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Fluorescence microscopes

หน่วยพิษวิทยาทางอาหารและโภชนาการ 2

ชั้น 3 ห้อง 347

โทรศัพท์ 326, 328

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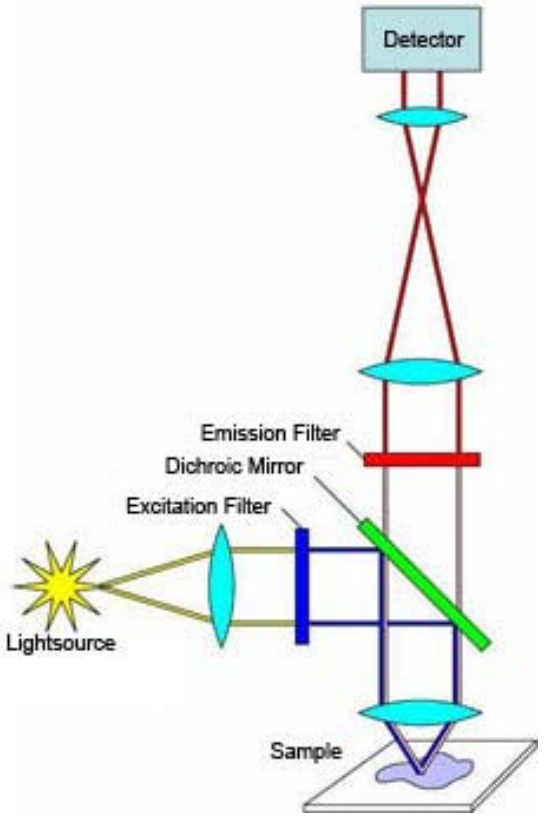
Fluorescence microscopes

- แหล่งกำเนิดแสง: UV, Violet หรือ Blue light
- ตัวอย่างย้อมด้วยสี Fluorochromes เช่น Immunophenotyping (Alexa), Nucleic Acid (Acridine Orange), Cell (DiO), Proteins (DsRed)
- กล้อง fluorescence (Nikon รุ่น Ci-L) พร้อมกล้องถ่ายภาพ

- หลักการทำงาน



Peripheral Blood Micronucleus Assay

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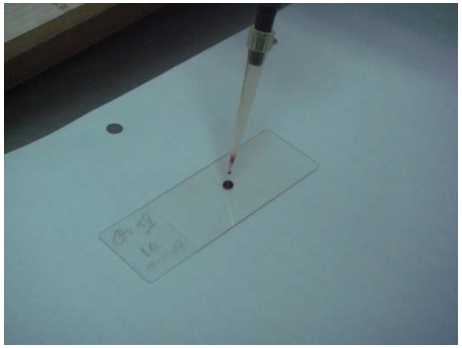
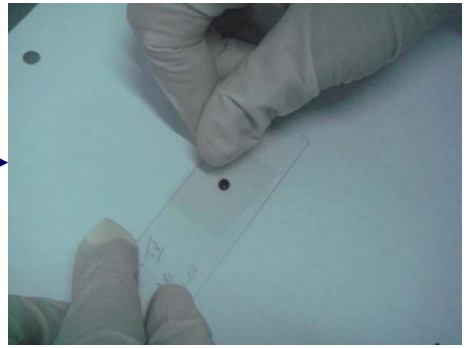
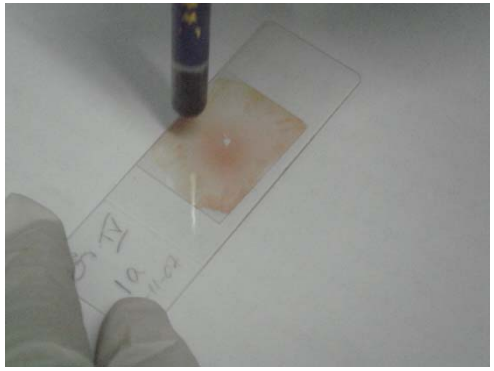
Acridine orange supravital staining method (developed by Hayashi *et al.*, 1990)





Blood withdrawal at orbital plexus

blood smears prepared on the AO coated microscopic slides



Peripheral blood smear

fluorescent microscope using a combination of a blue excitation and a yellow-to-orange barrier filter (40× objective)

Peripheral Blood Micronucleus Assay

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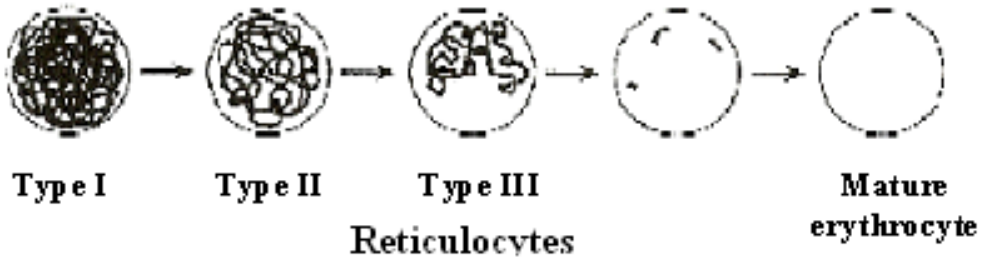
Reticulocytes can be classified into 4 groups represent as type I, II, III, and IV according to the degree of maturation corresponding to the amount of red fluorescing reticulum. Type I, II, and III reticulocytes are scored for the mouse.



The schematic presentation of the red fluorescing reticulum structure in each type of reticulocyte (From Hayashi M and Sofuni T, 1994).

Peripheral Blood Micronucleus Assay

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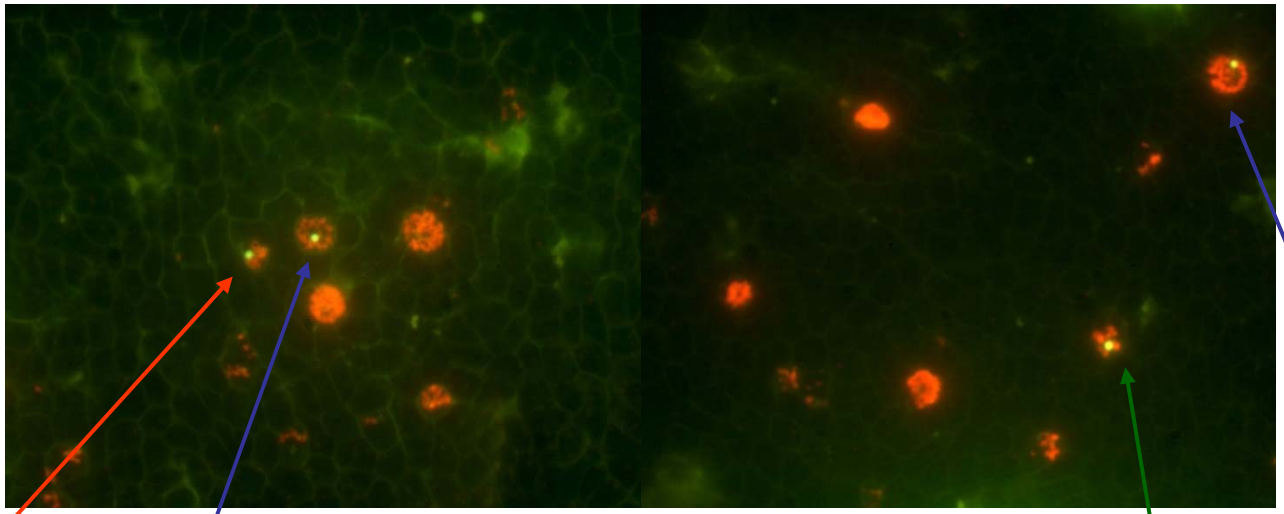

