Inside views: Immunofluorescent staining of cell structures

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Aim

Every cell in our body is an entire world itself. But it is extremely difficult to visualise this world: A cell itself shows little detectable structure under the visible microscope light. To gain an inside view, different cell organelles have to be stained separately, one by one. What we know about the shape and distribution of organelles within the cell is from adding up this separate information. In this course different staining methods are used, with emphasis being on immunofluorescence staining.

Each participant will stain cultured human fibroblasts (cells of the connective tissue). Different indirect immunofluorescent staining will be performed to visualize the Golgi-apparatus, the lysosome, the endosome and the microtubuli. Simultaneously the DNA or the actin filaments will be stained directly. During the incubation times a detailed introduction into organelles, cells and different responses of the immune system could be given.

The results can be seen under a fluorescence microscope equipped with the appropriate filter sets.

Methods of staining

Direct Staining

Chemical and biological substances are used for the direct staining of cell structures. These substances must possess two important characteristics:

1. They must only bind to those structures we want to stain
2. They must be able to give a signal.

The signal-giving substance can vary: it can be radioactive, bind to a colour producing enzyme or have a colouring effect itself.

In this experiment two fluorescent substances for direct staining will be used.

One of them is the fluorescent chemical substance called 'Hoechst' and binds only to the DNA of the cell nucleus. The nucleus fluoresces blue when excited with ultra violet light.

The second substance – Phalloidin – is the poison of the deadly amantia mushroom. It binds to actin, microfilaments of the cytoskeleton. The cytoskelton reacts with inflexibility and, as a consequence, the cell dies. For this experiment, Phalloidin is linked to the chemical substance Fluofor Alexa 488 (it fluoresces) in order to give a signal.

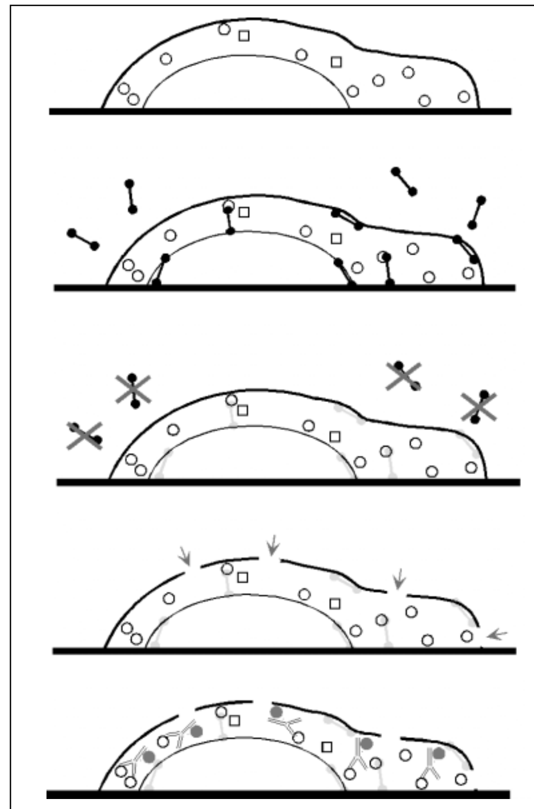
Immunofluorescence

Some frequently used staining agents are antibodies. Antibodies are generated by the different cells of our immune system to recognise foreign and possibly pathogen substances that has entered our body. They are proteins of a Y-like structure. With its branches ('variable region') each antibody recognises a certain foreign structure, while its stem ('constant region') initiates the immune responds of the body. Therefore nature provides a ready made biological substance to fulfil one of our staining agent demands: To bind specifically to one structure.

If we use an antibody to stain cell structures, it has to be produced in an animal (e.g. mouse) against the cell structure we want to stain (e.g. human Golgi-apparatus). Later on a signal unit has to be added

chemically. Various signal-giving substances (often called Fluophores) can easily be linked to their constant region. We distinguish between direct and indirect immunofluorescence.

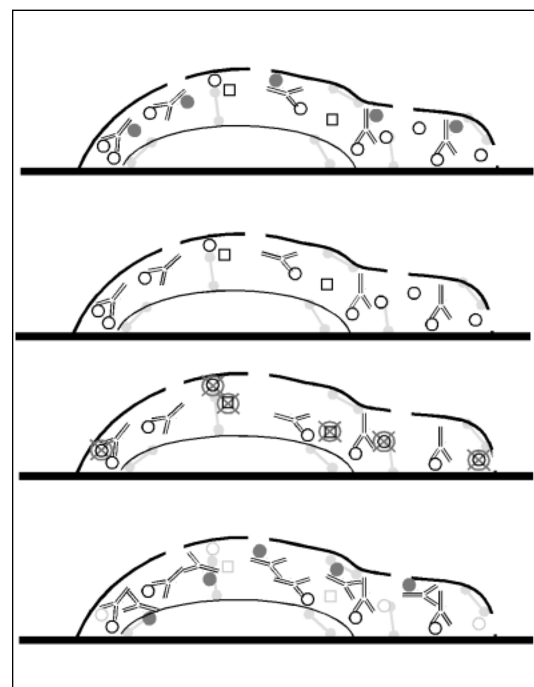
a) Direct Immunofluerescence



In direct immunofluerescence we operate as follows:

