Employment of laser induced fusion of living cells for the study of spatial structure of chromatin

Jan Ježek[a], Stanislav Palša[b], Emilie Lukášová[b], Stanislav Kozubek[b], Petr Jákl[a], Mojmír Šerý[a, c], Alexandr Jonáš[a], Miroslav Liška[c], Pavel Zemánek[a]

[a]Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Brno

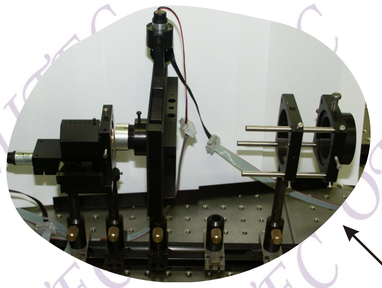
[b]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno

[c]Institute of Physical Engineering, Faculty of Mechanical Engineering, Brno University of Technology, Czech Republic

ABSTRACT

We study the transfer of the cell nucleus and individual chromosomes from one living cell to the other one during their fusion. To achieve this, the nuclei of the two fused cells are stained with different fluorescent dyes which serve as identification markers. The fusion itself is done in an inverted optical microscope by combined system that uses optical tweezers to bring two living cells into contact and optical scalpel to punctuate their membranes at the contact point. This process initiates a fusion of both cells into one hybrid cell containing two nuclei. If the fusion product is viable, these nuclei tend to mix together. The dynamics of the fusion process is then visualized by exciting the fluorescently labeled fusion product with a suitable light source. The time evolution of the mutual position of the fused cell nuclei and their final orientation is traced from a video record of the experiment. The spatial distribution of the nuclear material in the resulting hybrid nucleus is studied by analysis of positions of FISH (fluorescent hybridization in situ) signals of specific genetic loci in automated fluorescence microscope (high resolution cytometer). The obtained results are compared to the signals distribution of FISH in the original cells.

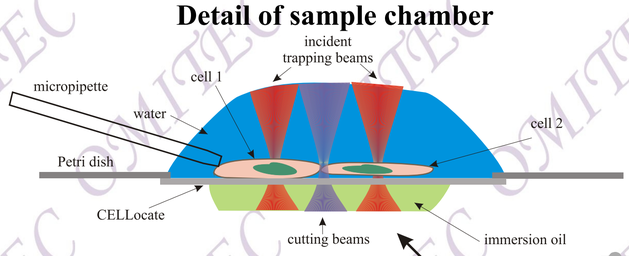
SET-UP



Telescopes

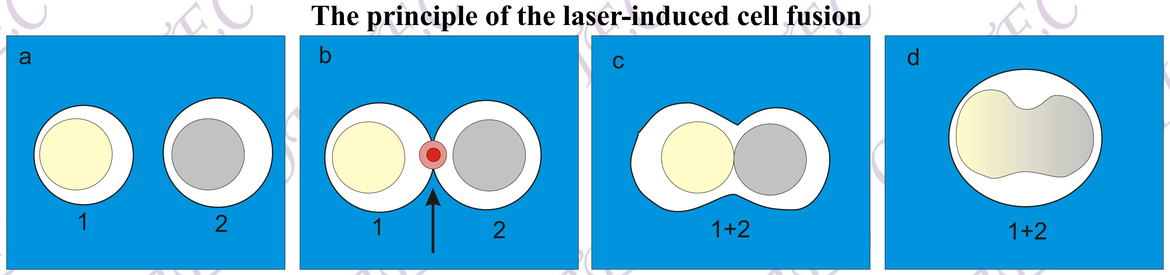
Spectra Physics laser T20-W-105c (Laser 1) with a maximal output beam power equal to 4 W and a wavelength

