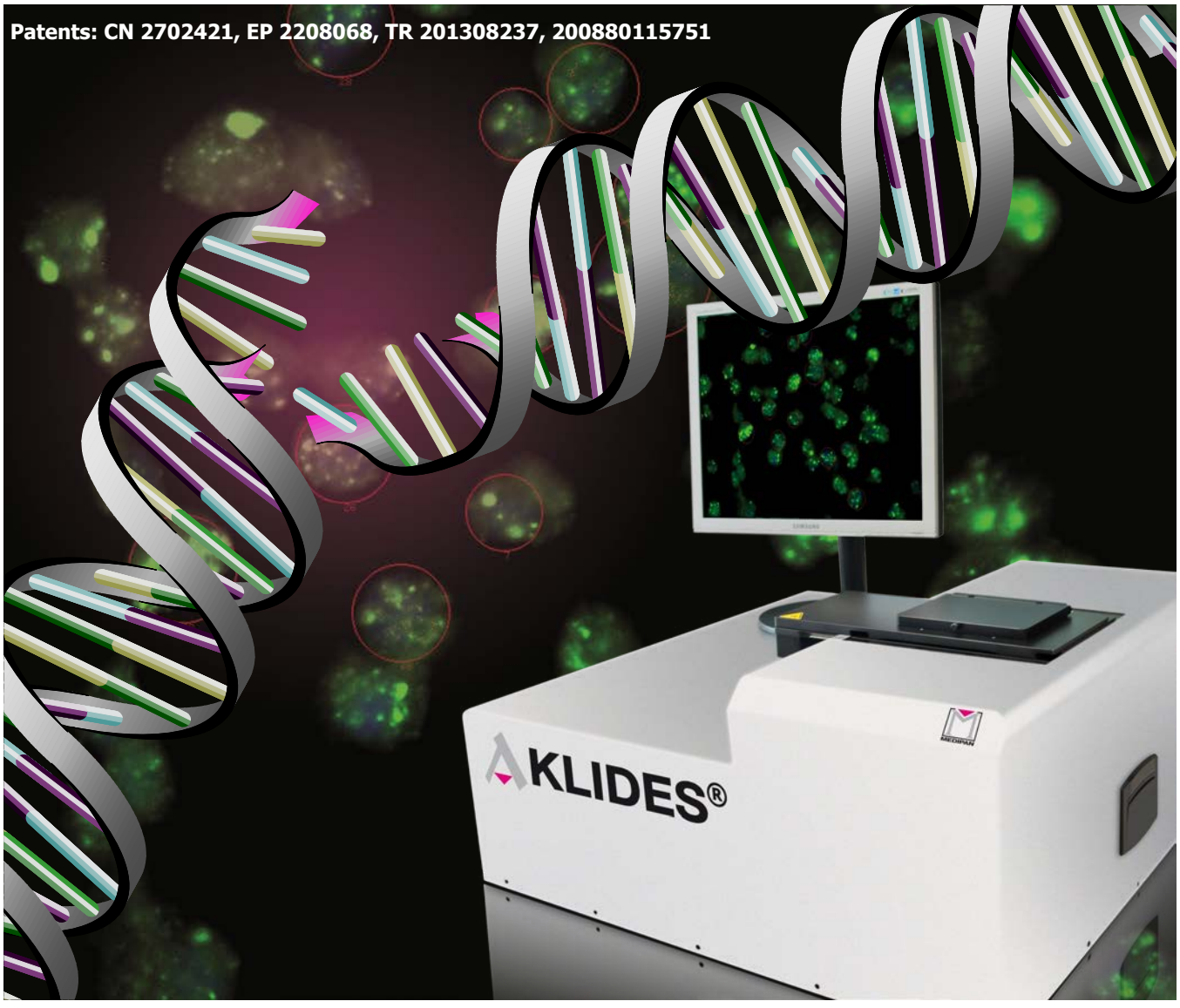
MEDIPAN

KLIDES®

Cell Damage

Standardized and automatic determination of DNA double strand breaks using immunofluorescence



