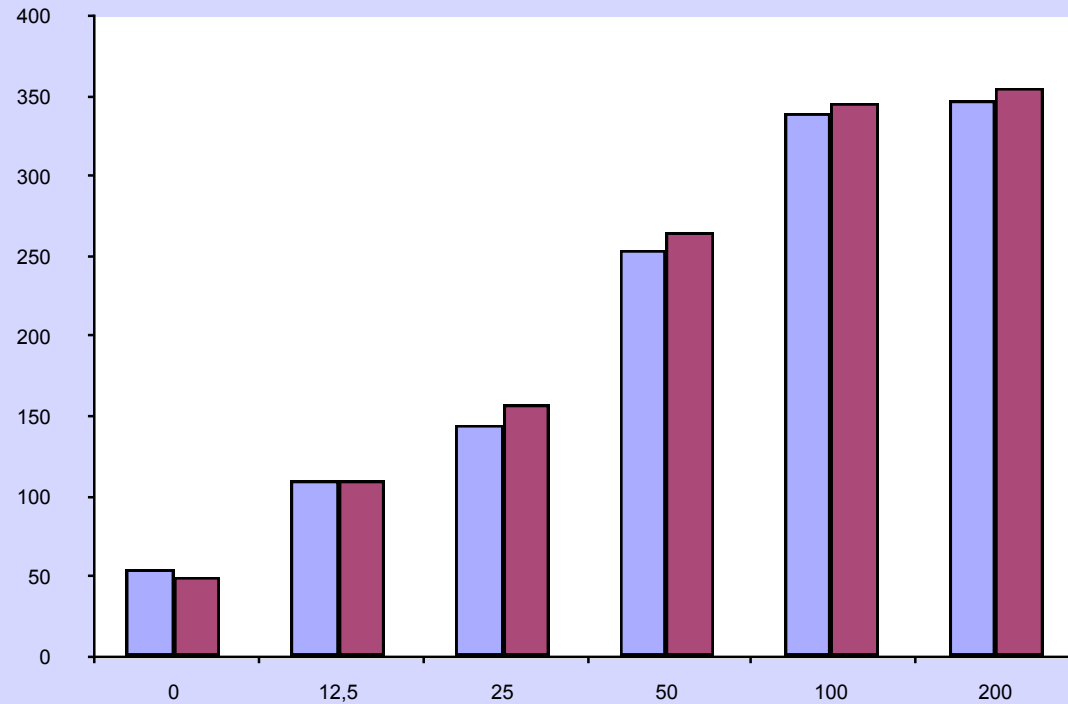
Perfect for testing different chemicals and for testing different cell extract in the in vitro repair assay



