Multiparameteranalytik für die moderne DNA-Diagnostik

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Brandenburgische
Technische Universität
Cottbus - Senftenberg

Overview

- Introduction
- Aim
- Methods and Technology
- Results
- Conclusions
- Outlook

PARMENIDes

INITIATIVE FÜR
PERSONALISIERTE
DIAGNOSTIK
UND MEDIZIN

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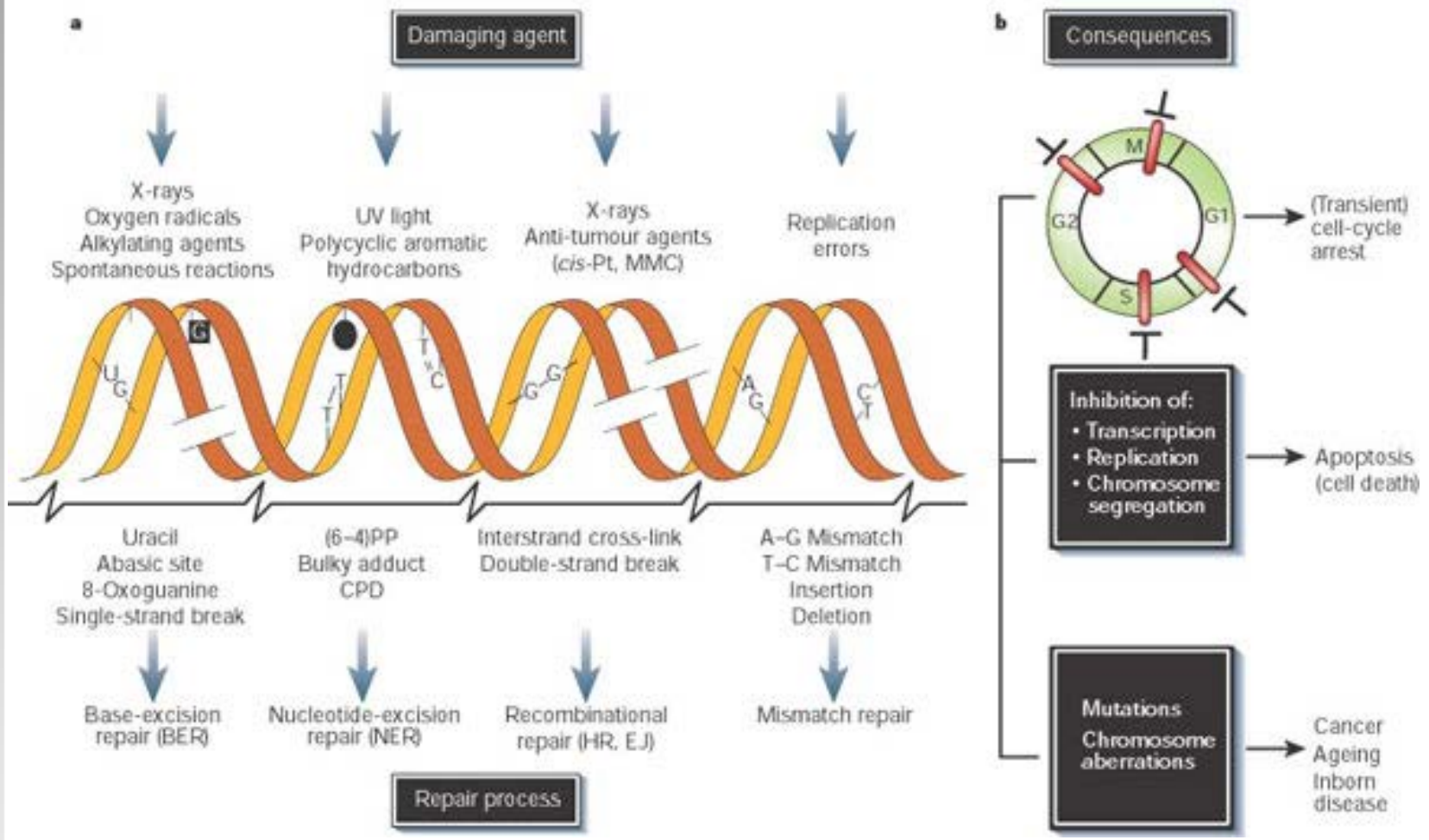
Introduction

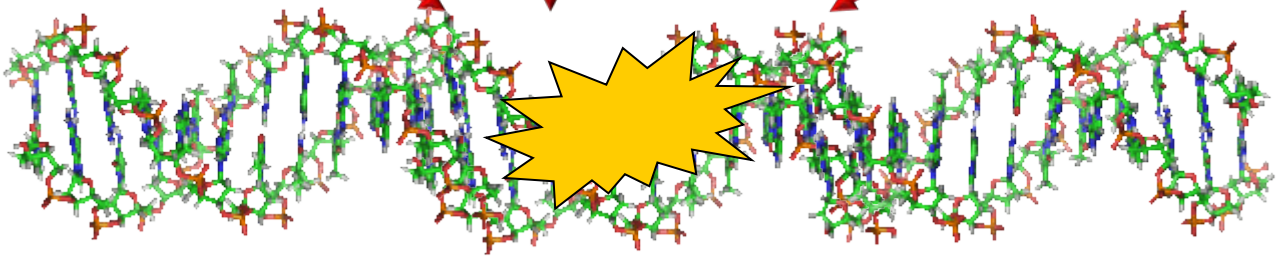


Hoeijmakers, J.H. Nature (2001) 411; 366-74

free radicals

radiation

chemotherapy



double strand break (DSB)

biosensors

Proteinkinase ATM

cell death

repair

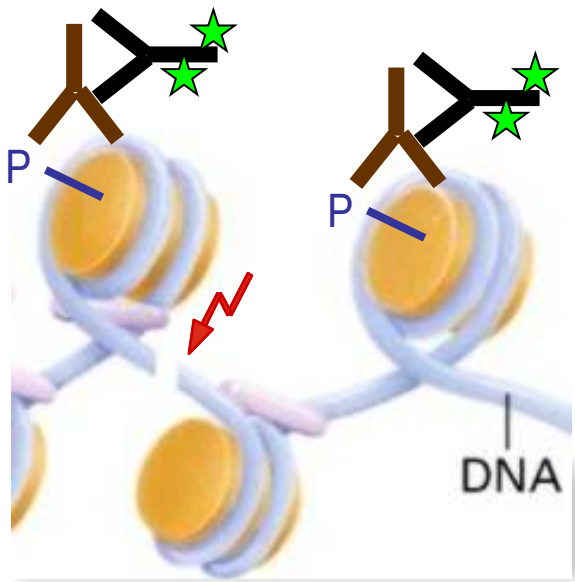
Introduction

Methods for detection of DNA damage

- Colony formation assays → clonogenic cell survival
- Comet assay → SSB, DSB
- γH2AX-assay → DSB