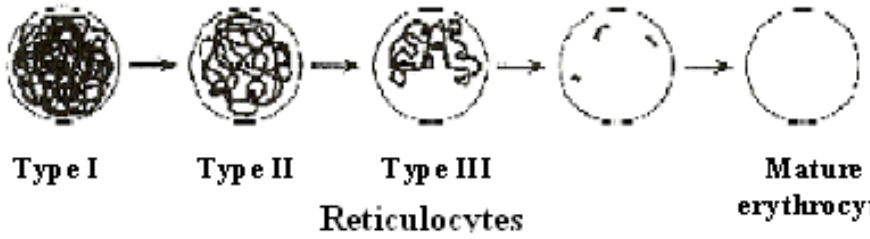

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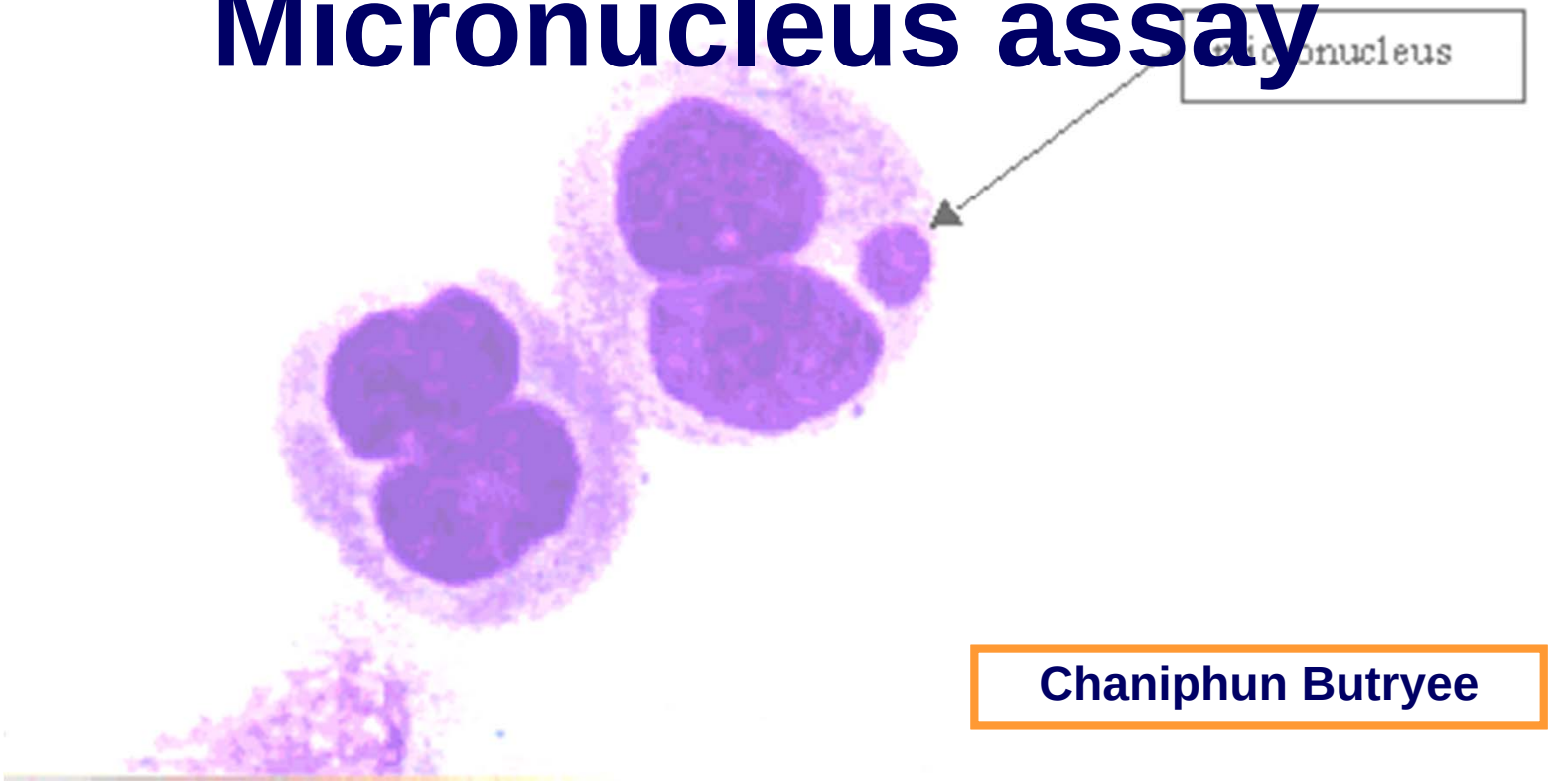
Micronucleus assay



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How Cytochalasin B works in CBMN cytome assay

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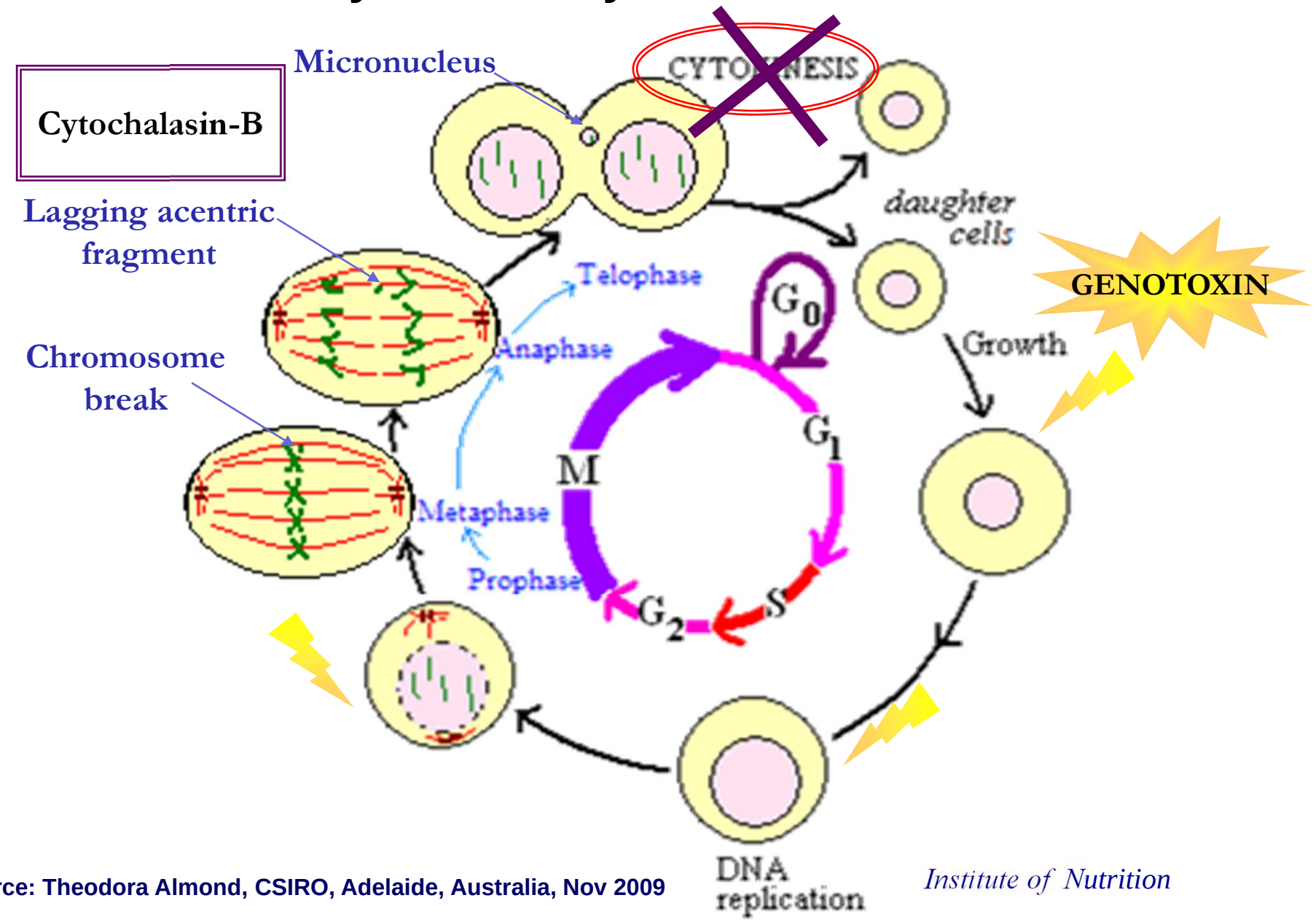