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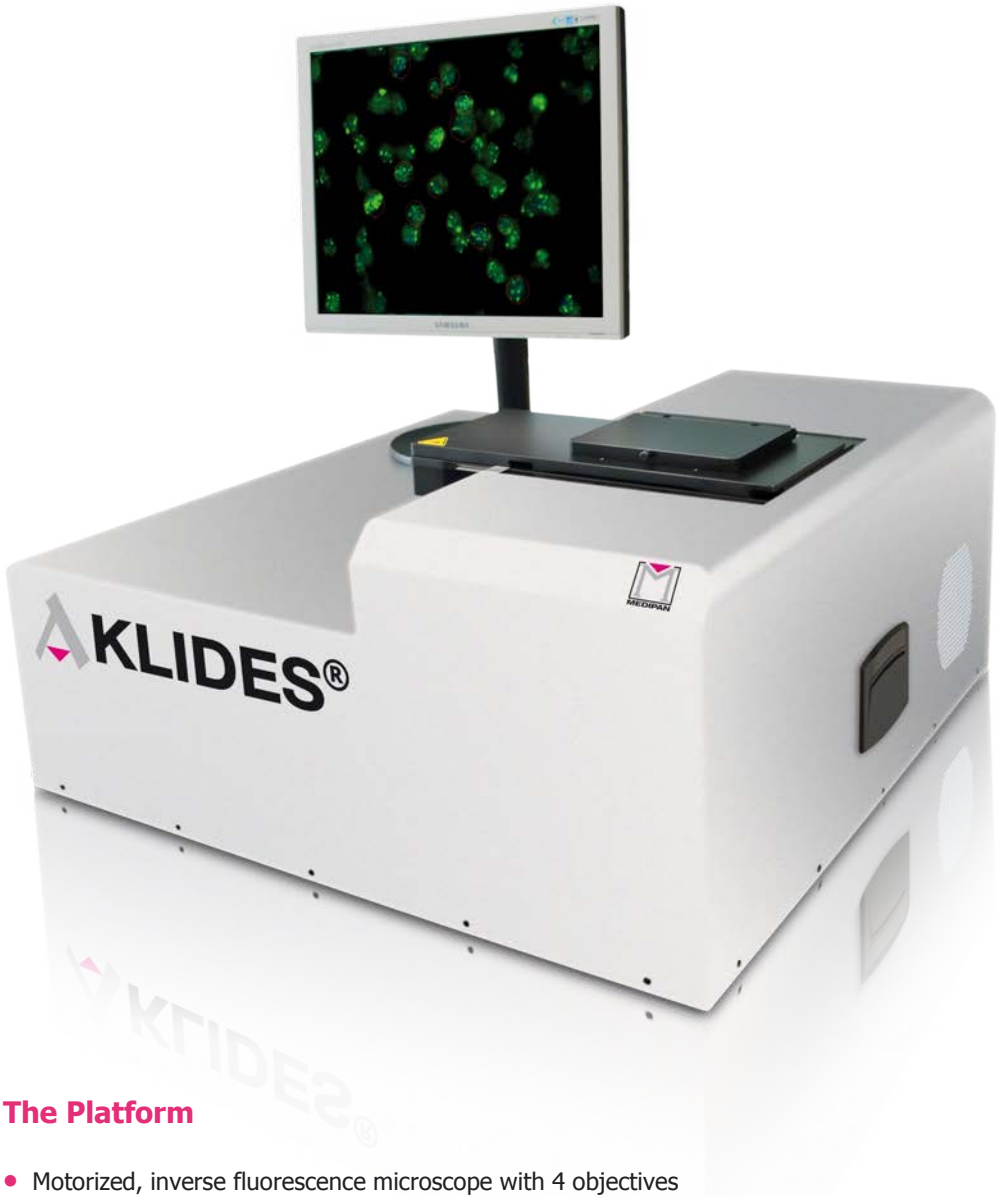

