Radiobiology in vitro

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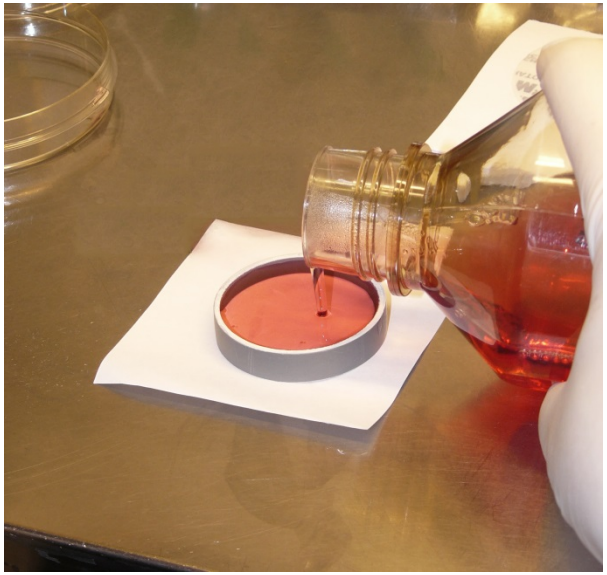
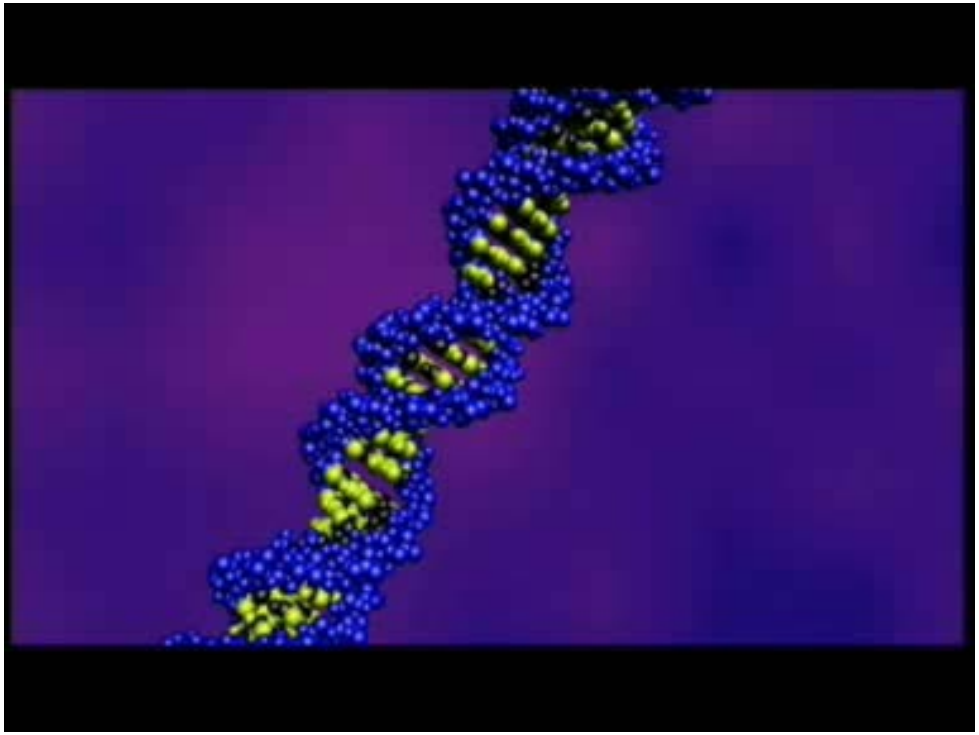
IFD-UW

- **Radiobiology** is the study of the action of ionizing radiation on living things
- **in vitro** experiments include work that uses culture cells