Pulse laser Minilite II: energy of up to



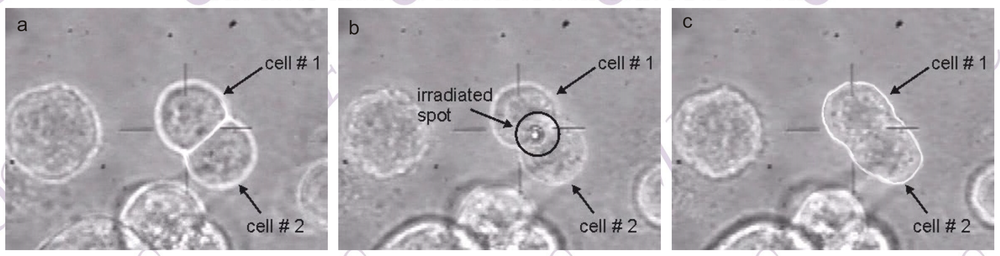
System set-up. Laser 1 (CW, 1053 nm) forms a beam that is used for optical trapping. The wavelength that pumps the laseris blocked by the filter (F) and the output beam polarization is rotated by half-wave plate (Q). The trapping beam is divided by thepolarizing beam splitter (PBS) into two beams. These beams pass through couples of telescopes T1-T2 and T3-T4 , they are coupledtogether by PBS and directed to the microscope Olympus IX 70 where they form two optical traps. These traps can be independently positioned axially and laterally by movement of the first lenses of the telescopes (arrows near the lenses). Laser 2 (pulse, 355 nm) isused for the laser-induced fusion of cells. Its optical path consists of two telescopes T5 and T6 that position the beam focus in the microscope field of view. Trapping and cutting beams are coupled together by dichroidic mirror DM2 and reflected to the objective by DM3.

The principle of the laser-induced cell fusion



(a) Two cells (denoted as 1, 2) are brought into contact using optical tweezers. (b) Laser pulses are applied to perforate the cell membrane at the point of contact (denoted by arrow). (c) The content of both cells is mixed. (d) The nuclei fuse together and the fusion product takes round shape again.

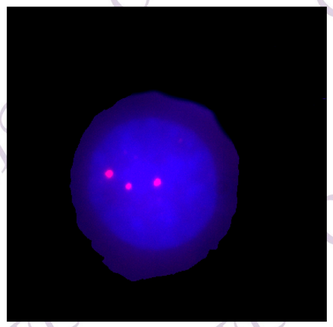
The laser-induced fusion of adherent MCF 7 cells



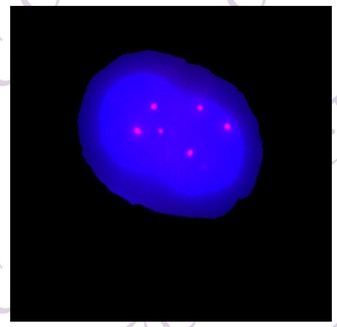
Two MCF 7 cells (denoted as 1, 2) form a cluster (see subfigure **a**). Laser pulses are applied to perforate the cell membrane at the point of contact (light spot in subfigure **b** denoted by circle). The content of both cells is mixed (see subfigure **c**).

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 m) 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 chromosome 12 centromere. We found out that non-fused single MCF7 cells had three signals corresponding to the chromosome 12 (trisomia of chromosome 12), meanwhile six signals were found in fused cells. Using high-resolution cytomery, 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.

Single MCF7 cell with three signals



Fused MCF7 cells with six signals



CONCLUSIONS

We succeeded in laser-induced fusion of adherent MCF 7 cells. To compare nuclear location of chromosomes in fused and normal nuclei, fused cells were fixed and hybridized with specific chromosome probes. The chromosome location was observed in the progress of time after the cell fusion. We found that non-fused MCF7 cells had three signals corresponding to chromosome 12 and we observed six signals in fused cells. We analyzed the position of the FISH signals in the fused cell and we conclude that the homologous chromosomes do not merge together but occupy their separate positions in the fused nuclei of both cells.

ACKNOWLEDGEMENT

The work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic, projects No: A1065203 and by the Academy of Sciences of the Czech Republic, projects No: S5004010.