Introduction

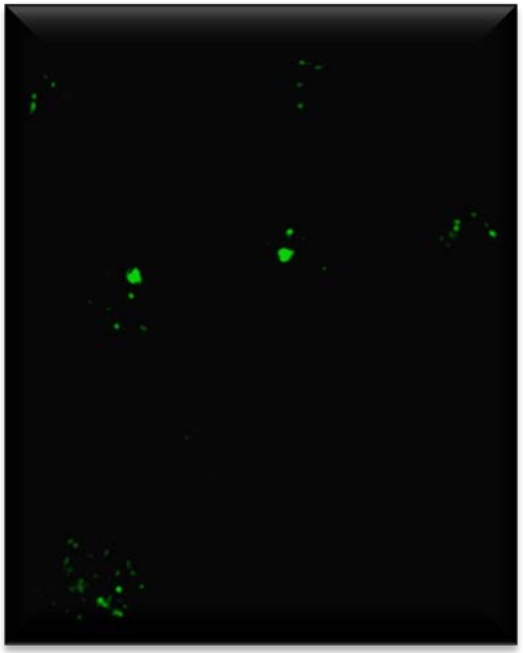
γH2AX assay



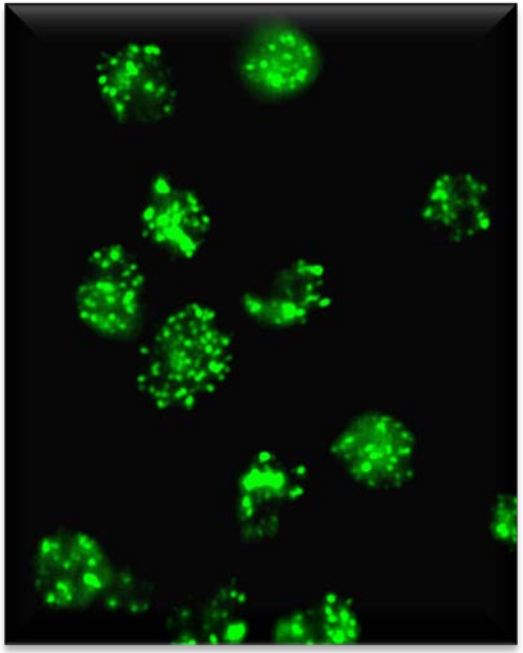
- Phosphorylation of histone subunit H2AX ⇒ γH2AX
- Recognition by specific antibodies
- Detection via fluorescence

The γH2AX Foci assay is the most sensitive and specific test available in the area of the detection of DSB at the moment.

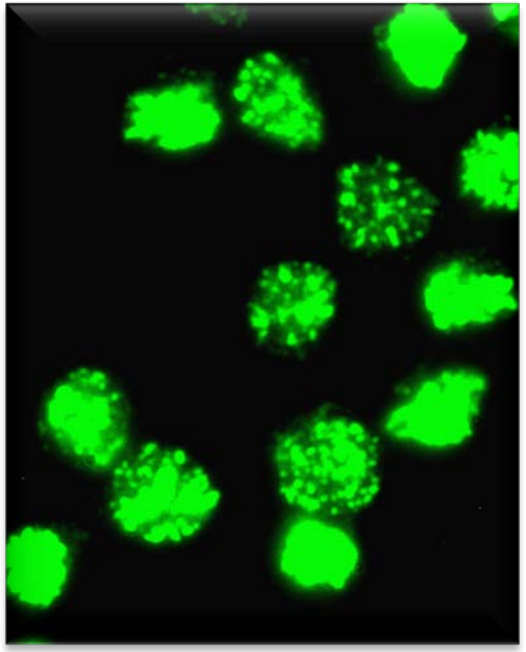
Introduction



Dose: 0 Gy
(no radiation)



Dose: 2 Gy



Dose: 6 Gy

Recent status: manual evaluation (counting of the spots)

„The evaluation is time consuming and requires a lot of routine and experience.“

→ No standardisation, very subjective, no automatisation

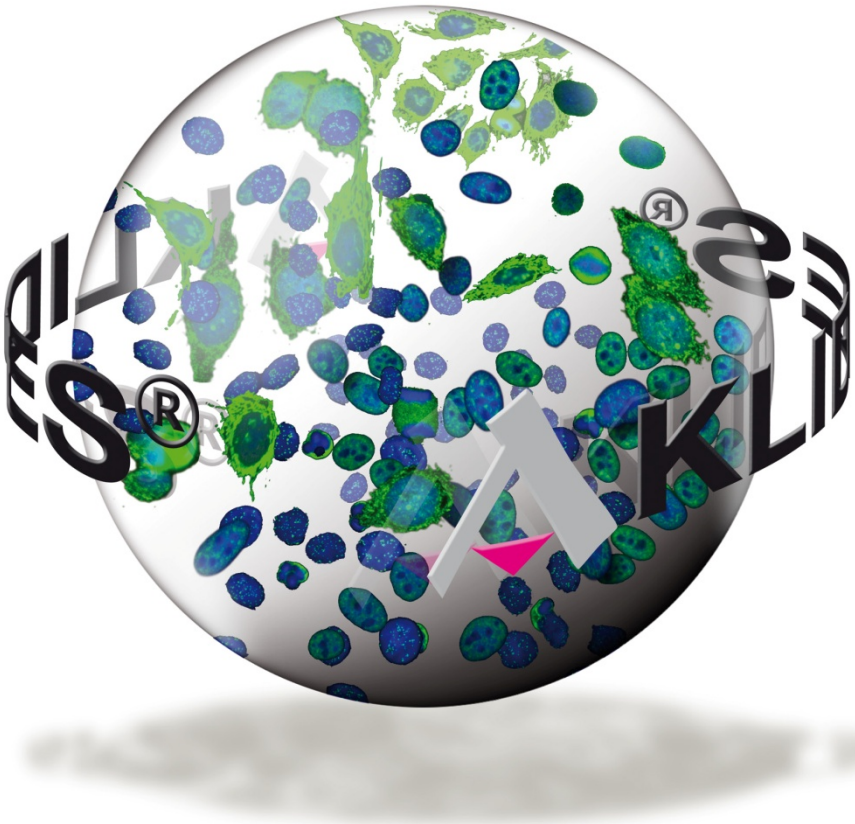
R.Runge et al., Int J Radiat Biol 2012

Aim

Goal: Fully automated evaluation of DSB via γH2AX-foci assay

- To facilitate your workflow
- Standardisation for comparable and reproducible data
- Objectivity
- More flexibility

AKLIDES® Technology



New innovative analysis platform technology

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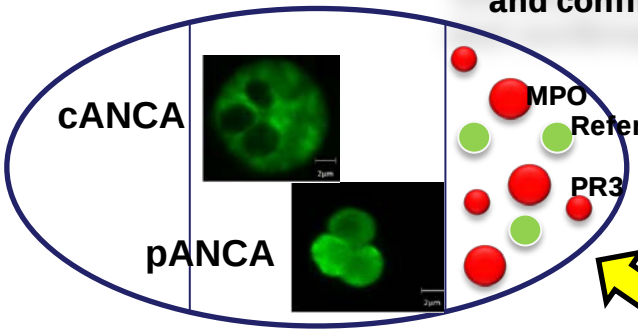
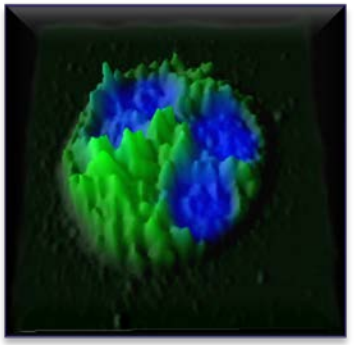
Multiplexing

Simultaneous screening and confirmatory testing



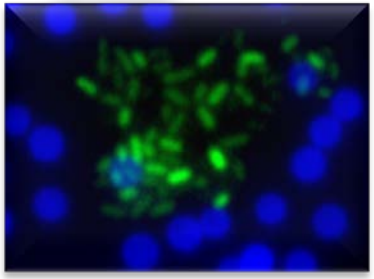
Autoimmunity

Automation and standardization



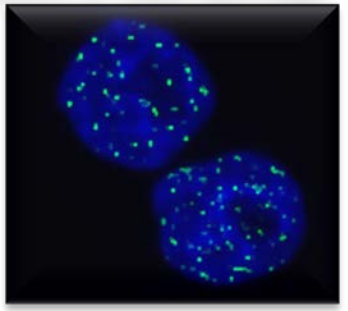
Microbiology

Analysis of bacterial growth



Oncology

Analysis of DNA damage



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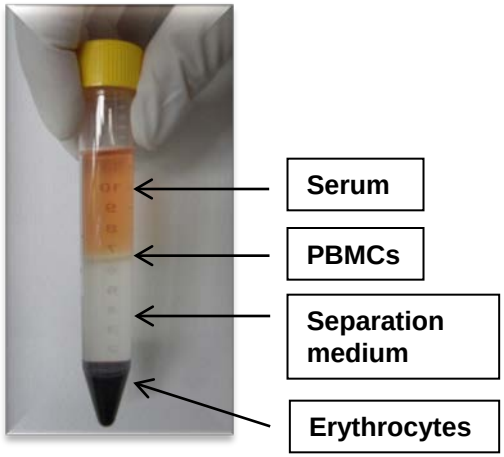
Content of our HLC kit