Team

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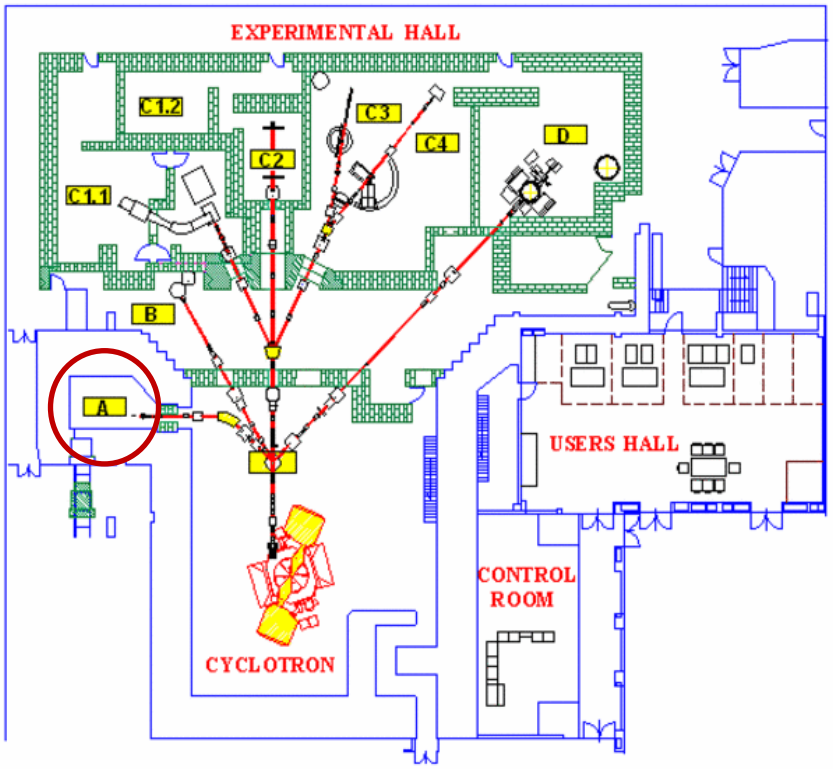
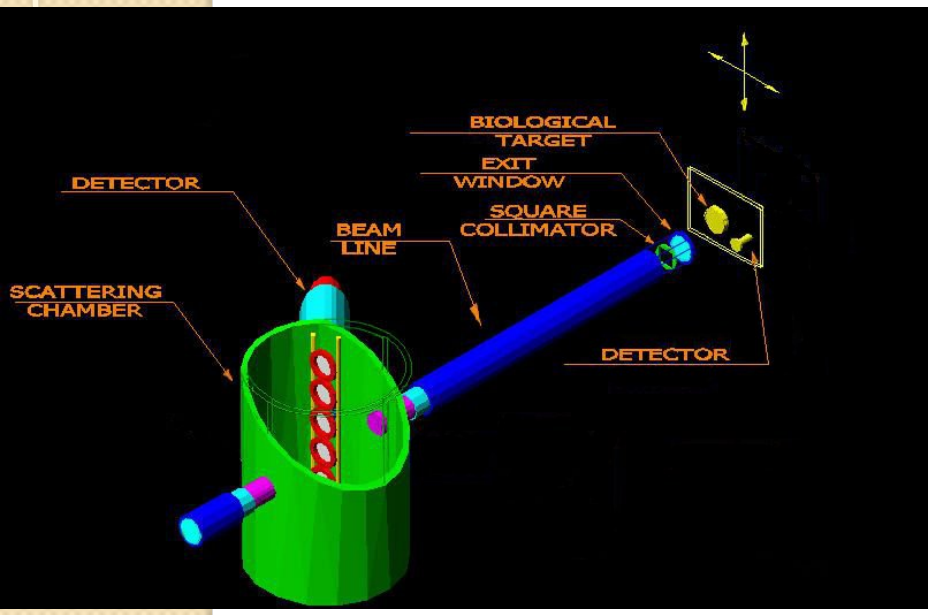
Institute of Nuclear Chemistry and Technology, Warsaw, Poland

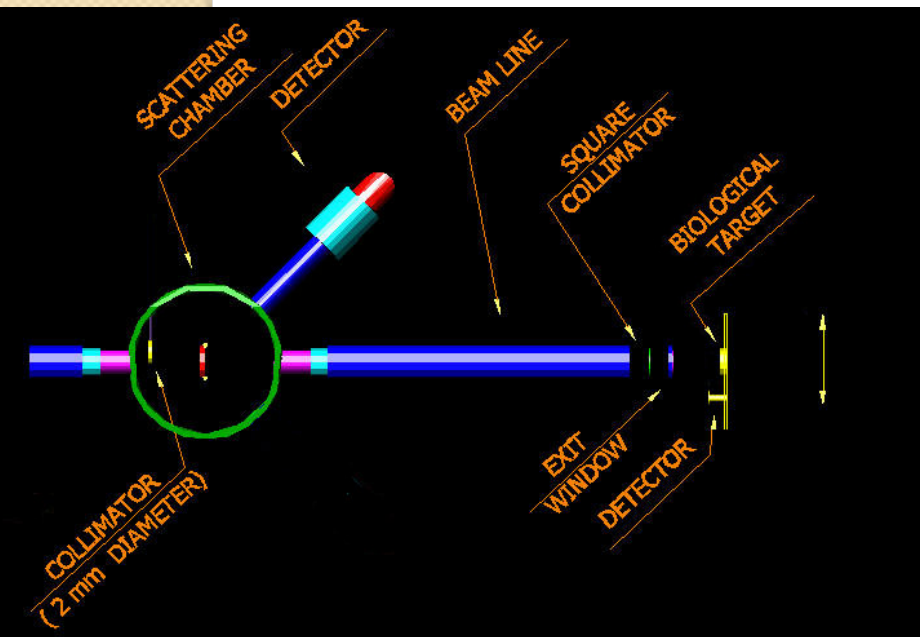
Experimental setup

- Beam is delivered to the position A in the experimental hall of cyclotron



Experimental setup





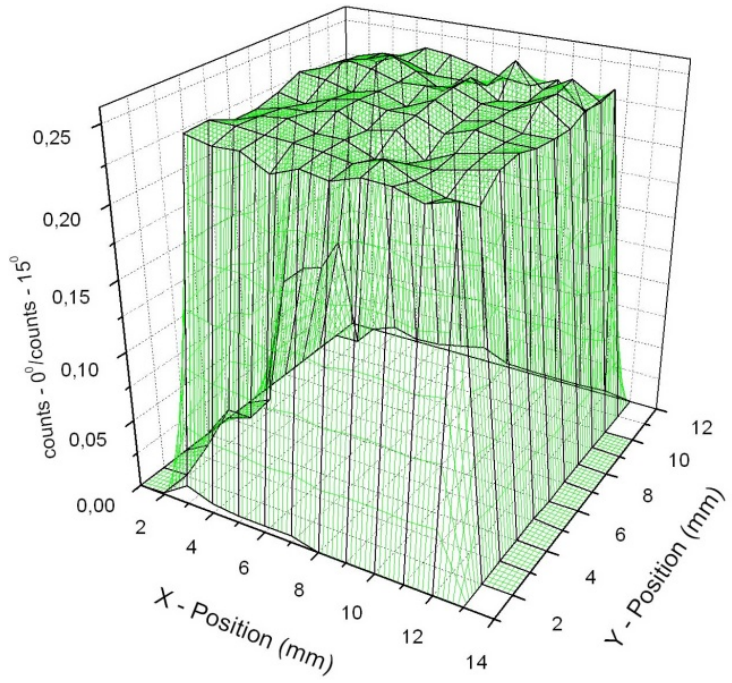
- beam is scattered on the gold target to obtain square beam size of 1 cm x 1 cm

