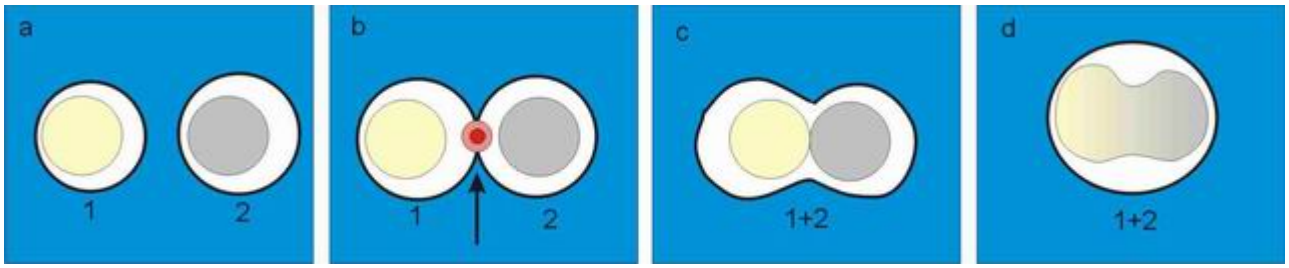
Cells Fusion

There are known three methods of cell fusion. The first one uses immersion of cells into chemical solution (e.g. 50% PEG 1500)[1], the second method employs external electric field for the cell perforation[2]. Unfortunately both these methods cannot be easily used for the fusion of individually selected cells. The third method of cell fusion uses focused laser beams that evaporate tiny volume of the cell membrane[3, 4]. Laser induced cells fusion takes place under an objective of a microscope and easily enables the study of the fusion dynamics.

The principle of the laser-induced cell fusion:



Two selected cells (No. 1 and 2) are brought into contact by using optical tweezers (see subfigure **a**). Bigger cells, which are not taken by optical tweezers, are transported by mechanical micromanipulator (Eppendorf TransferMan® NK) and micropipette (Eppendorf CellTram Air). A sequence of pulses is applied at the point of cells contact (subfigure **b** denoted by arrow), the membranes in contact are perforated and the content of both cells is mixed (subfigure **c**). A few minutes later the fusion product takes round shape again and cell nuclei start to mix.

Experiment:

The first experiment with cells fusion we provide with human lymphocyte cells HL60. Now we work with adherent MCF 7 cells that were placed on the micro grid cover slip (CELLocate, square size 55 μm) for easier localization under the microscope, because MCF 7 cells were found to be easier to fuse.

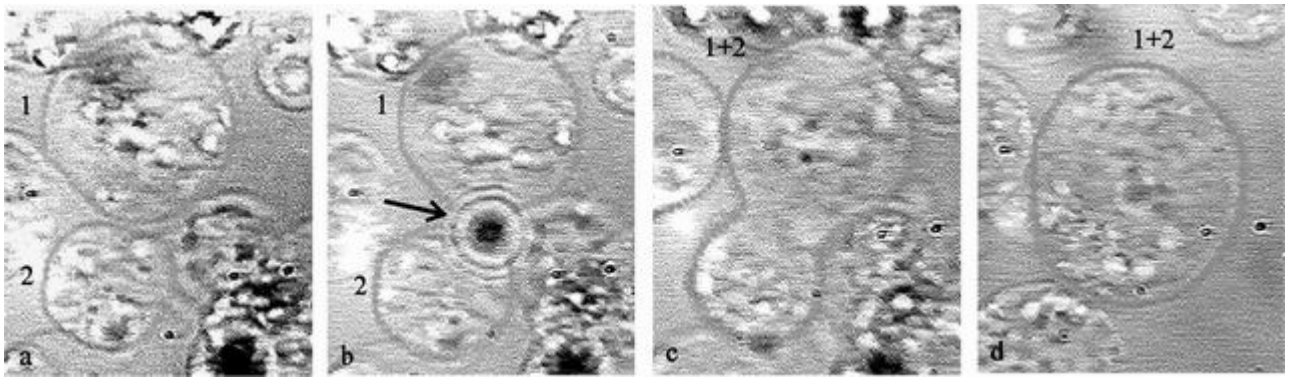
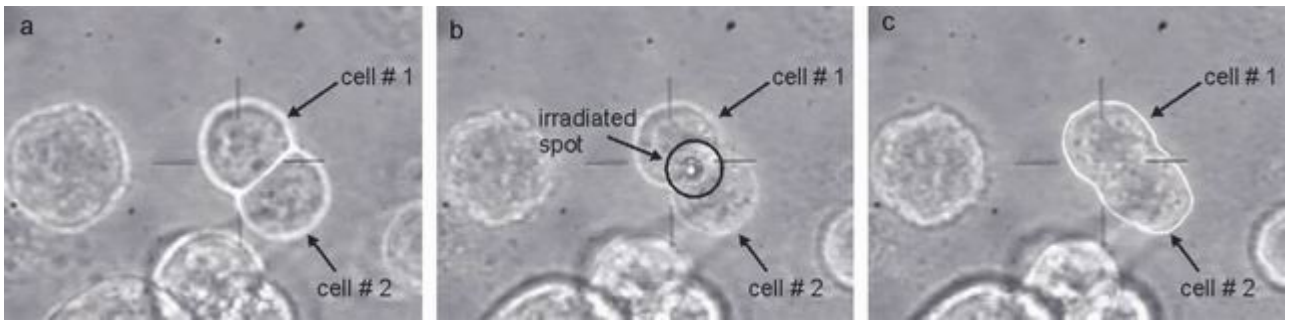