Isolation of lymphocytes

Blood sample

Isolation of the peripheral blood cells (PBMCs)



Fixation of the cells on slides

Permeabilization of the cells

Addition of the primary antibody

Addition of the secondary antibody with the fluorochrome

Automatic evaluation with the Aklides® system

Method and Technology

Modular Hardware: Microscope

Modular Hardware: LED light source

Modular Hardware: Sample stage for slides and microtiter plates

Modular Hardware: CCD Grey scale camera

Software

Image Generation

Fully automated control of x- and y-levels and fully automated focussing

Image Processing

Algorithms calculate antibody concentration via fluorescence intensity (arbitrary units = AU) and calculate fluorescence patterns

Analysis

Automatic generation of assay results

Image Storage

Assures digital archiving of the results over years.

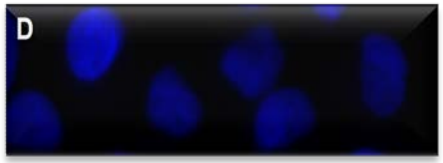
Export Function

Image export via pdf- and excel-reports.

Software

Image Processing

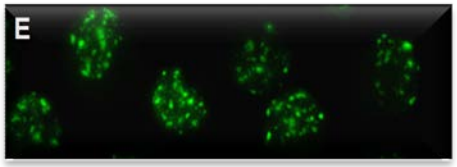
1. Excitation in DAPI channel



Focussing by permanent signal and cell/nucleus identification

+

2. Excitation in FITC channel



Signal is depending on immunocomplex (Determination of intensity)

=

Overlay of both pictures

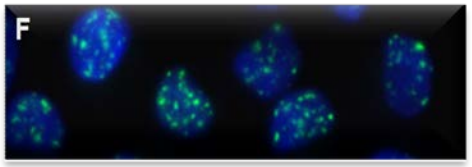
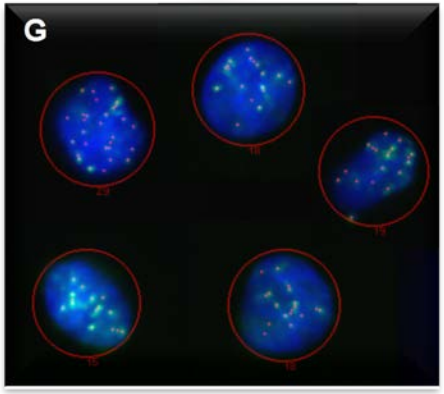
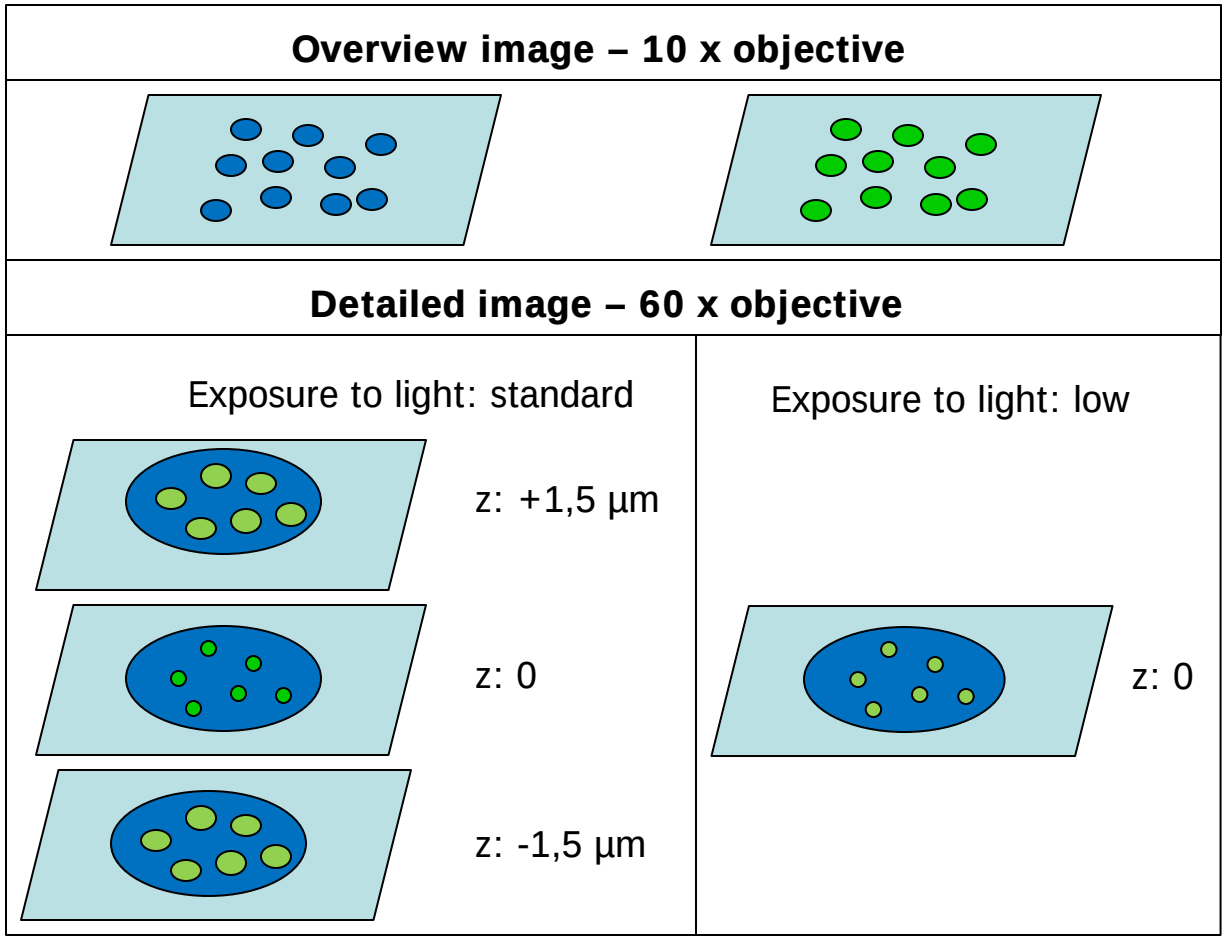