(at a distance of 233 cm from target)

- then, the beam is delivered in the air to irradiate the cells in Petri dish

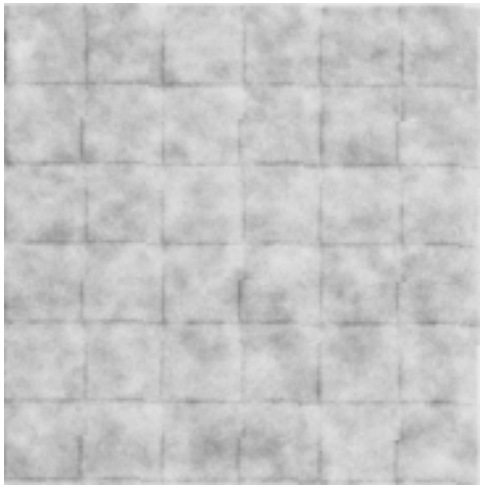
IMPORTANT:

- beam is horizontal
- biological sample is mounted vertically
- special detector is mounted at an 20° angle



Measured two dimensional plot of the 12C ions intensity scattered over the 1x1 cm^2 exit window at the cell container position

The irradiated area of 6x6 cm^2 at the cell container position with dose equal to 1.8 Gy registered by the X-ray film

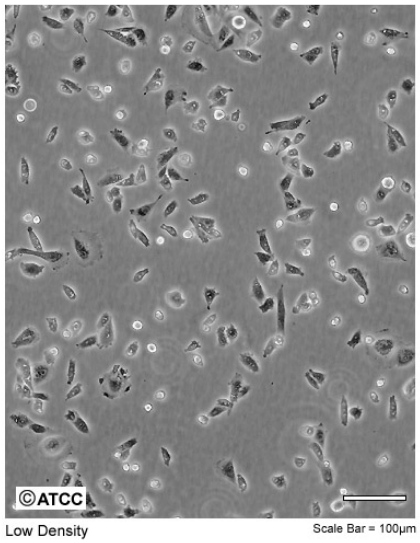


CHO-K1 cells

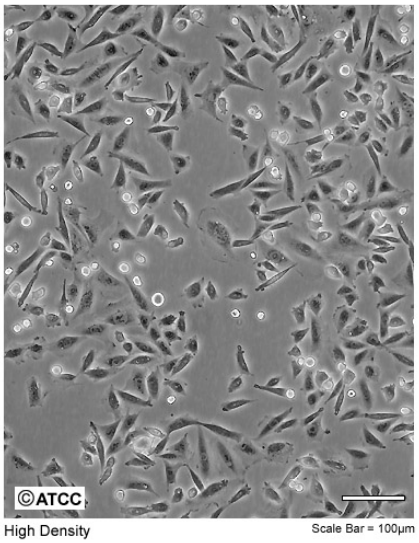
Chinese hamster ovary cells

- they are typically used in radiobiological studies
  - easily stick to the mylar foil
    - easy in culture