Figure shows the employment of the trapping and cutting beam for the cell fusion HL60. Two human lymphocyte cells (No. 1 and 2) were brought into touch by means of the optical tweezers (see subfigure **a**). Laser pulses of the cutting beam (dark spot in subfigure **b** denoted by the arrow) perforated the outer cell membrane and both cells fused together (**c, d**). Subfigure **c** is taken 40s and subfigure **d** 160s after the wall perforation.



Two MCF 7 cells (denoted as 1, 2) form a cluster (see subfigure **a**). Laser pulses are applied to perforate the cell membrane at the point of contact (light spot in subfigure **b** denoted by circle). The content of both cells is mixed (see subfigure **c**).

The cell nuclei were dyed by low flourochrome concentration for their easier identification in the fused cell. One of MCF7 cell was colored by Hoechst 33342 which provides blue fluorescence and the other by Propidium Iodide which provides red fluorescence. The cell nuclei fusion dynamic was visualized by fluorescent microscopy and recorded for several times during 24 hour. Fused cells were fixed by paraformaldehyd after fusion and centromerus of chromosomes 12 and 7 were observed by fluorescent hybridization in situ (FISH).

Conclusions:

We tested experimentally the laser-induced fusion of adherent MCF 7 cells that were placed on the micro grid cover slip (CELLocate, square size 55 μm) for easier localization under the microscope. Optical tweezers or micropipette was used to bring both cells to contact and series of 4-5 pulses from UV laser (an average energy per pulse was equal to 8 μJ) perforated the membrane. To achieve this, it was necessary to move the sample vertically so that the point of contact coincided with focal plane of the UV beam. The plasmatic membranes disrupted by thermal ablation and their ends immediately joined to form a single hybrid cell. After the fusion, the cells were cultured in a fresh medium. In the intervals of 4, 8, 12, and 24 hours fused cells were fixed by paraformaldehyde and afterwards they were studied by fluorescence in situ hybridization using the specific DNA probe for chromosomes 12 and 7 centromere. We found out that non-fused single MCF7 cells had three signals corresponding to the chromosomes 12 and 7 (trisomia of chromosomes 12 and 7), meanwhile six signals were found in fused cells. Using high-resolution cytomery[5], the dynamics of the chromosome arrangement in the progress of time after the cell fusion was studied. We observed that the homologous chromosomes in the fused cells do not merge together but occupy their separate positions in the fused nucleus.

References:

1. G. A. Neil and U. Zimmermann, "Electrofusion", *Methods Enzymol.* **220**, pp. 174-196, 1993.

2. A. Strömberg *et al*, " Manipulating the genetic identity and biochemical surface properties of individual cells with electric-field-induced fusion", *PNAS* **97** (1), pp. 7-11, 2000.

3. S. Sato *et al*,"Achievement of laser fusion of biological cells using UV pulsed dye laser beams", *Appl. Phys. B* **54**, pp. 531-533, 1992.

4. R. Wiegand *et al*, "Laser-induced fusion of cells and plant protoplasts", *Journal of Cell Science* **88**, pp. 145-149, 1987.

5. M. Kozubek *et al*, "Combined confocal and wide-field high-resolution cytometry of FISH-stained cells" *Cytometry* **45**, pp.1-12, 2001.