The Platform

- Motorized, inverse fluorescence microscope with 4 objectives
- Motorized x-y stage holding up to 5 slides
- LED light source (4 wavelength)
- CCD grey scale camera
- PC including AKLIDES® software

Applications for γ-H2AX as a biomarker

- Radiation biology / Biodosimetry
- Assessment of individual Sensitivity to Radio- and Chemotherapies
- Autoimmune and Age-related Diseases
- Environmental Genotoxicity/Drug Development
- Genome Integrity / DNA repair pathways

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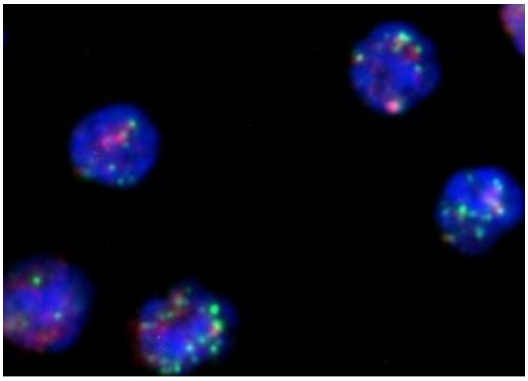

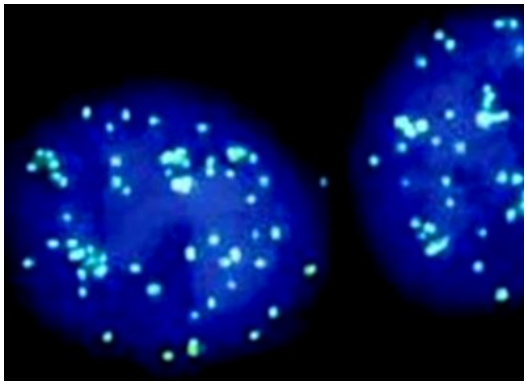

Biomarkers for Cellular Stress Response

Quantitative determination of DNA double-strand breaks (DSB) via immunofluorescence

- **Standardized and automatic** image analysis for the determination of DNA double-strand breaks
  - **for the first time** comparability of examination results possible
  - suitable for medical screening
- **Automatic** determination of foci with **different** fluorescent dyes
- **Combined** determination of foci with different dyes for the evaluation of DNA **repair mechanisms**
- **Modular** software for the analysis of various **cell lines** and human lymphocytes

Advantages automatic analysis

- Standardized and reliable results between different instruments due to intensity calibration of LED light source
- Standardized assay results due to novel microbead based reaction control
- Integrated dark chamber
- Detailed results via readout of various parameters
  - Number of cells measured
  - Number of cells with foci
  - Average number of foci per cell
  - Mean intensity of foci
  - Percentage of cells with foci = total cell damage
  - Average diameter of foci and cells
- Sample specific reports in pdf and xls format
- Database and archiving of results
- 8 minutes analysis/well with 100 cells counted
- 5 times faster analysis compared to manual reading
- Time, staff and cost efficient analysis

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Scientific background

DNA damage such as DNA double-strand breaks (DSBs) have been observed following exposure to ionizing radiation or mutagenic substances such as cytostatic chemicals. Cells are able to recognize this damage and repair it. This DNA repair is usually accurate and has no consequences for the cell. However, excessive DNA damage can lead to cell death or even oncogenic transformation of the cell. The knowledge of the formation and of the repair of DNA damage in particular are an important requirement to avoid pathological changes and to assure optimal application of ionizing radiation in medicine and technology.

DNA double-strand breaks are recognized very quickly and efficiently by the cell. The kinase ataxia telangiectasia mutated (ATM) is responsible for this. Serine 139 of the histone subunit H2AX is phosphorylated, and is then named γ-H2AX. The p53 binding protein 53BP1 binds the phosphorylated H2AX and regulates the repair of DNA double-strand breaks.