ATCC Number: **CCL-61**
Designation: **CHO-K1**



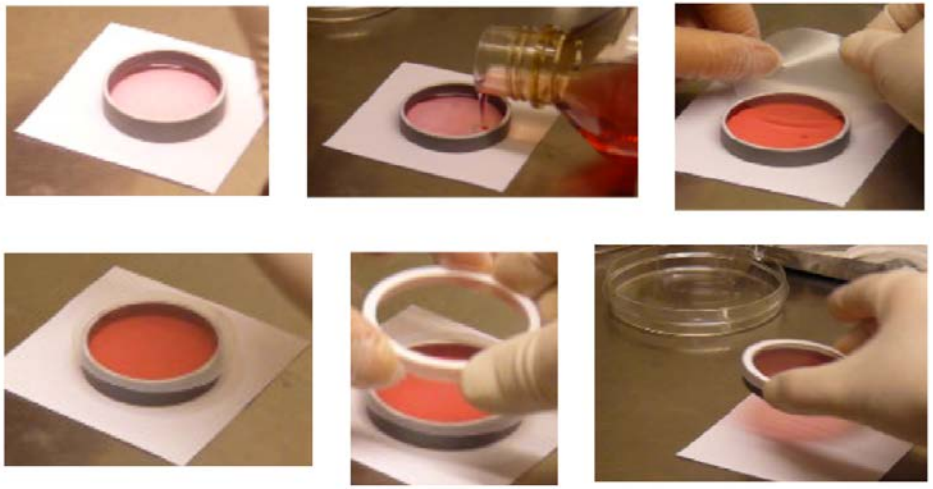
Low Density Scale Bar = 100µm



High Density Scale Bar = 100µm

www.atcc.org

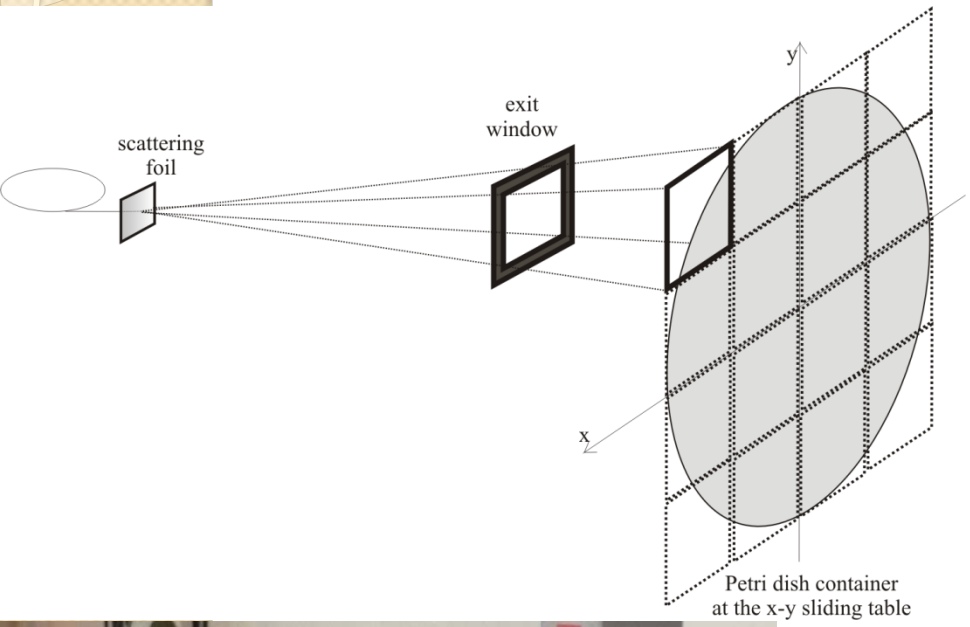
Preparation of the cells to the irradiation

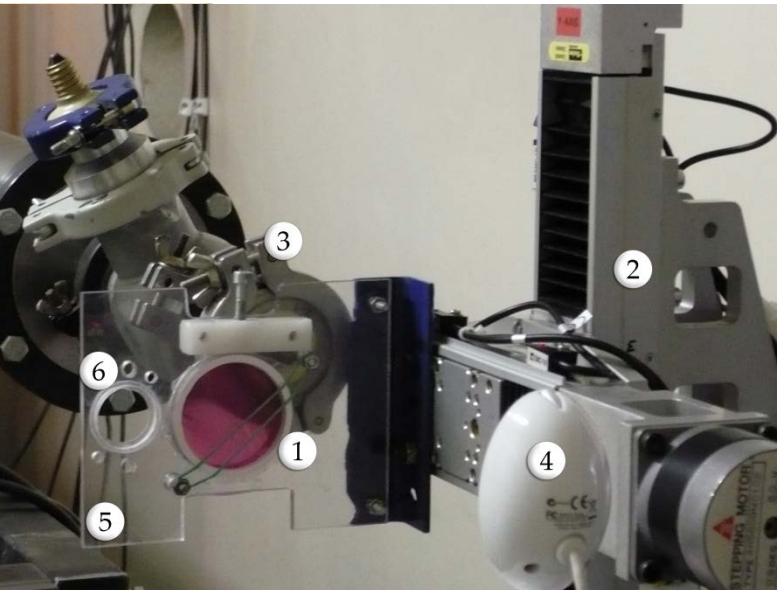


- Stick mylar foil as the bottom of plastic ring
- Seed cells – 24h before irradiation
- Pour nourishment
- Fix parafilm by plastic ring as the cover

Preparation of the cells to the irradiation

Cell irradiation



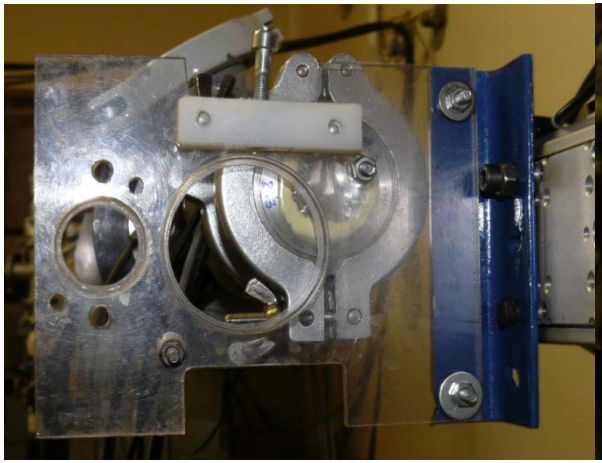


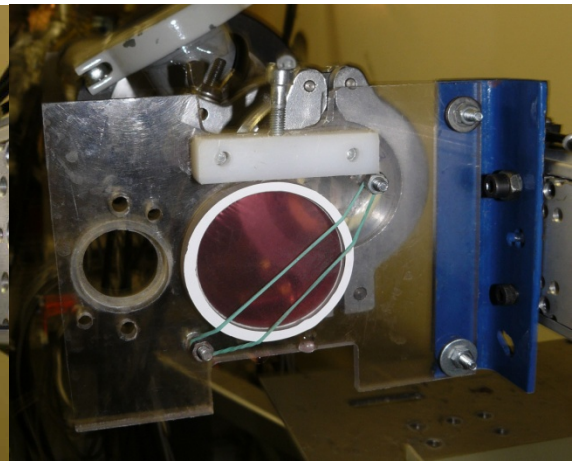
No. 1: Petri dish No. 2: sliding table

- beam size of 1 cm x 1 cm irradiate the cells in Petri dish with a diameter of 5 cm

- irradiation procedure is as follows:
  - beam is stationary,
  - Petri dish with cells is shifted by 1 cm using the sliding table,
  - Table changes position when it receives an impulse from the detector at an angle of 20^0,
  - Impulse is generated when the detector registers a sufficient number of particles (proportional to the absorbed dose)

Irradiation





Survival test

- survival test is performed to determine the degree of cells survival after irradiation with ions (surviving fraction)
- figure shows survival test technique
- based on data obtained from survival test (surviving fraction) we plot the survival curve