Image generation, localisation of signal, foci determination

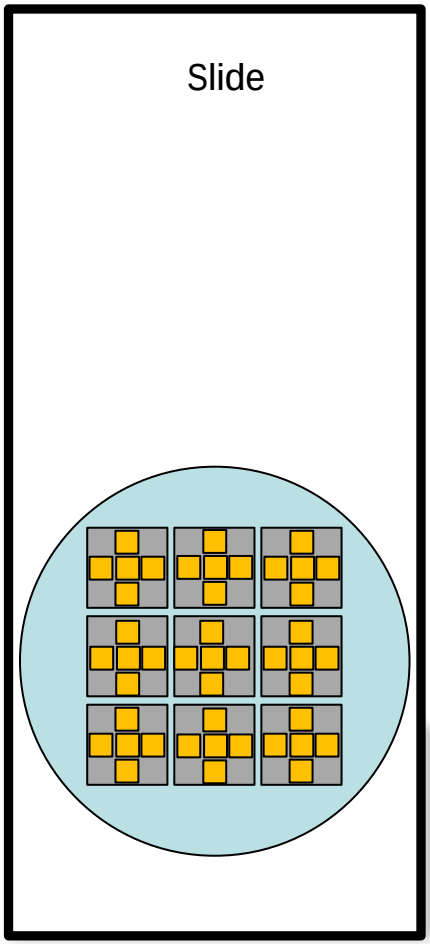


Analysis

Software



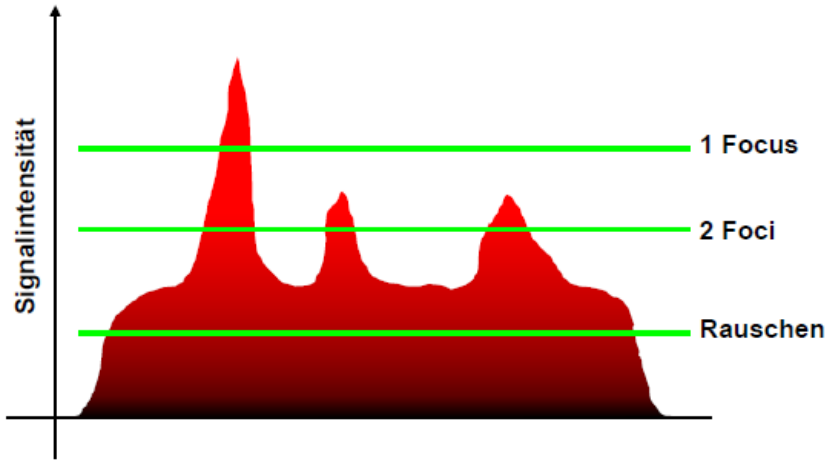
Software



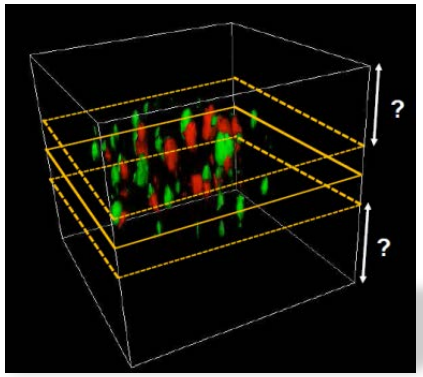
- 9 positions for measurement
- 5 scenes
- 3-7 focus levels per scene
- up to 1000 cells counted

Software

Image Analysis



Z-levels

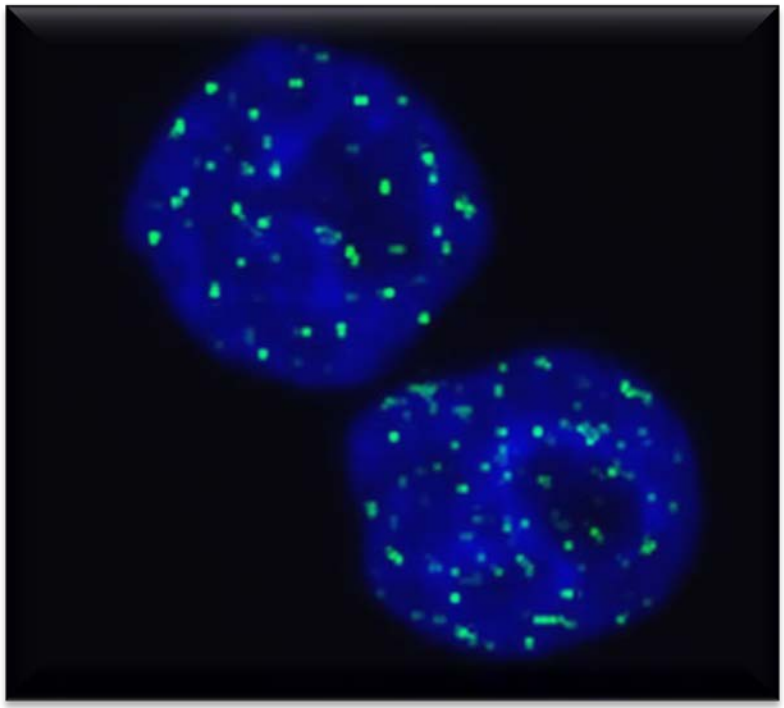


Default: 3 z-levels (lymphocytes).
z-levels must be adapted for each cell line and could be increased.

AKLIDES® Nuk

Fully automated interpretation of ionizing radiation-induced γH2AX foci by the novel pattern recognition system AKLIDES®

Roswitha Runge, Rico Hiemann, Maria Wendisch, Ulla Kasten-Pisula, Katja Storch, Klaus Zoephel, Christina Fritz, Dirk Roggenbuck, Gerd Wunderlich, Karsten Conrad & Joerg Kotzerke. *Int J Radiat Biol, 2012*



Source: Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, USA

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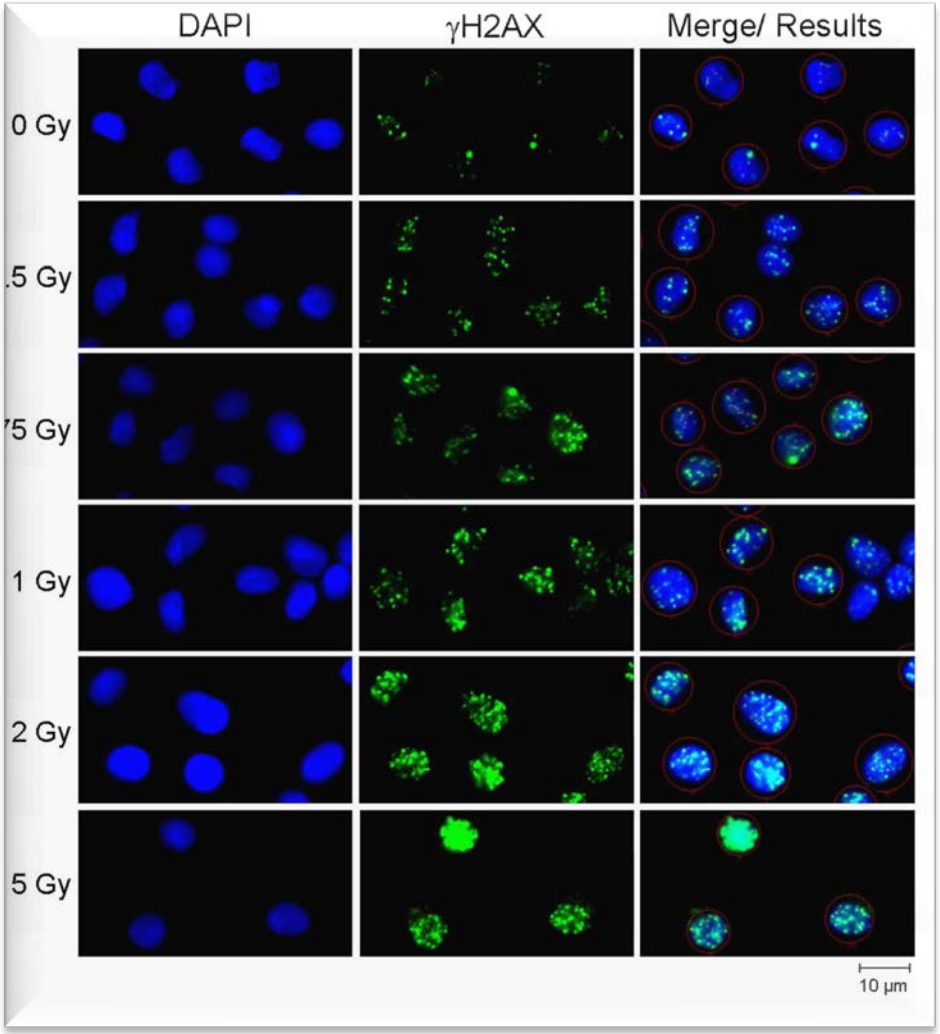
Results



Immunofluorescence microscopy of PCCL3-cells after radiation with ^{188}Re (60 x).