Source: Theodora Almond, CSIRO, Adelaide, Australia, Nov 2009

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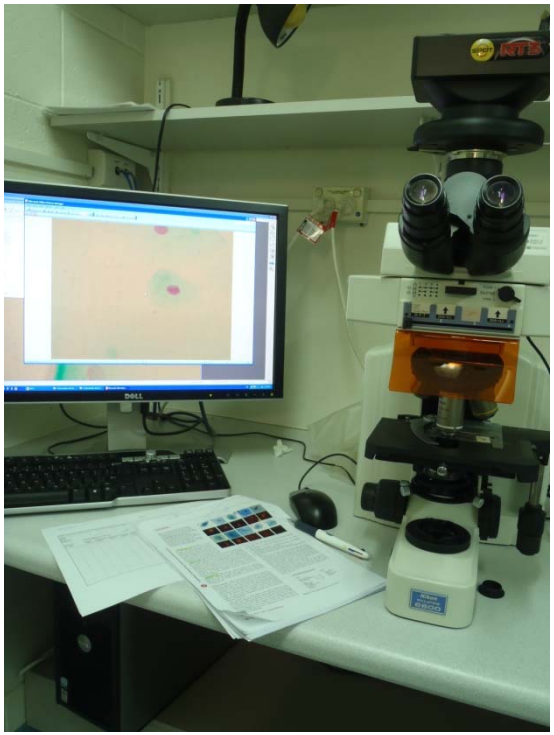

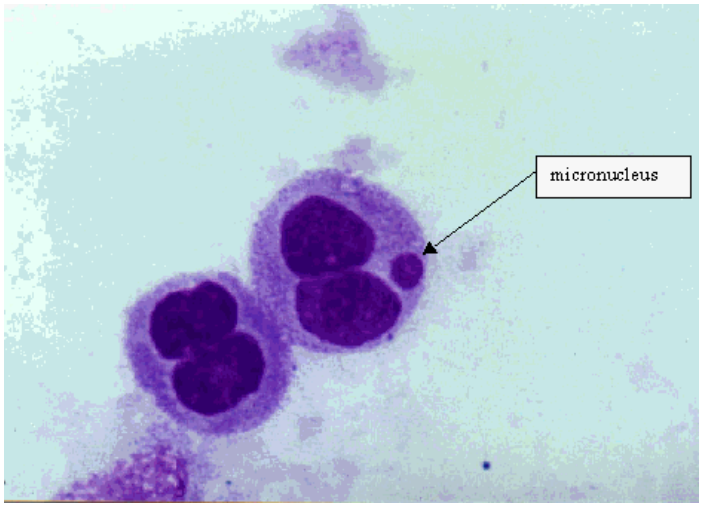

Scoring of micronuclei on different cell types: relevant for human biomonitoring

- **Lymphocytes, fibroblasts and exfoliated epithelial cells, without an extra *in vitro* cultivation step**

Overview of Scoring

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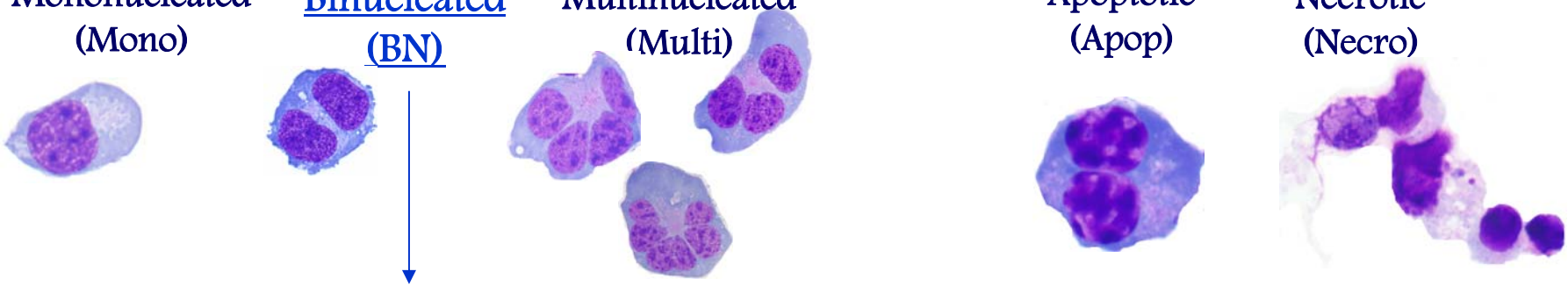
$$\text{NDI} = \frac{(\text{MONO}) + 2(\text{BN}) + 4(\text{MULTI})}{\text{Total no. of MONO, BN and MULTI cells scored}}$$

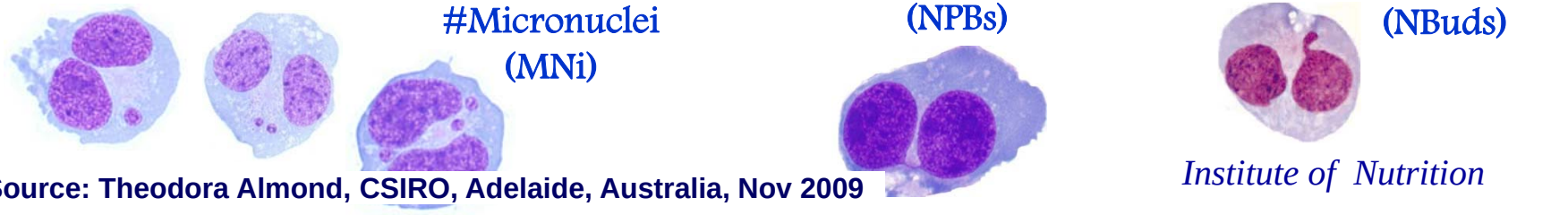
Score at least 150-250 cells

- Viable Cells
  - Mononucleated (Mono)
  - Binucleated (BN)
  - Multinucleated (Multi)
- Non-Viable Cells
  - Apoptotic (Apop)
  - Necrotic (Necro)



Genome Damage (Score 1000 BN cells)

- #BN cells with Micronuclei (MNi)
  - #Micronuclei (MNi)
- #BN cells with Nucleoplasmic Bridges (NPBs)
- # BN cells with Nuclear Buds (NBuds)