Specific antibodies detect γ-H2AX as well as 53BP1 and specific secondary fluorochrome labeled antibodies visualize each marker (figure 1). The resulting fluorescence signal can be visualized as a focus (pixel) under a fluorescent microscope. γ-H2AX as well as 53BP1 form foci which colocalize to a certain degree.

After exposure to radiation or genotoxic substances an increase in foci numbers depending on dosage can be seen (figure 2).

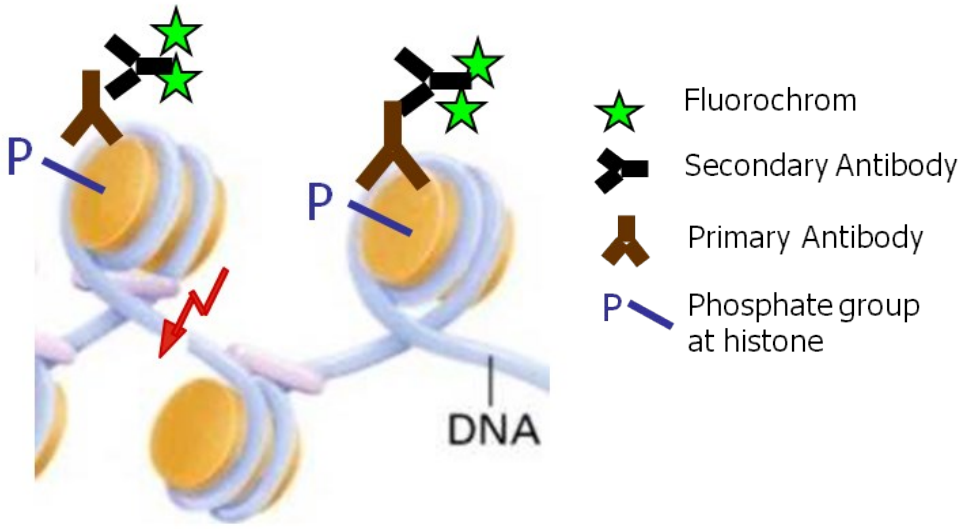

Figure 1:
Schematic illustration of DSB detection via immunofluorescence staining of γ-H2AX foci.

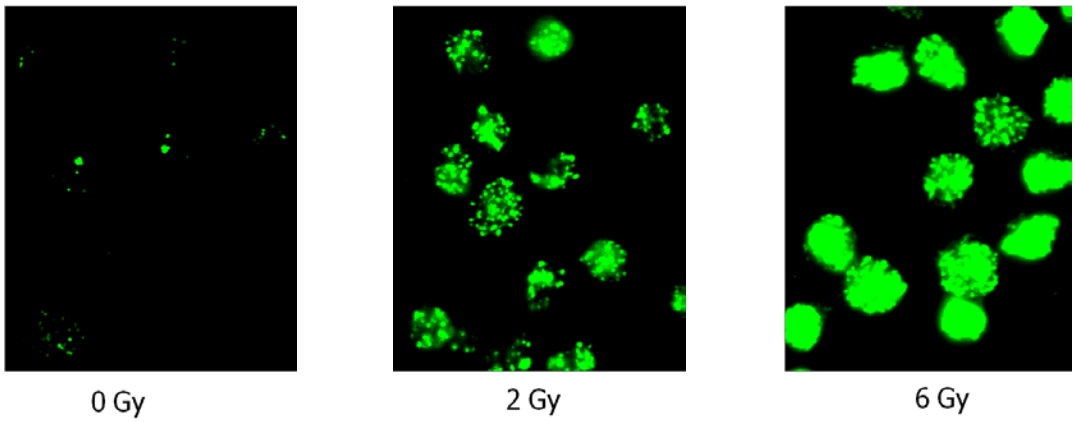

Figure 2:
Dose-response of γ-H2AX foci in human cells after irradiation (Runge et al. Int J Radiat Biol, May 2012; 88(5): 439-447)

Analysis of γ-H2AX foci is an established marker of DNA double-strand breaks and therefore cell damage, whereas DNA repair mechanisms can be evaluated using the fraction of p53 binding protein 53BP1. Combining the analysis of γ-H2AX with the determination of 53BP1 and analyzing their colocalization can be used to evaluate cellular repair mechanisms (figure 3). Using immunofluorescence to analyse these foci is a very sensitive method evaluating single cells as well as the localization of foci. These parameters can be measured fully automatically using the AKLIDES® Nuk Software.