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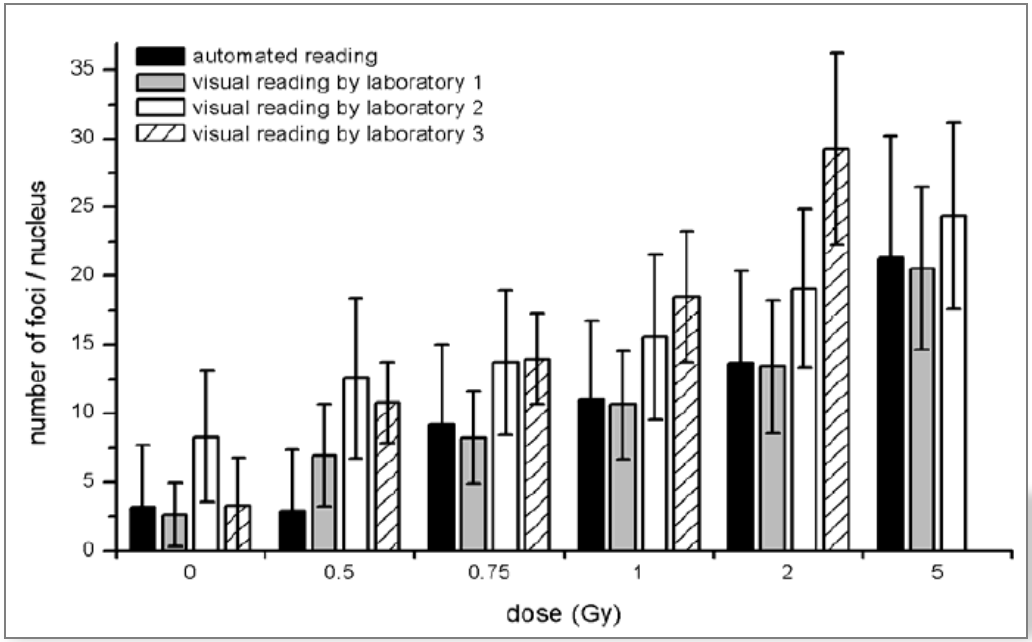
Results

Comparison of automatic and visual analysis by three different laboratories



R.Runge et al., Int J Radiat Biol 2012

In cooperation with

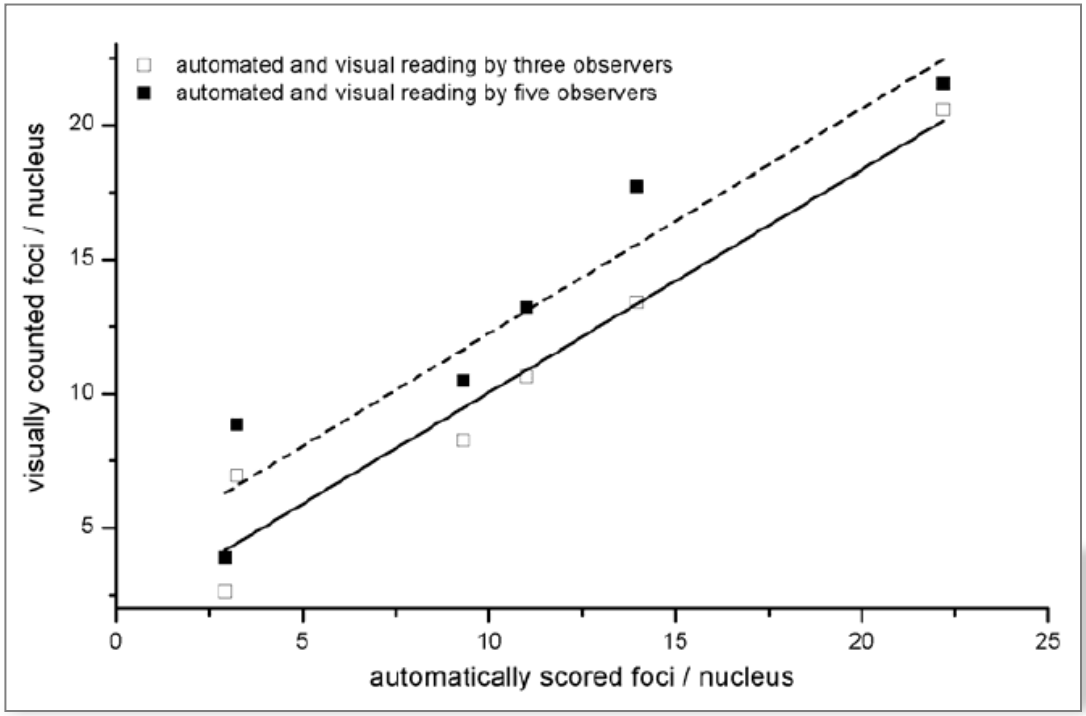
Nuklearmedizin

Results

- Good correlation between γH2AX-foci and doses
- High variability between the three visual analyses
- Good correlation between visual and automatic analysis

Results

Comparison of automatic and visual analysis by three different laboratories



- Laboratory 1 and automatic → $R^2 = 0.931$
- Laboratories 1, 2, 3 and automatic → $R^2 = 0.889$