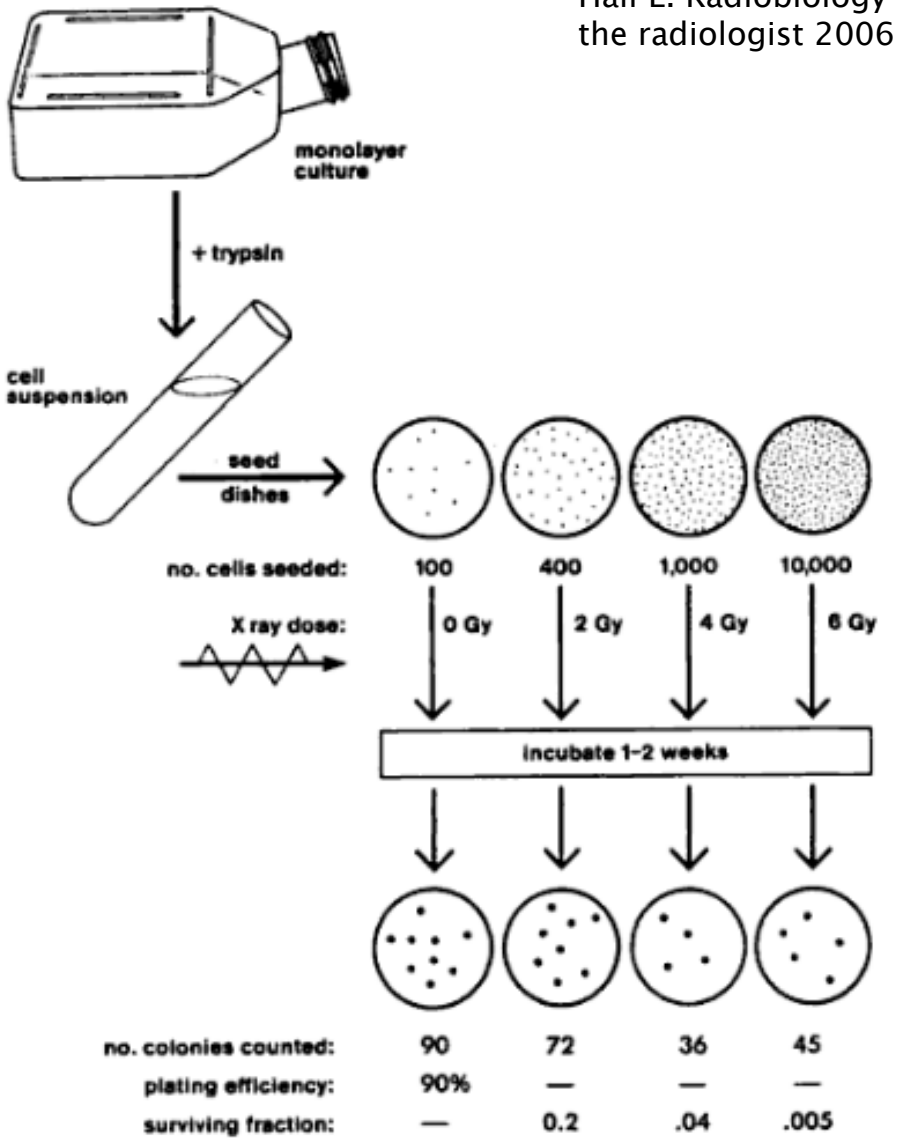
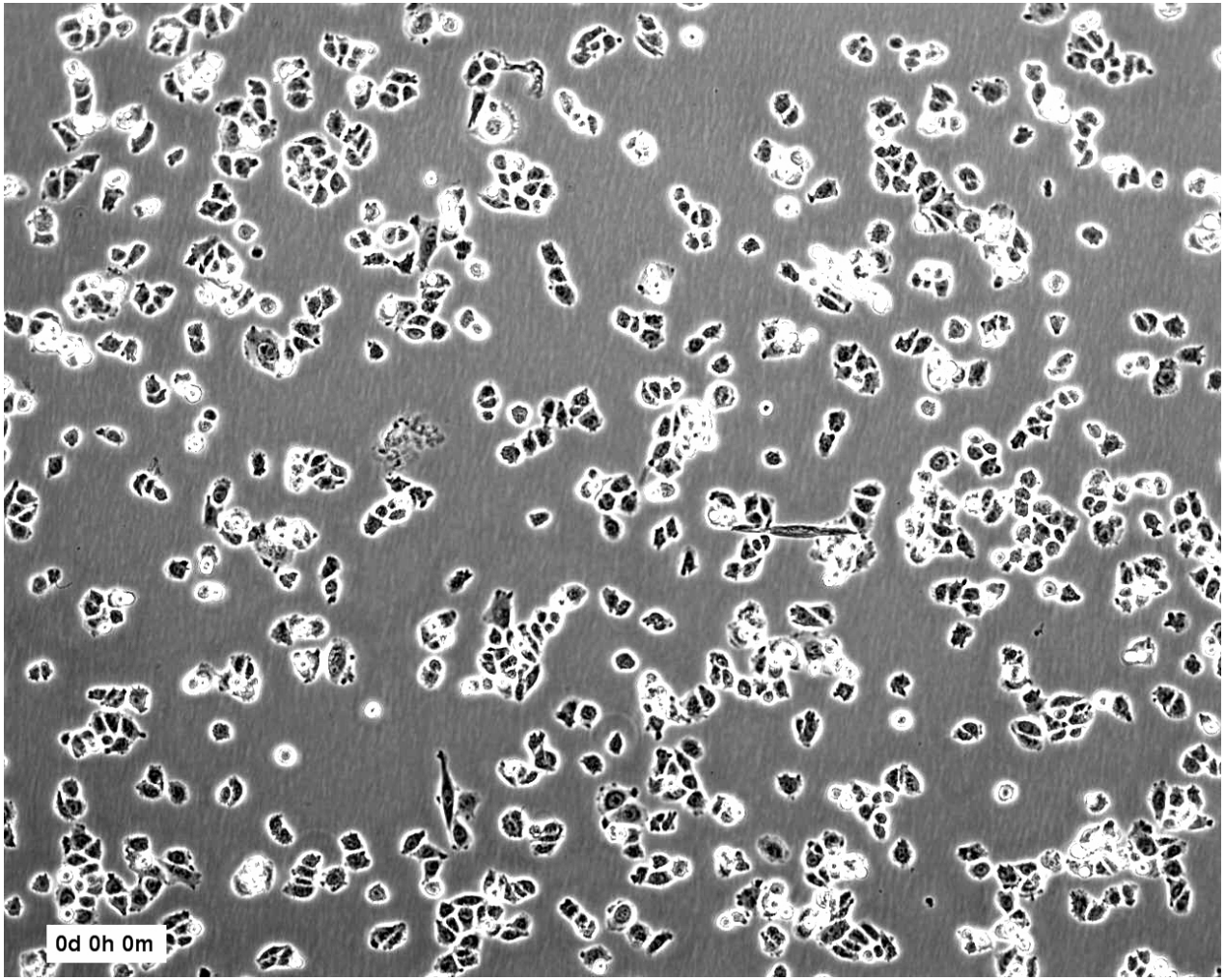