Source: Theodora Almond, CSIRO, Adelaide, Australia, Nov 2009

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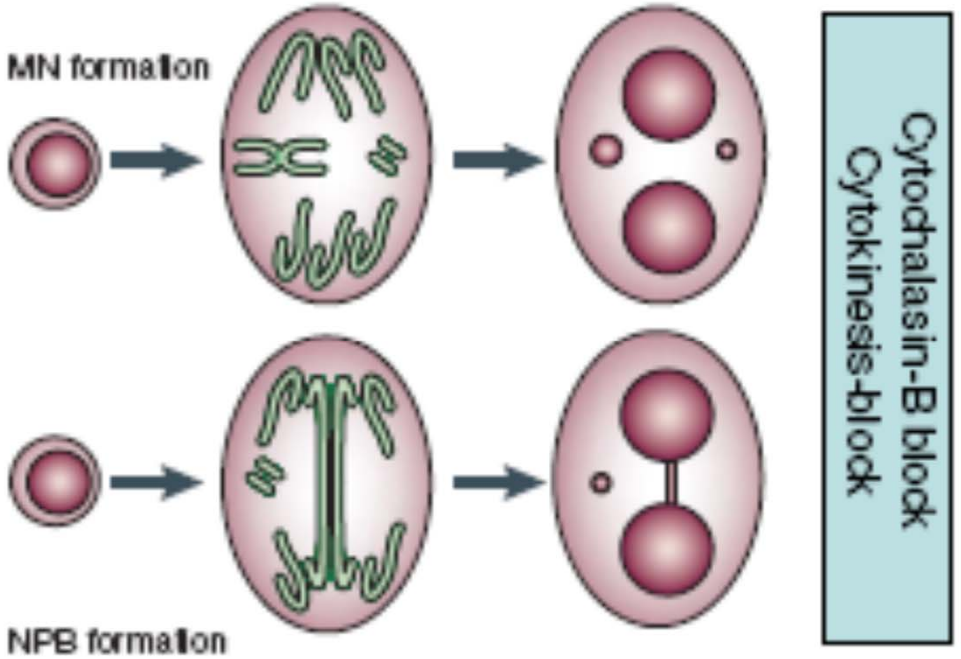
PROTOCOL

Cytokinesis-block micronucleus cytome assay

Michael Fenech

Genome Health Nutrigenomics Laboratory, CSIRO Human Nutrition, Food Science Australia, PO Box 10041, Adelaide 5000, South Australia, Australia. Correspondence should be addressed to M.F. (michael.fenech@csiro.au).

Published online 3 May 2007; doi:10.1038/nprot.2007.77



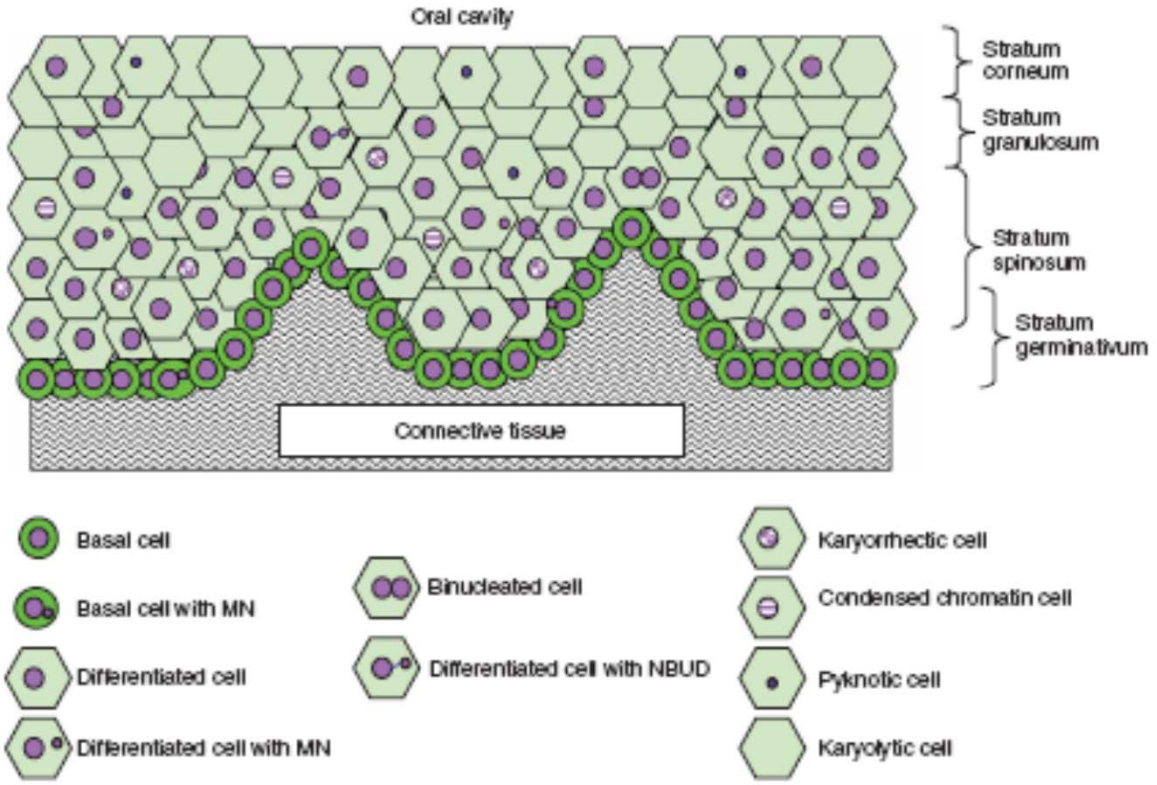
Source: Nature protocol/ vol. 2 no. 5/ 2007

Buccal micronucleus cytome assay

Philip Thomas[1], Nina Holland[2], Claudia Bolognesi[3], Micheline Kirsch-Volders[4], Stefano Bonassi[5], Errol Zeiger[6], Siegfried Knasmueller[7] & Michael Fenech[1]

[1]CSIRO Human Nutrition, Adelaide, South Australia. [2]School of Public Health, University of California, Berkeley, CA, USA. [3]Unit of Environmental Carcinogenesis, National Cancer Research Institute, Genoa, Italy. [4]Laboratory for Cell Genetics, Vrije Universiteit, Brussels, Belgium. [5]Unit of Molecular Epidemiology, National Cancer Research Institute, Genoa, Italy. [6]Errol Zeiger Consulting, Chapel Hill, NC, USA. [7]Institute of Cancer Research, Inner Medicine I, Medical University Vienna, Austria. Correspondence should be addressed to P.T. (philip.thomas@csiro.au) or M.F. (michael.fenech@csiro.au).

Published online 7 May 2009; doi:10.1038/nprot.2009.53



Source: Nature protocol/ vol. 4 no. 4/2009

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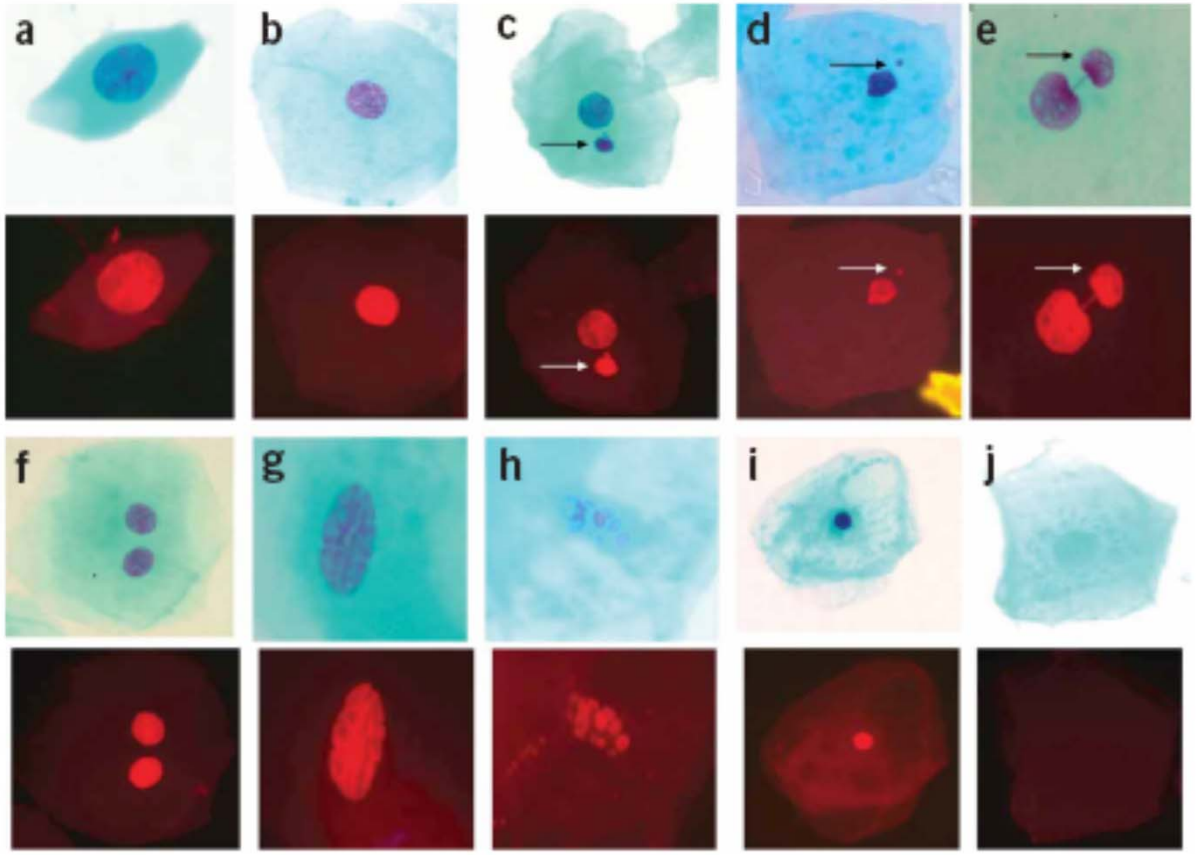