In cooperation with

Carl Gustav Carus 1789 - 1869

Nuklearmedizin

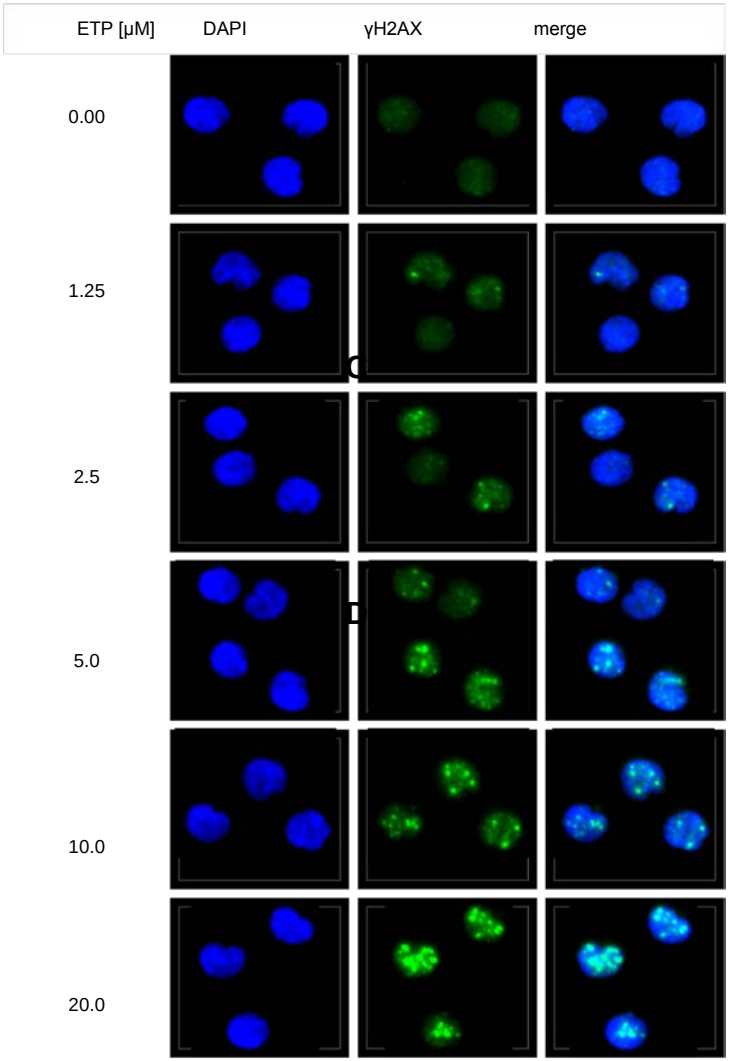
ORIGINAL ARTICLE

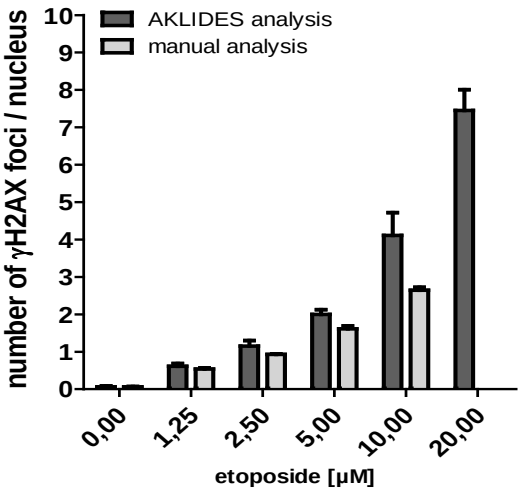
Cytometry
PART A
Journal of the International Society for Advancement of Cytometry

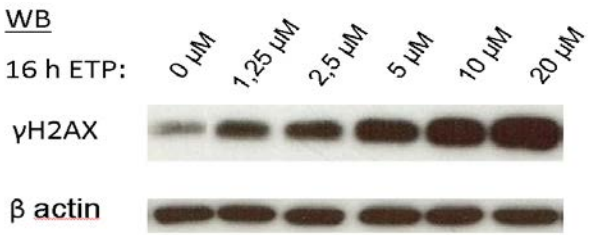
Fully Automated Analysis of Chemically Induced γH2AX Foci in Human Peripheral Blood Mononuclear Cells by Indirect Immunofluorescence

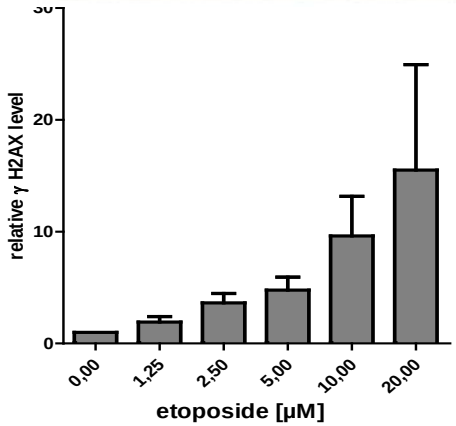
Annika Willitzki,[1] Sebastian Lorenz,[2] Rico Hiemann,[3] Karina Guttek,[1] Alexander Goihl,[1] Roland Hartig,[1] Karsten Conrad,[4] Eugen Feist,[5] Ulrich Sack,[6] Peter Schierack,[3] Lisa Heiserich,[2] Caroline Eberle,[2] Vanessa Peters,[2] Dirk Roggenbuck,[2,3]* Dirk Reinhold[1]

Results









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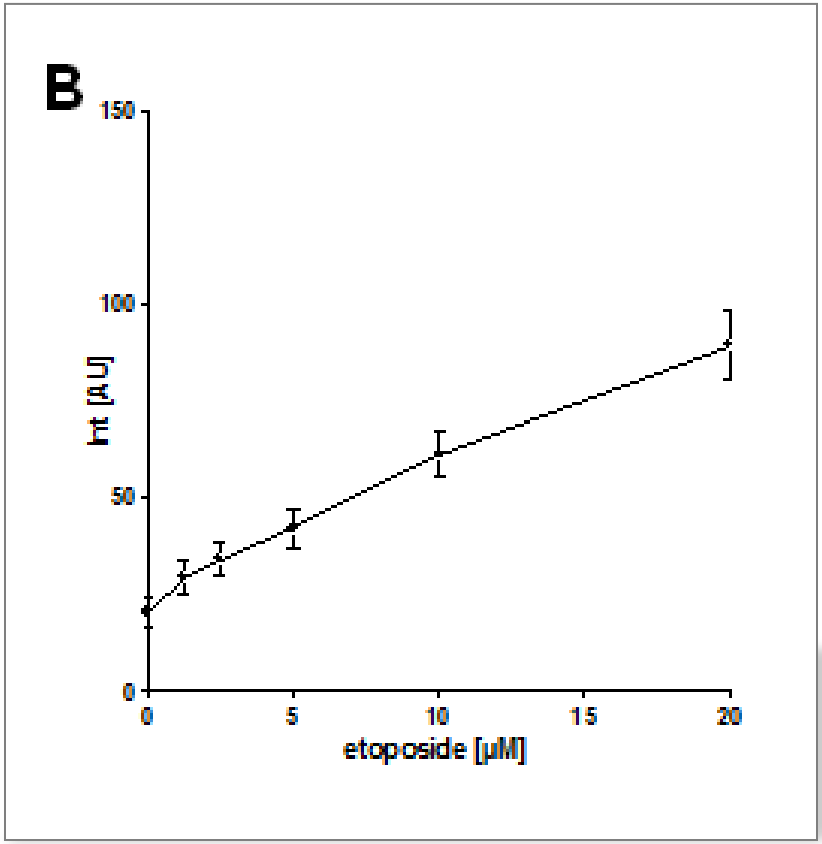
Results

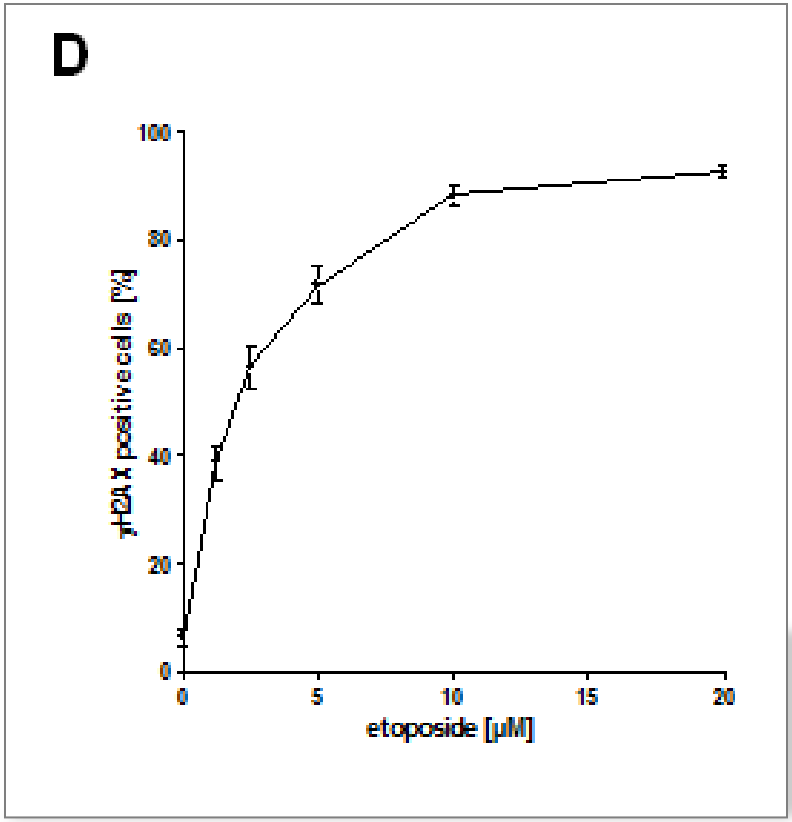
Automatic γH2AX foci analysis of PBMCs treated with indicated concentrations of etoposide (ETP) for 16 hours.



fluorescence intensity of etoposide treated cells.