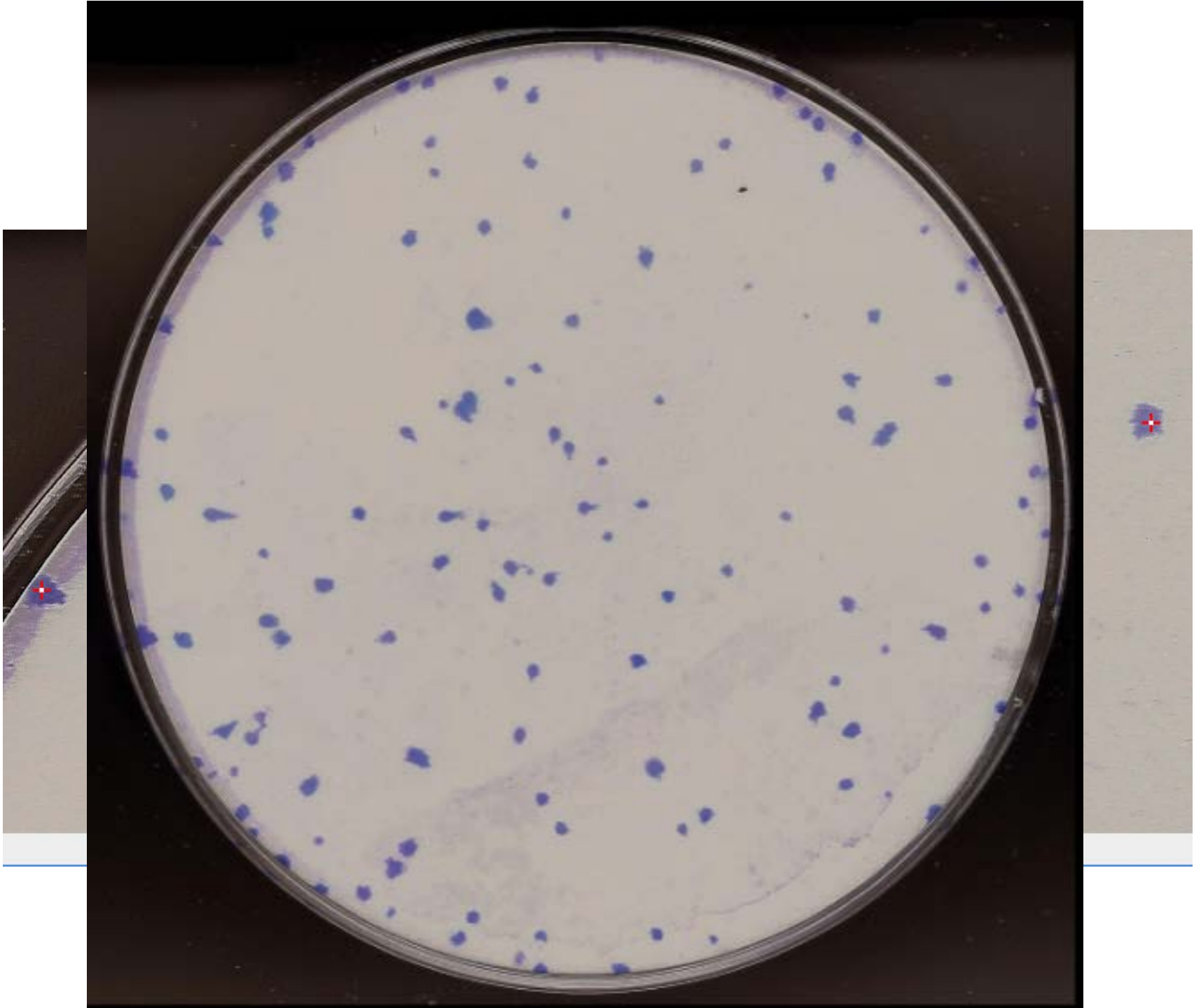
FIGURE 3.2 ● The cell culture technique used to generate a cell survival curve. Cells from a stock culture are prepared into a single-cell suspension by trypsinization, and the cell concentration is counted. Known numbers of cells are inoculated into petri dishes and irradiated. They then are allowed to grow until the surviving cells produce macroscopic colonies that can be counted readily. The number of cells per dish initially inoculated varies with the dose so that the number of colonies surviving is in the range that can be counted conveniently. Surviving fraction is the ratio of colonies produced to cells plated, with a correction necessary for plating efficiency (i.e., for the fact that not all cells plated grow into colonies, even in the absence of radiation).