## MEDIUM THROUGHPUT ASSAY: H<sub>2</sub>O<sub>2</sub> dose response



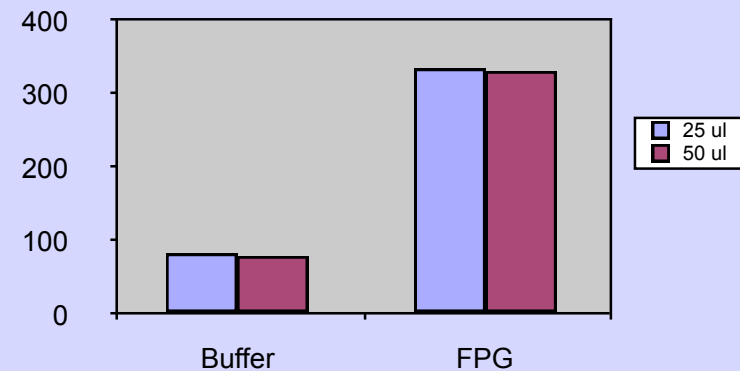
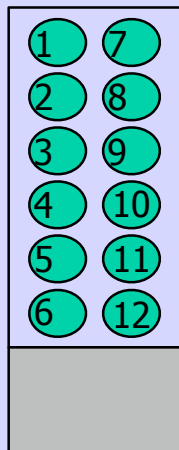
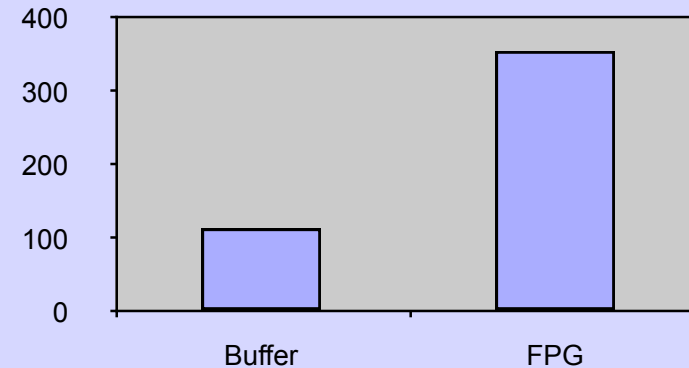
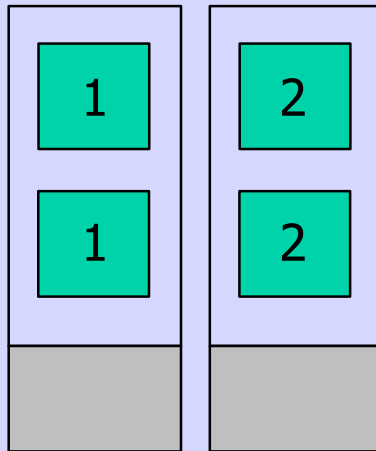
1 and 12: 0  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>  
2 and 11: 12,5  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>  
3 and 10: 25  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>  
4 and 9: 50  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>  
5 and 8: 100  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>  
6 and 7: 2000  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>



Cells were treated after embedding in agarose.

- No leakage between wells
- Good dose response
- Excellent reproducibility between duplicates

## MEDIUM THROUGHPUT ASSAY: comparison with standard assay



Good agreement between the two systems  
25  $\mu$ l are enough to incubate the minigels in the well

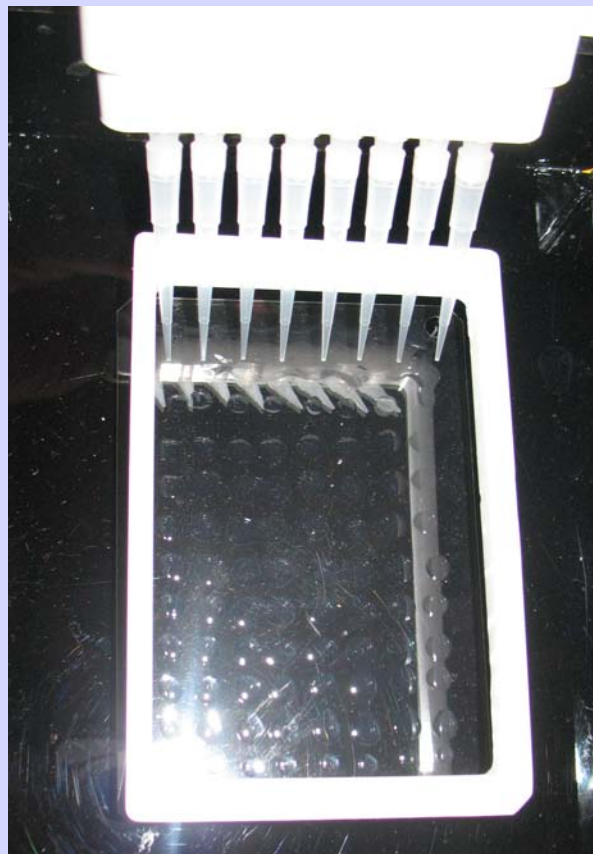
## MEDIUM THROUGHPUT ASSAY

### Next steps:

- Decrease the amount of extract/compounds/enzymes for incubating minigels
- Validation of the new medium-throughput assay (12 gels/slide) and the high-throughput assay (96 gels/Gelbond film)