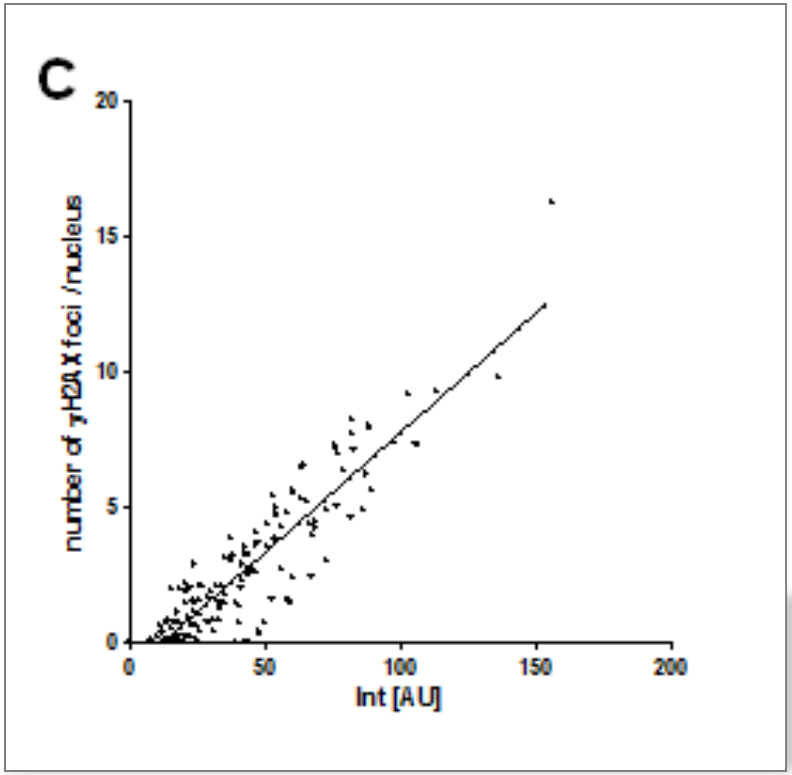
Results

Dose amount of γH2AX foci positive cells after treatment with different concentrations of ETP



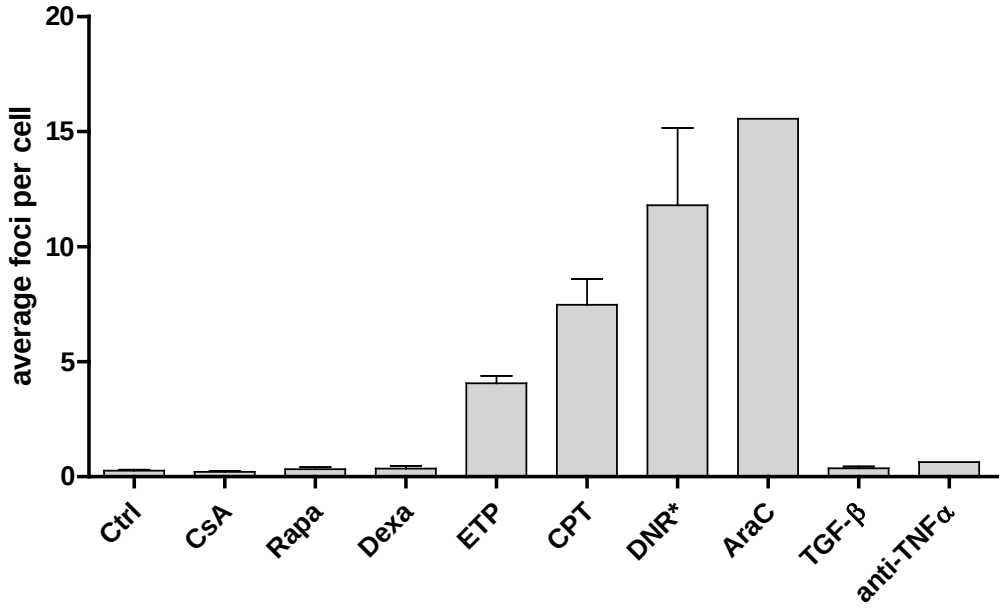
Results

Correlation between γH2AX foci number per cell and average cell fluorescence intensity..



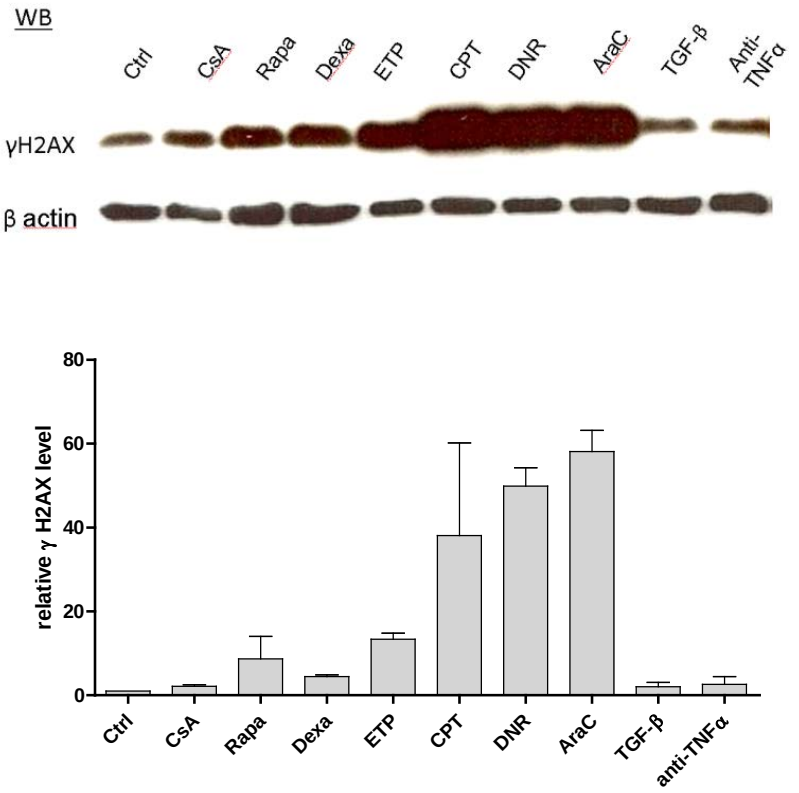
Results

Immunofluorescence staining and quantification of γH2AX foci



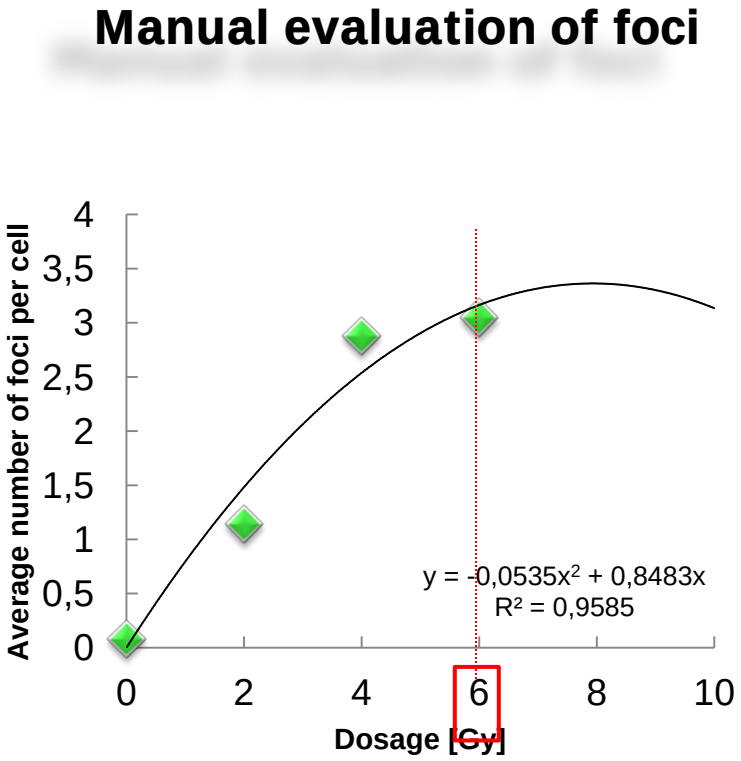
Induction of γH2AX foci in PBMCs after treatment with different immunosuppressive reagents. PBMCs were treated over night with either 10 µM cyclosporine A (CsA), rapamycin (Rapa), dexamethason (Dexa), etoposide (ETP), camptothecin (CPT), daunorubicin (DNR), cytarabin (AraC), 10 ng/ml active TGF-β1 or 100 µg/ml anti-TNF-α.