Analyzing the kinetics of DNA damage and repair individual differences or repair defects can be detected. After exposure to irradiation the number of γ-H2AX foci show a steep increase, followed by a continuous decrease back to basal levels for complete repair (figure 4). Cells with impaired DNA repair show increased foci numbers even after 24 hours of recovery.

This approach can also be used to screen the genotoxicity of various substances.

Measuring DNA damage and DNA repair capacities of cells can be used as a diagnostic tool in various applications in basic research as well as clinical settings.

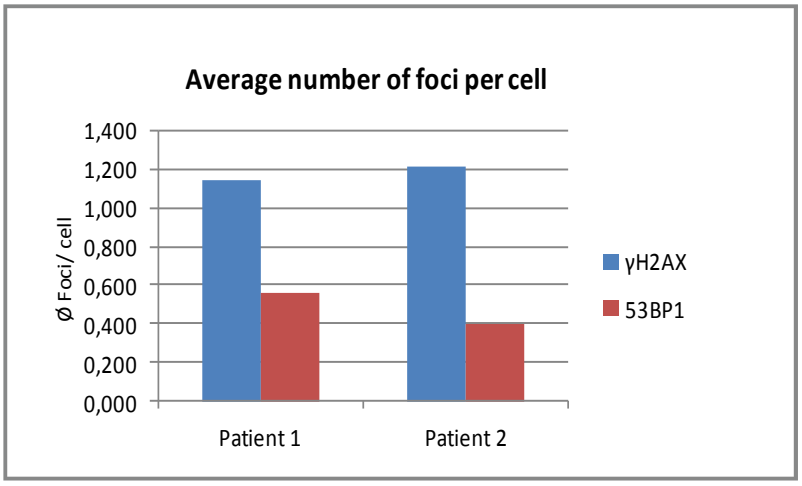

Figure 3:
Assessment of γ-H2AX and 53BP1 foci

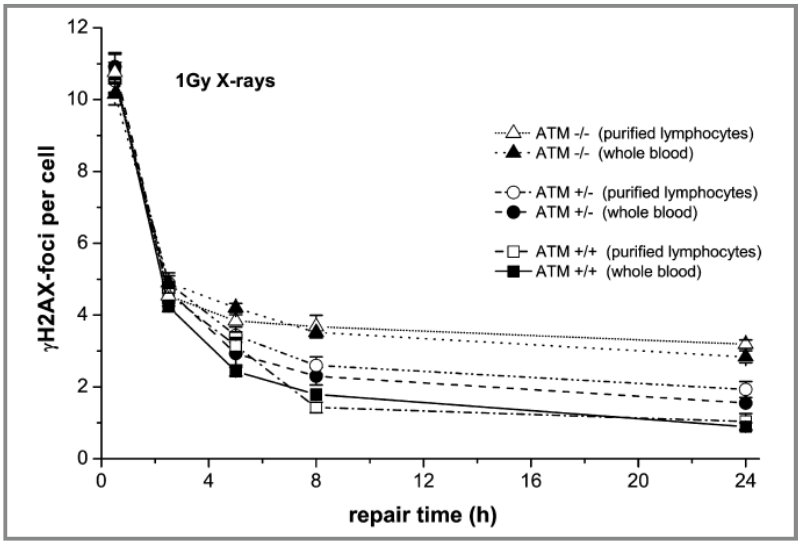

Figure 4:
Kinetics of γ-H2AX foci in cancer patients after irradiation with 1 Gy (Rübe et al., Int J Radiat Oncol Phys 2010 Oct 1; 78 (2): 359-69)

References

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(4) Valdiglesias V, Giunta S, Fenech M, Neri M, Bonassi S. γH2AX as a marker of DNA double strand breaks and genomic instability in human population studies. Mutat Res 2013 Jul;753(1):24-40.