Figure 5 | Images of the different cell types stained using Feulgen and Light Green scored in the BMCyt assay viewed by transmitted light or under fluorescence with a far red filter; (a) basal cell; (b) differentiated cell; (c) early differentiated cell with micronucleus (arrow); (d) late differentiated cell with micronucleus (arrow); (e) differentiated cell with nuclear bud (arrow); (f) binucleated cell; (g) condensed chromatin cell; (h) karyorrhectic cell; (i) pyknotic cell; (j) Karyolytic cell. Upper panels light microscopy, lower panels fluorescence microscopy. All images were taken at ×1,000 magnification.

Source: Nature protocol/ vol. 4 no. 4/2009

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Lymphocyte micronucleus and buccal micronucleus cytome assay training at Genome Health Nutrigenomics Laboratory, CSIRO Human Nutrition, Adelaide, South Australia

11-22 April 2011

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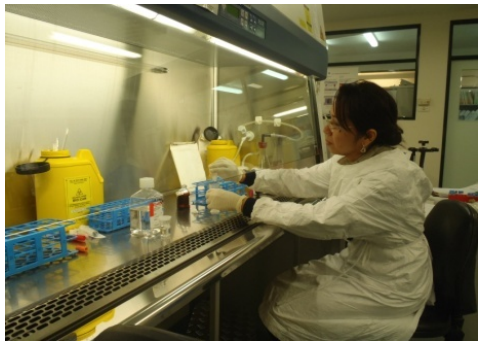

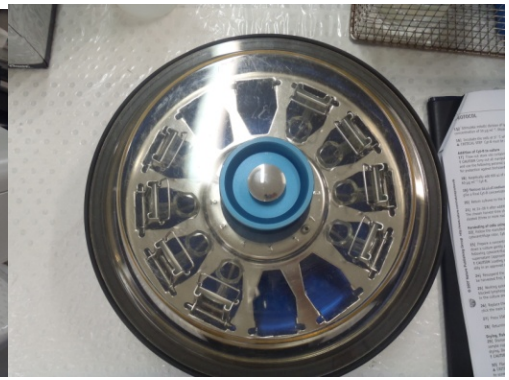

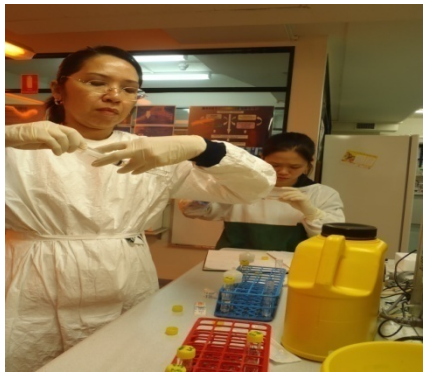

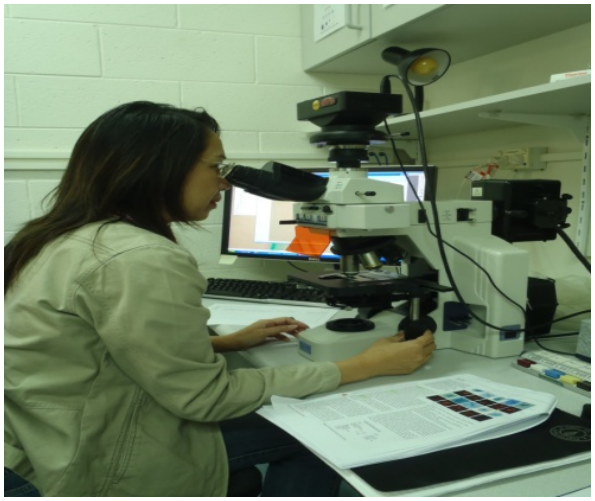

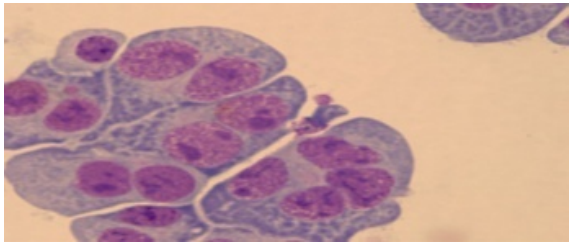

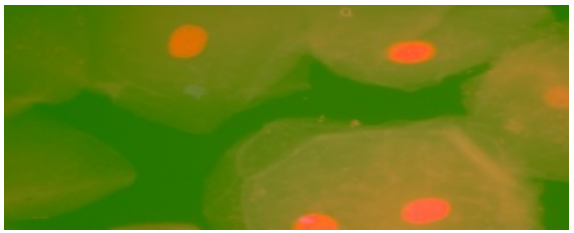

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Project on Use of micronucleus buccal cells as biomonitor of exposure to environmental toxic substance in Map Ta Phut industrial complex

- **Folate, vitamin B12, homocysteine status and buccal micronucleus frequency in Map Ta Phut Industrial Estate population**

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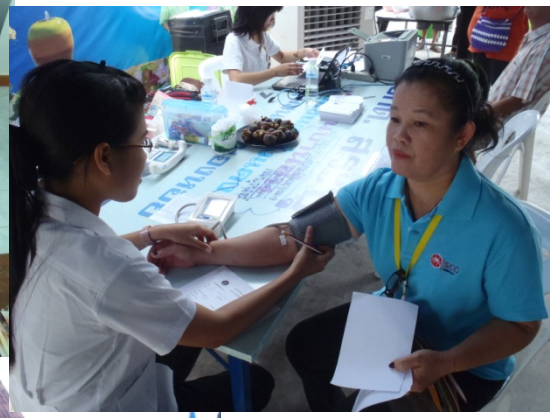
Project on Use of micronucleus buccal cells as biomonitor of exposure to environmental toxic substance in Map Ta Phut industrial complex

- Folate, vitamin B12, homocysteine status and buccal micronucleus frequency in Map Ta Phut Industrial Estate population