Results



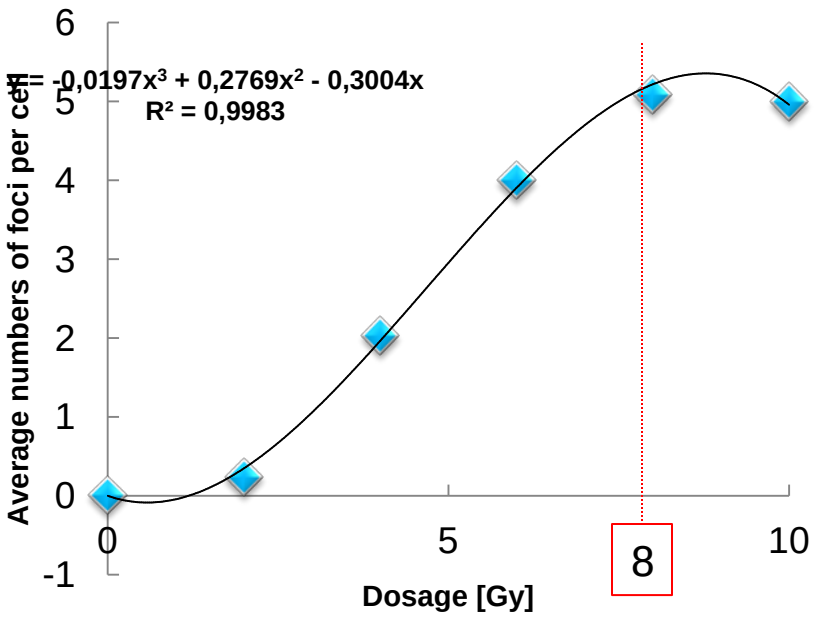
Determination of γH2AX level in PBMC lysates normalized to γH2AX level of untreated cells.

Results: Increased range of analysis



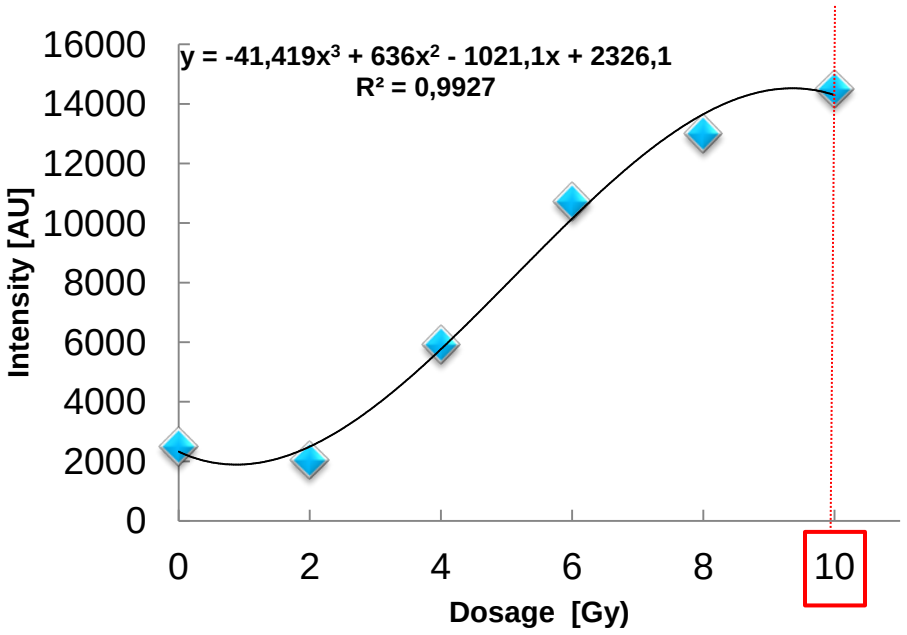
Results: Increased range of analysis

Automatic evaluation of foci



Results: Increased range of analysis

Automatic evaluation of foci



Conclusions

Advantages of automatic reading with AKLIDES®

Results include 9 different parameters, which indicate cell damage:

1. Number of cells counted
2. Average intensity per cell in [AU] = Total intensity
3. Average intensity of foci in [AU]
4. Average number of foci per cell

All results are given in Excel-format and PDF-format.

Advantages of automatic reading with AKLIDES®

5. Overall numbers of foci in all cells counted
6. Number of cells with foci/double-strand breaks
7. Percentage of cells with foci = total damage
8. Average diameter of cells
9. Average diameter of foci

All results are given in Excel-format and PDF-format.

Conclusions

Advantages of automatic reading with AKLIDES®

- Integrated dark room
- Analysis of a well takes 6 minutes with

  100 enumerated cells → 4 to 5 times faster analysis time
- Time-, staff-, and cost-efficient

Conclusions

Advantages of automatic reading with AKLIDES®

- Sample specific reports in pdf. and csv. Formats
- Archiving of results (data base)
- Standardized analysis of γH2AX foci in human lymphocytes, PCCL3, FaDu
- Colocalization with 53BP1 and other markers possible
- Adaptation available for all cells, cell lines and tissues

A high throughput analysis is now possible !

Outlook

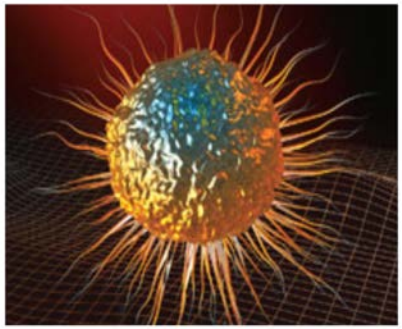
1 Peripheral Blood Mononuclear Cells PBMC‘s

Test for individual damage by radiation, e.g., stay in radioactive contaminated areas, flight personnel and other

2 Cell lines

Research : DNA repair, radio oncology, radiation biology

Clinical application: individual radiation therapy of cancer patients

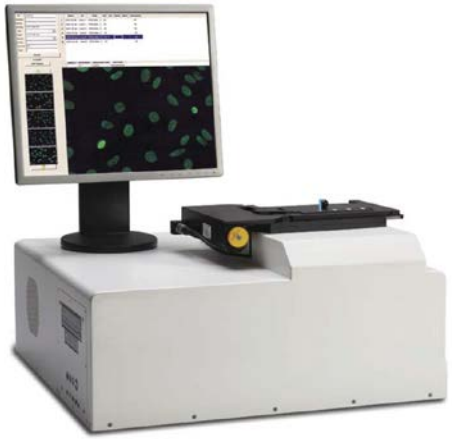


Outlook

Detection of genetic damage

- Biological dosage measurement for X-ray investigations
  - in vivo quantified by lymphocytes
  (Radiation sensitive people show more DSB)
- Tissue specific biomarker for the susceptibility of the DNA
  - basic research in knockout-mice
- Pharmacological research
  - screening of substances
- Aging research

KLIDES



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Thank you for your attention!

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