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The software module provides fully automated measurement

Automatic focussing and cell identification are carried out with 60 times magnification in the DAPI channel (figure 5A).
The sensitive determination of the γ-H2AX fluorescence signal takes place in the green FITC channel and in several z-levels (figure 5B). The same applies for 53BP1 measured in the red APC channel.
For the **automatic analysis** the signals of DAPI, FITC and APC channels will be overlayed and the implemented algorithms generate a 3-dimensional matrix from the x, y and z coordinates of the detected foci.
Every focus will be described by detailed parameters like size and intensity.

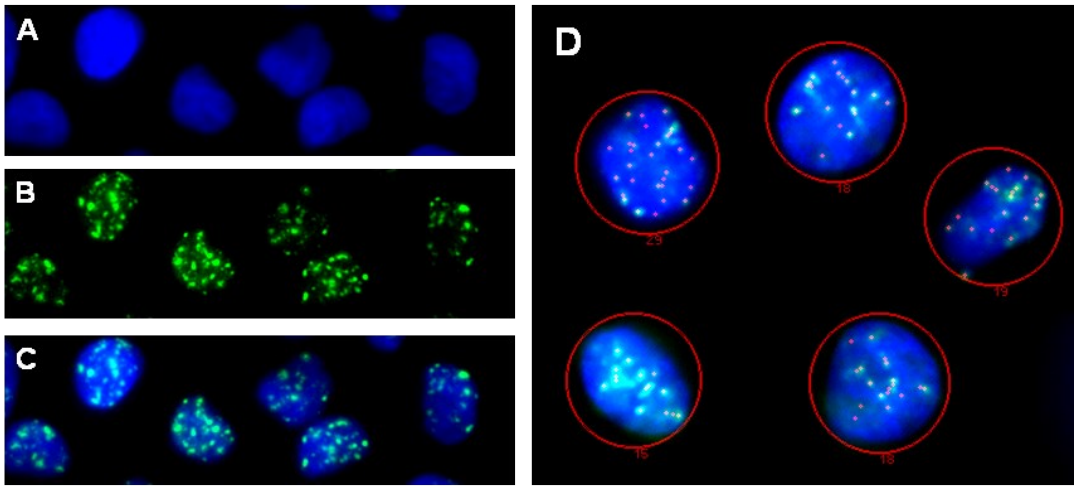


Figure 5:
Measurement of initial foci of PC Cl3 thyroid cells under radiation of 1 Gy ^{188}Re.

A: Focussing and cell identification in the DAPI channel.

B: Determination of fluorescence signal in the FITC channel.

C: Overlay of both fluorescence signals.

D: Analysis of foci parmeters (Runge et al., Int J Radiat Biol, May 2012; 88 (5): 439-447).

Increased foci determination range

The automatic analysis of foci using the AKLIDES® Nuk software increases the range of foci determination. A comparison of manual and automatic foci determination using PC Cl3 thyroid cells shows a limited measurement range from 6 Gy for the manual analysis (figure 6A). In contrast automatic reading expands the range up to 8 Gy (figure 6B) and it could be increased additionally up to 10 Gy when total intensity is considered (figure 6C).