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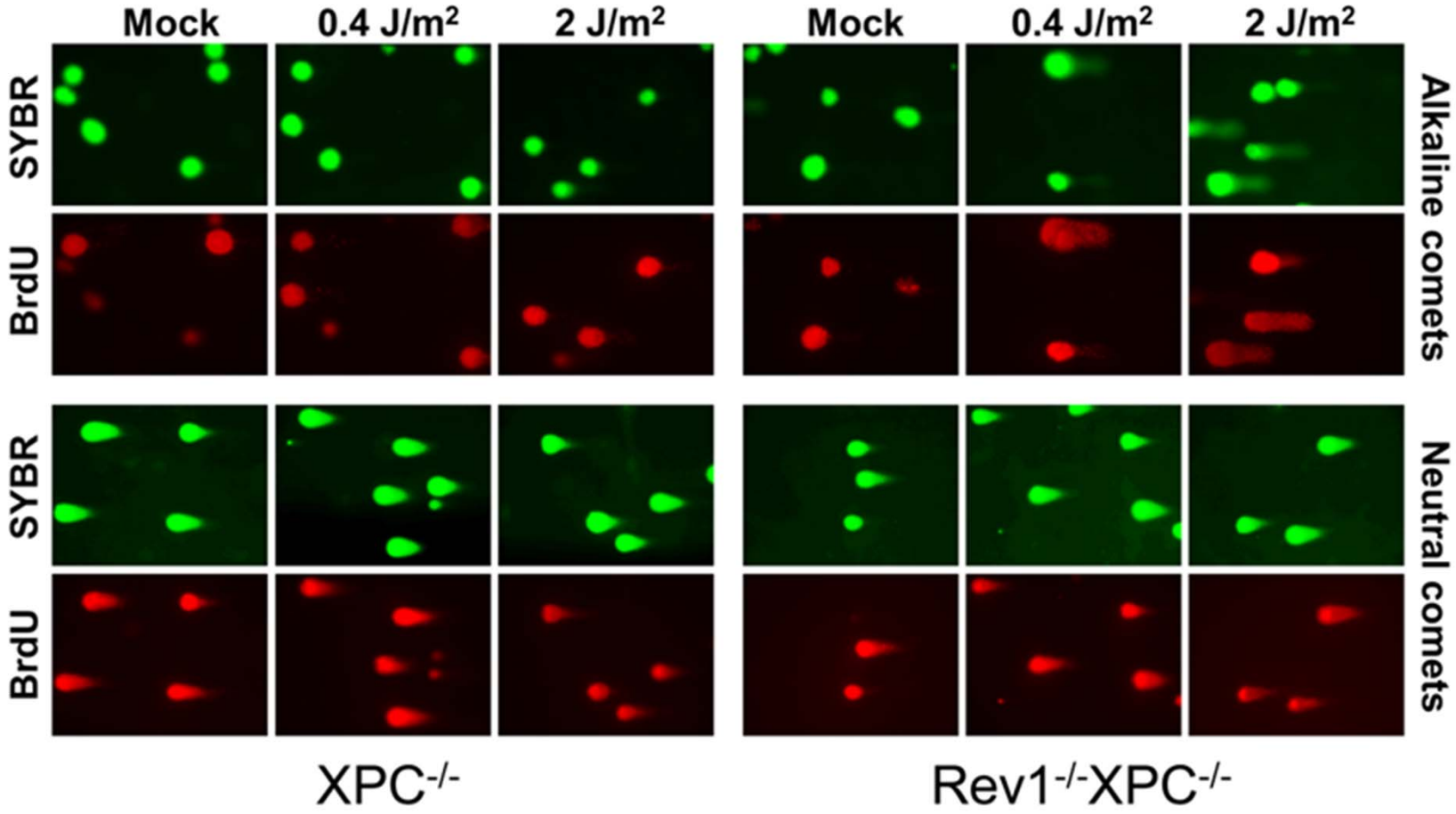
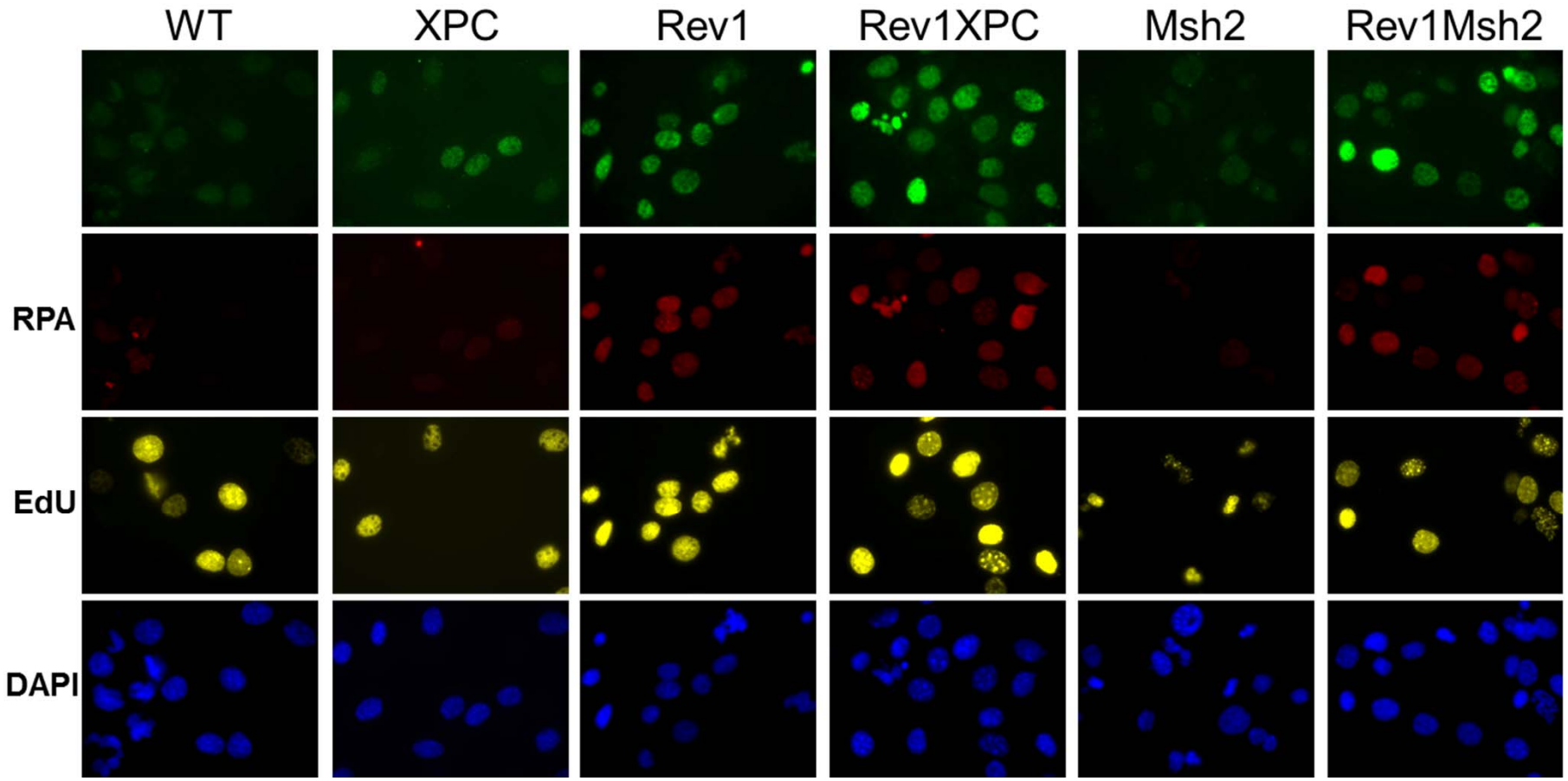
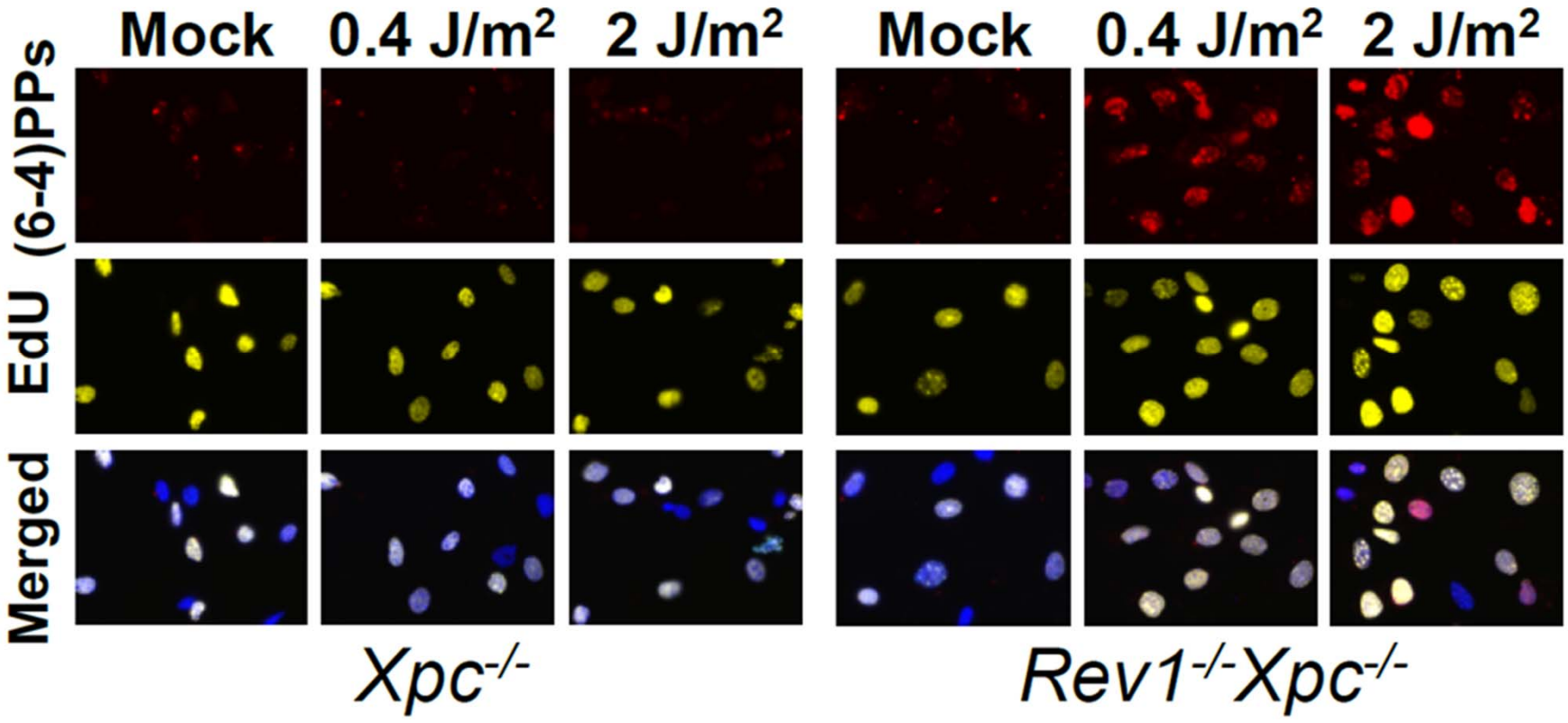
Comet assay