Hall E. Radiobiology for the radiologist 2006

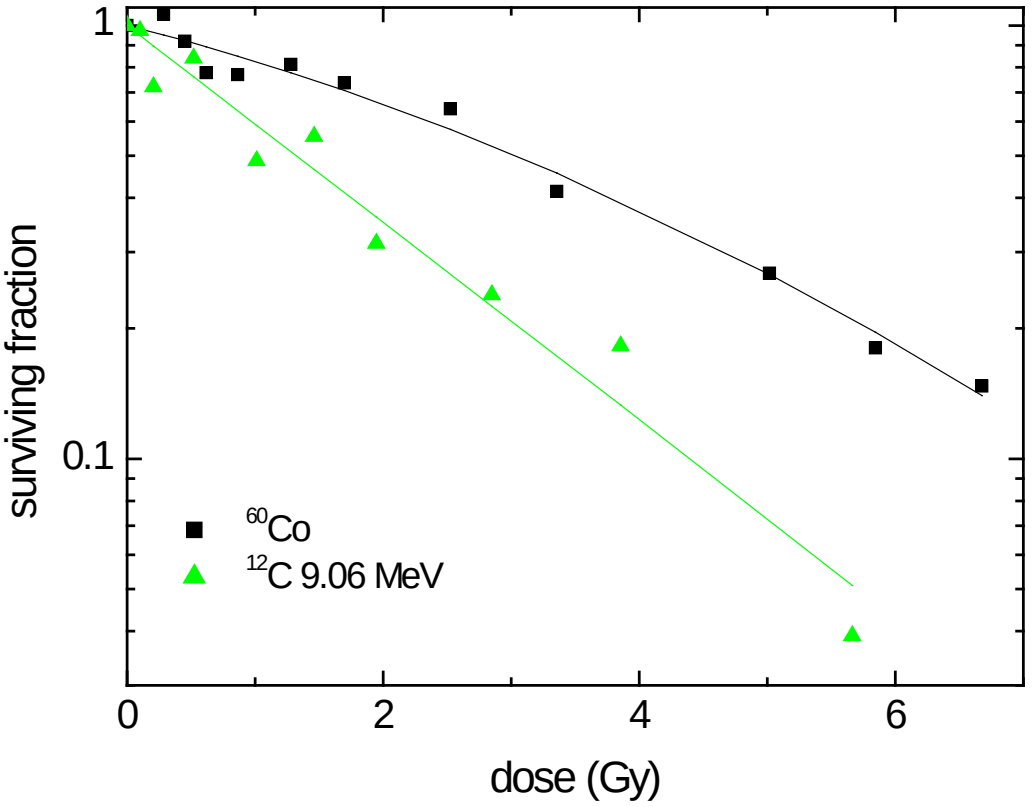


http://www.hpacultures.org.uk/products/celllines/generalcell/detail.jsp?refId=85051005&collection=ecacc_gc