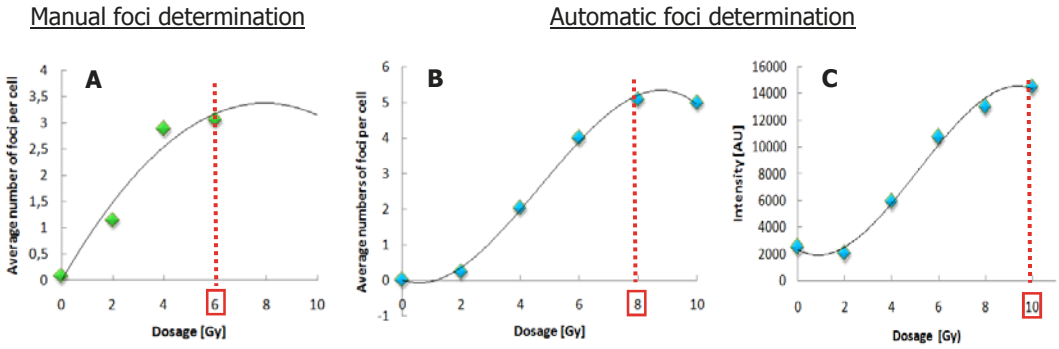
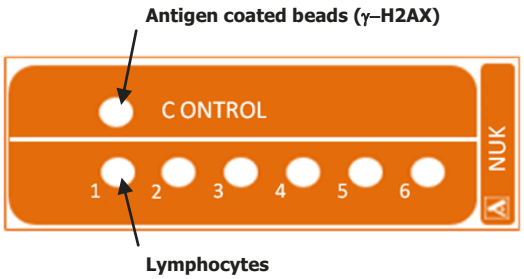


Figure 6: Dose-response curves of manual and automatic analysis of irradiated PC Pl3 cells.
A: Average number of foci per cell analyzed manually.
B: Average numbers of foci per cell analyzed automatically with AKLIDES®.
C: Total intensity of foci analyzed with AKLIDES®.

Standardized results due to integrated reaction control and intensity calibration of instruments



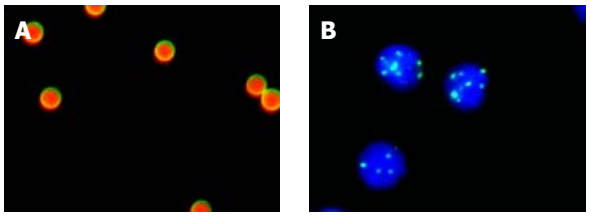
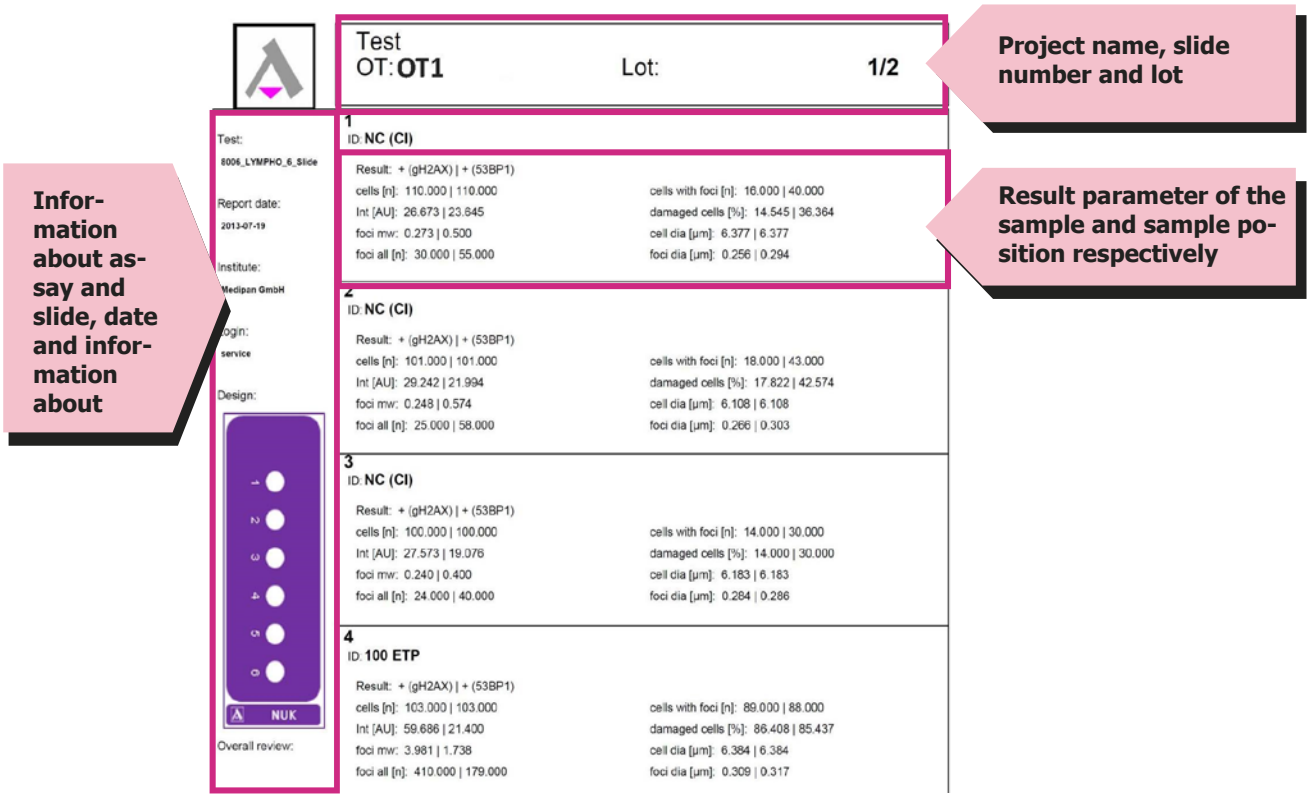