## HIGH THROUGHPUT ASSAY

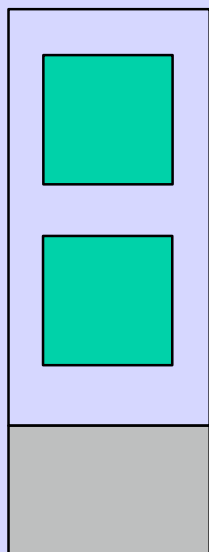
Gelbond film with 96 gels/film



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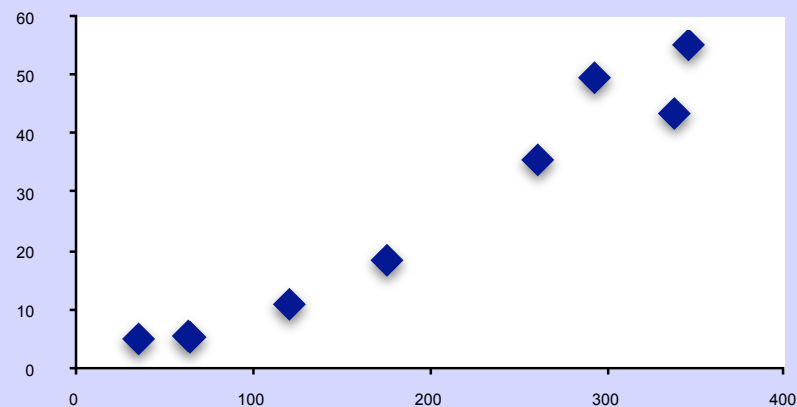
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# AUTOMATED SCORING (IMSTAR) vs VISUAL SCORING

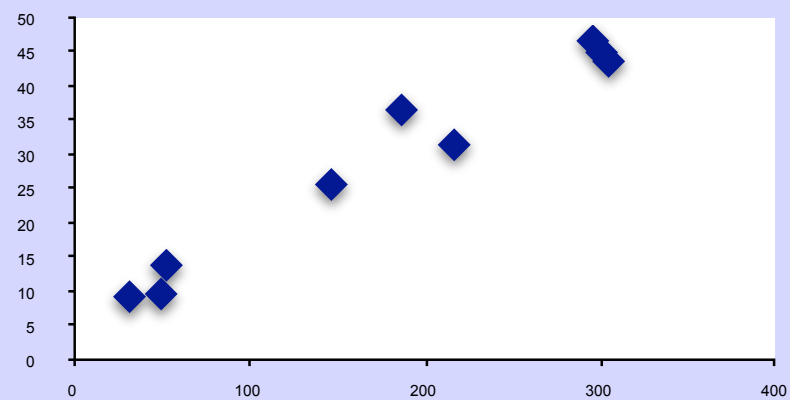


Cells treated with different concentrations of H<sub>2</sub>O<sub>2</sub>

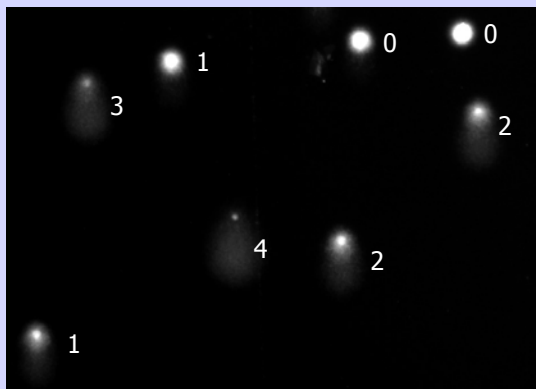
## Lymphocytes



## HeLa cells



## Visual categories



## AUTOMATED SCORING (IMSTAR)

Next steps:

-Adapt the IMSTAR system to the new prototypes: medium-throughput assay (12 gels/slide) and high-throughput assay (96 gels/Gelbond film).

Until now - very promising!!!

Scoring will be even faster if we manage to find a staining method to give a direct measure of total tail DNA

THANK YOU FOR YOUR ATTENTION!!!