Mock
0.4 J/m²
2 J/m²
Mock
0.4 J/m²
2 J/m²
SYBR
BrdU
SYBR
BrdU
Alkaline comets
Neutral comets
XPC-/-
Rev1-/-XPC-/-

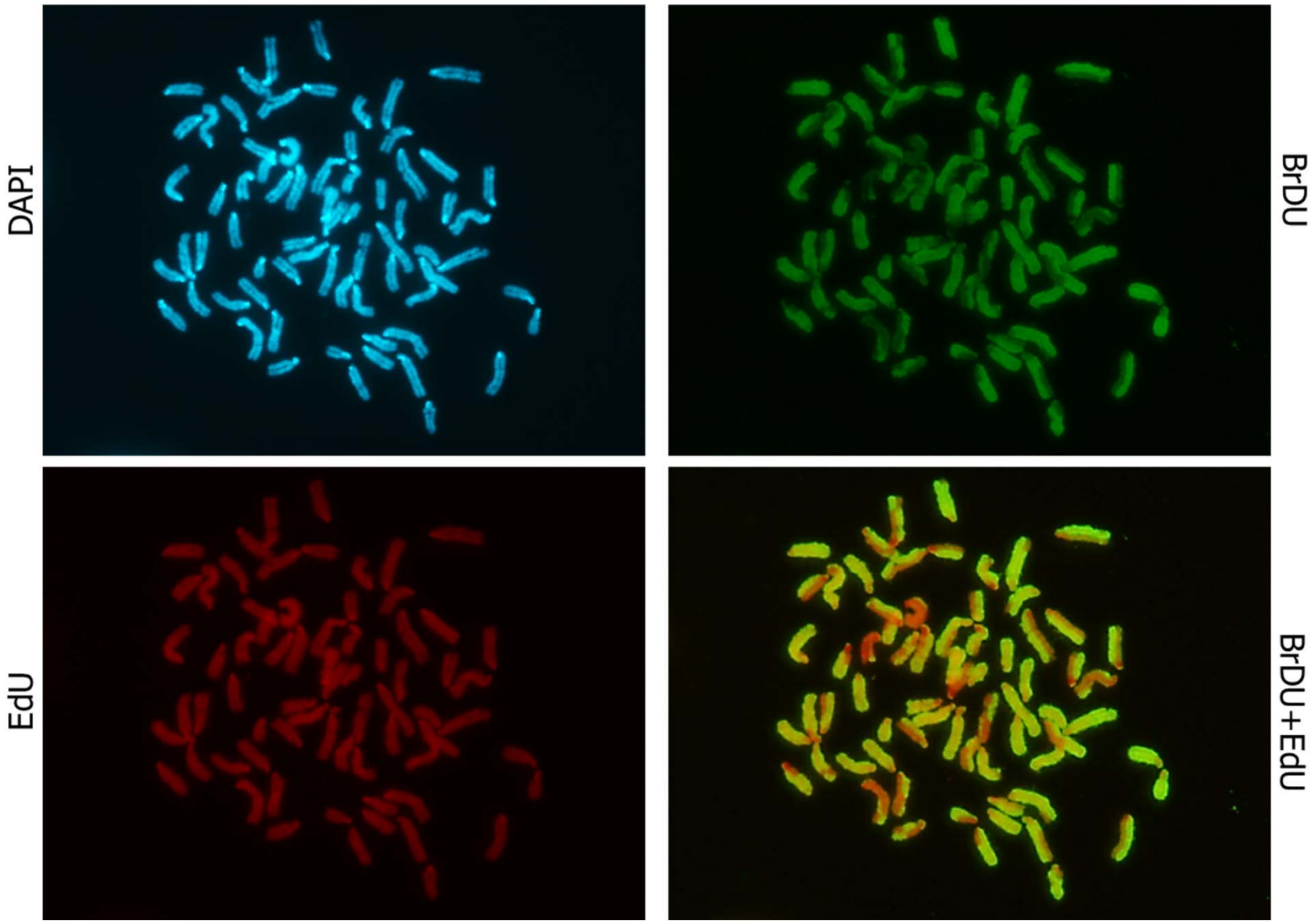
Immunostaining for protein detection



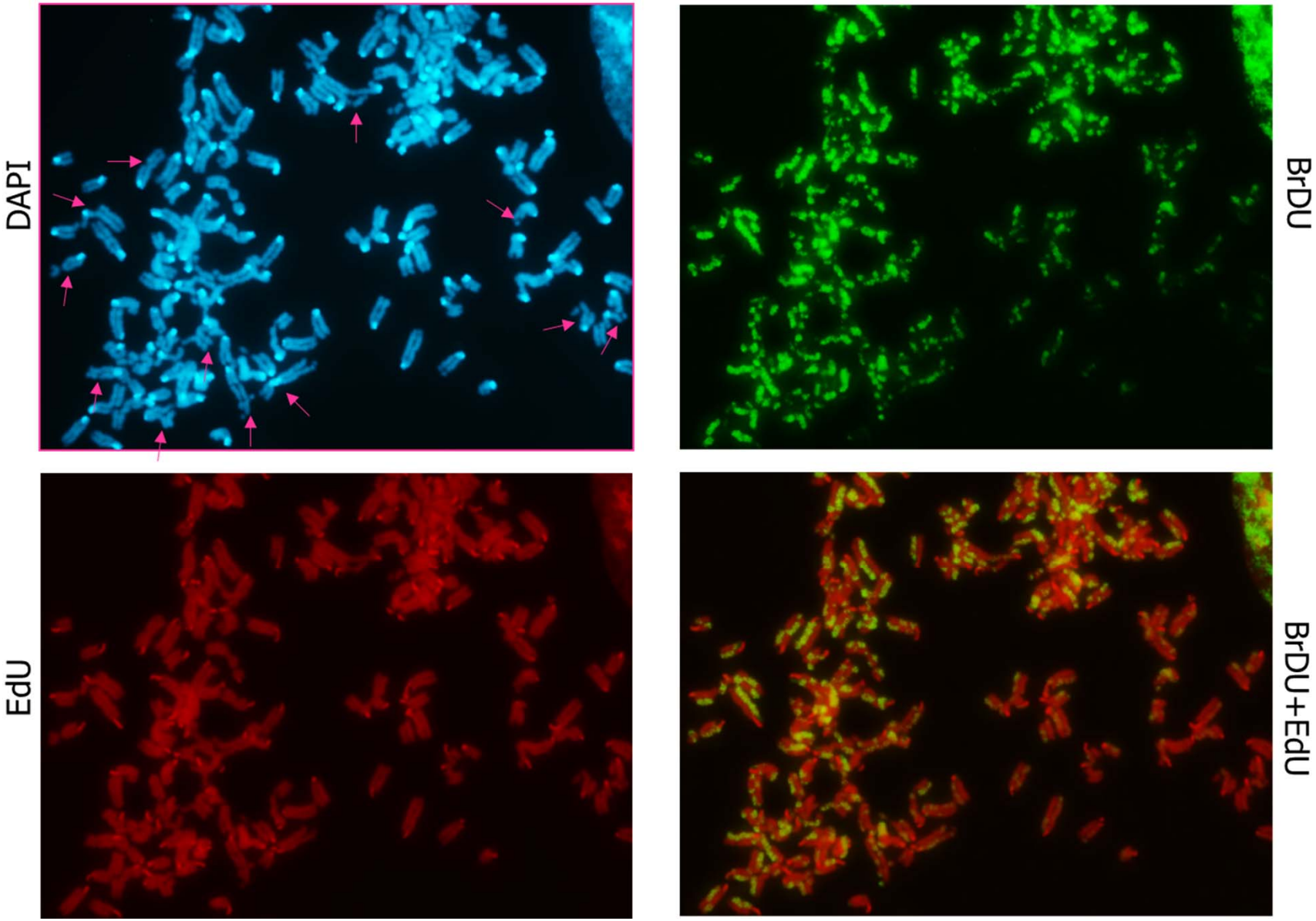
Immunostaining for probing DNA damages