Figure 7: Differences between assays can be abolished using an integrated reaction control
A: Antigen coated microbeads
B: Irradiated lymphocytes

The immunostaining procedure of assay control and lymphocytes are performed on the same slide so that differences between preparations can be abolished. γ–H2AX coated microparticles serve as a reaction control producing intensities in a defined range. In combination with the calibration of the LED light source standardized and reliable results can be produced for this parameter.
This means that for the first time comparison of results from different studies from all over the world are possible which is an important step for the clinical evaluation of γ–H2AX.

Archiving and result report



The detailed result report gives all the information necessary for the archiving and the construction of a result database.

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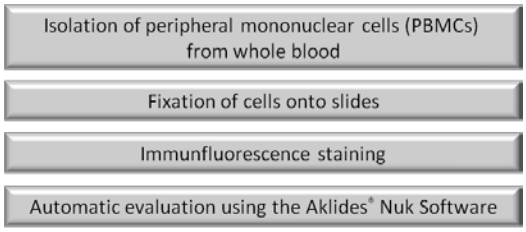
Overview kits

AKLIDES®Nuk* Human Lymphocyte Complete **Order code 4162**
- contains all reagents to detect γ-H2AX foci
- 10 slides for 30 duplicates or 60 single determinations

AKLIDES®Nuk* Human Lymphocyte Complete Combi **Order code 4268**
- contains all reagents to detect γ-H2AX and 53BP1 foci simultaneously
- 10 slides for 30 duplicates or 60 single determinations

AKLIDES®Nuk* Human Lymphocyte Isolation **Order code 4278**
- contains all reagents to isolate PBMCs from whole blood
- 10 slides for 30 duplicates or 60 single determinations

The assays above use a standardized protocol for lymphocyte isolation and staining



⇒ One technician can process 5 patient samples within 8 hours

Storage of lymphocytes

- A minimum of 3 ml venous blood taken in 6 ml EDTA tubes should be processed immediately
- It is possible to store isolated lymphocytes as follows:
  - Fixation of lymphocytes on slides and storage at 4 °C
  - Freezing of lymphocytes in specific media and storage in liquid nitrogen

Determination Service

Isolated lymphocytes can be stored fixed onto slides and then send to the MEDIPAN GMBH for analysis.

* Nuk = nuclear cell damage

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