Survival test



Survival curve



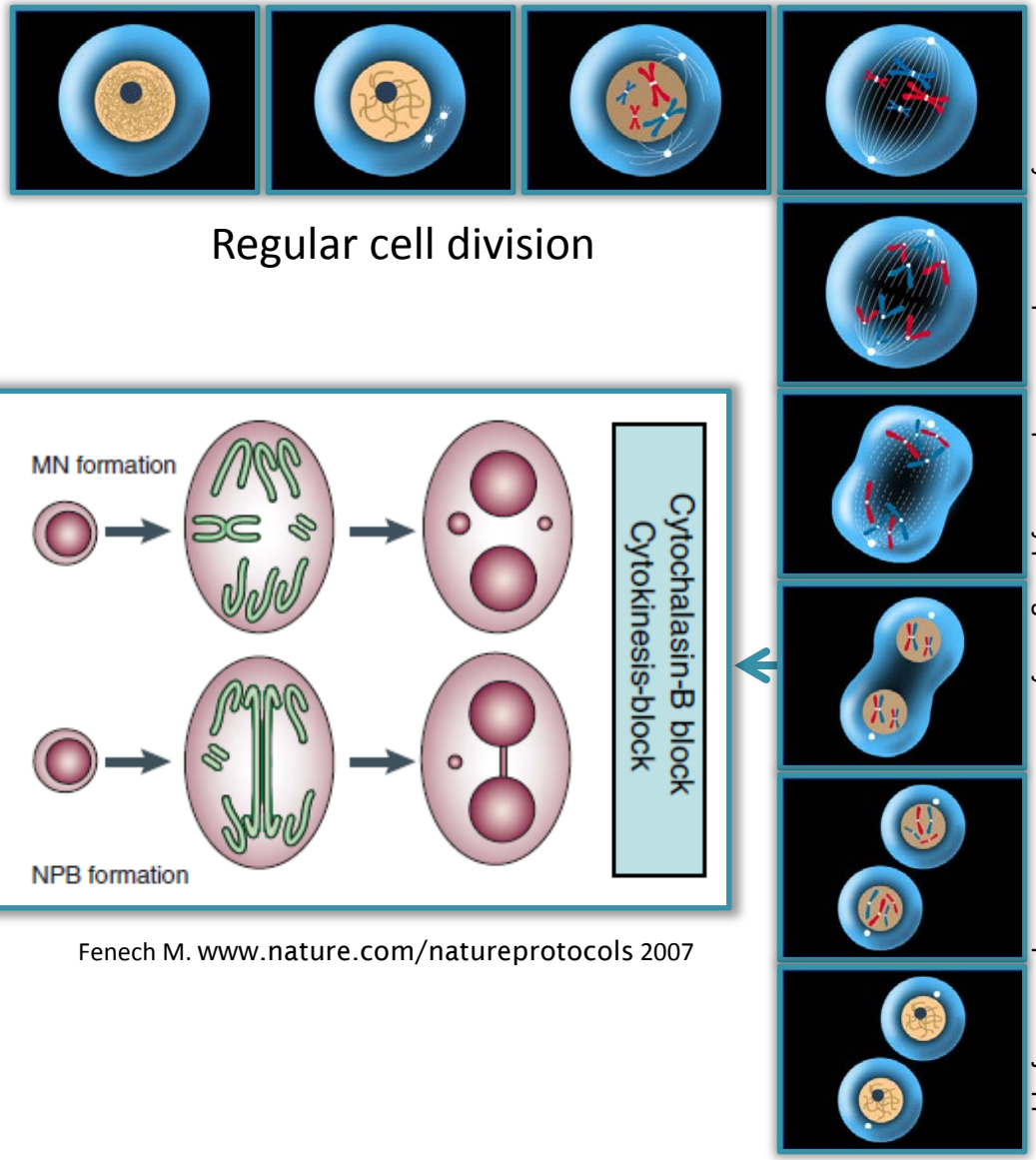
Czub J. et al. App. Rad.Isotop. 2009

- survival curve is a function of the degree of cell survival after irradiation (surviving fraction) and the absorbed dose

Micronucleus assay

Micronucleus – small structure seen in cytoplasm created from:

acentric chromosome fragment (fragment from chromosome breakage)



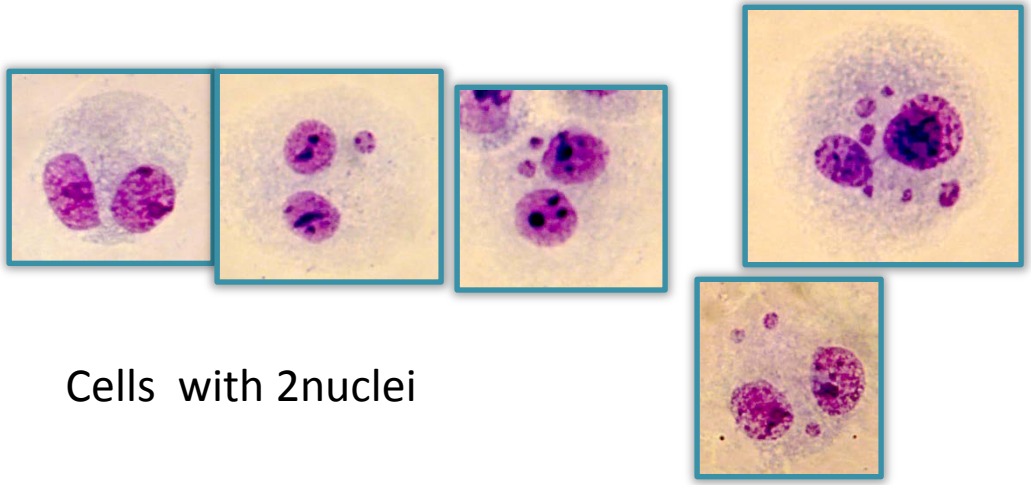
Regular cell division

Fenech M. www.nature.com/natureprotocols 2007

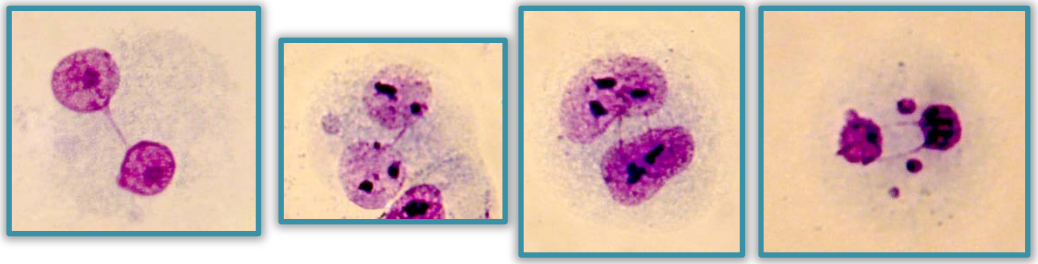
Krawczuk-Rybak M. Współczesne podstawy patogenezy nowotworów u dzieci prezentacja ppt

Micronuacleus assay (MN)

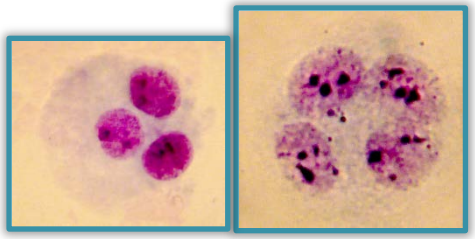
- Standard protocol - Fenech 2007
  - cell irradiation
  - add cytochalasin B
  - after 20-24 h – add trypsin
  - place drop on microscope glass
  - add Giemsa (20%)
  - analysis on microscope



Cells with 2nuclei



Cells with nucleoplasma bridge and MN



Multinucleus cells

Fenech M. www.nature.com/natureprotocols 2007


THANK YOU!