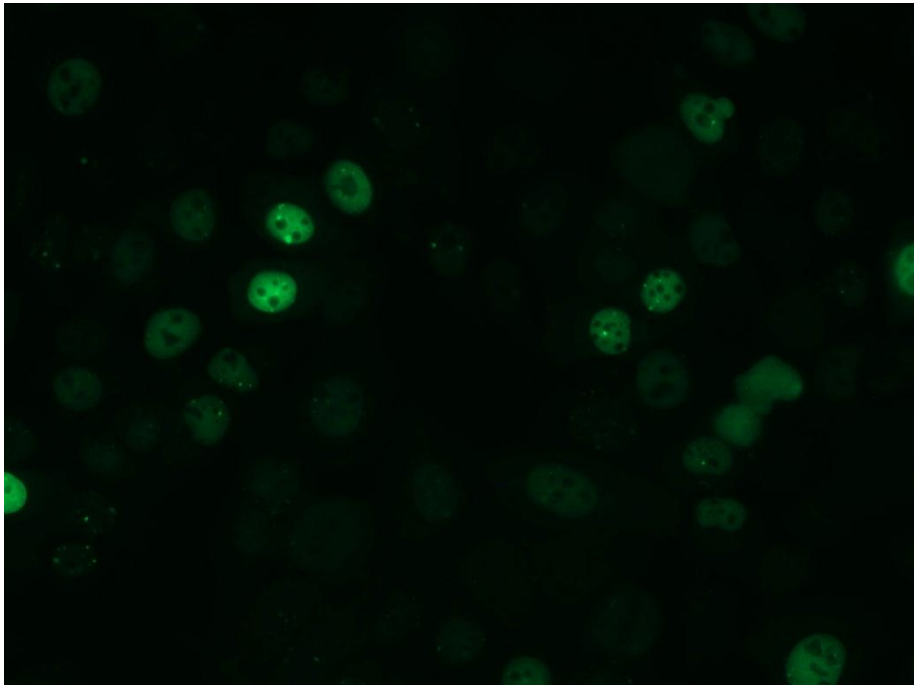
Chromosomal aberration ; XPC$^{-/-}$ 0.4J



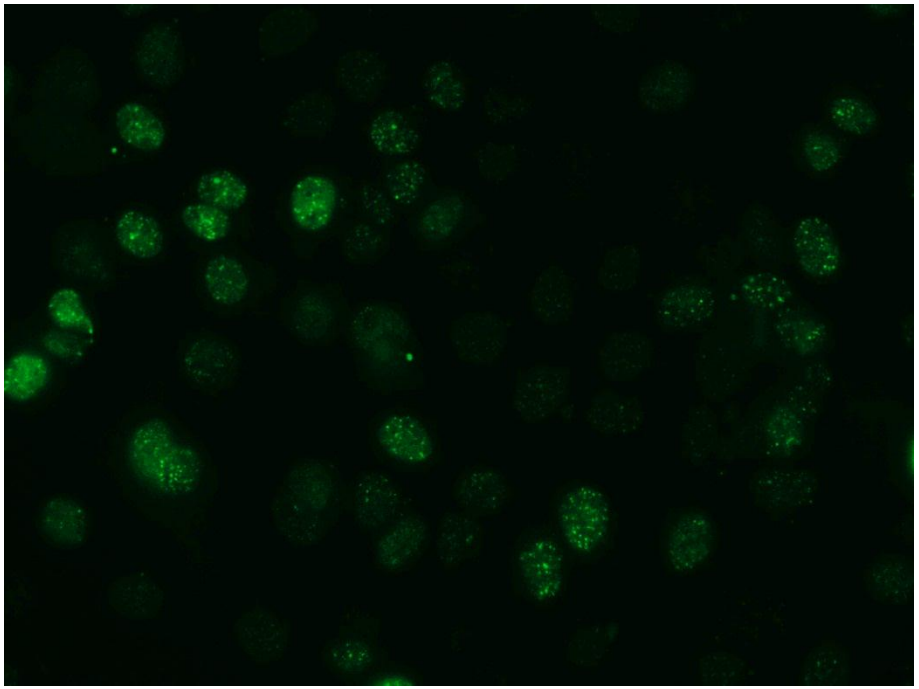
Chromosomal aberration ; XPC$^{-/-}$ 5J



U2OS untreated, transfected with YFP-53BP1



U2OS + 10 Gy, transfected with YFP-53BP1



Live cell imaging: Time-Lapse