- The cell we are going to stain is from a cell culture of fibroblasts. These are cells that adhere to the surface of glass
- First the cell culture is fixed with paraformaldehyde (PFA). PFA links proteins. As a consequence the cell dies.
- Surplus PFA must be removed to prevent it from linking the antibodies that will be added later. (Antibodies are proteins too.) For this we use ammonium chloride.
- Now the cells must be perforated to allow entry of the antibodies to structures inside the cell (permeabilization). For this we use a detergent such as Triton X-100.
- Now we add the antibody with Fluophore linked to it. It only binds to the cell structure we want to stain

b) Indirect Immunofluerescence



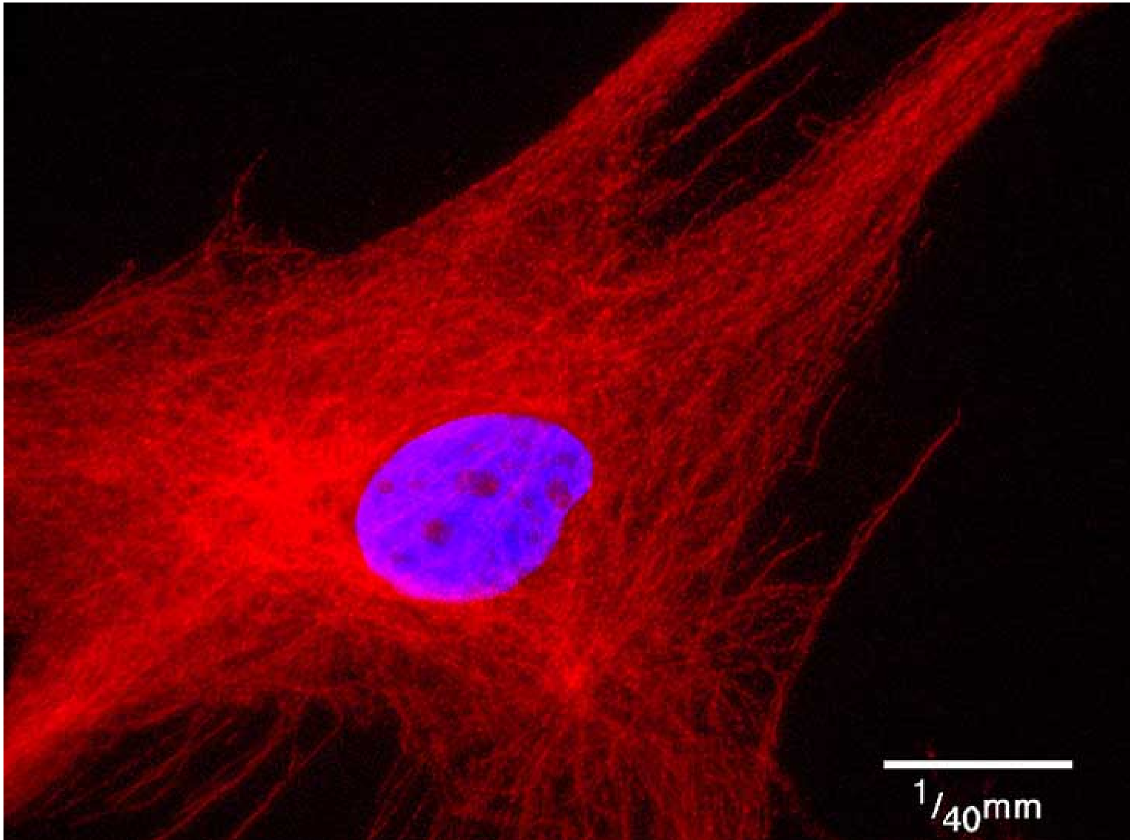
More common and better developed is indirect immunofluerescence. It is more complex but stains more effectively. This method works as follows:

- First we proceed as in direct immuno-fluorescence.

BUT:

- We add antibodies without Fluophore (first antibody). This binds to the structure we want to stain.
- To avoid non-specific binding of the second antibody to proteins that we do not want to stain, we treat the cells with serum. This blocks all the other proteins.
- Now we add the second antibody with Fluophore linked to it. It recognizes the constant region of the first antibody and binds to it.

With this method we gain a more intense staining because often several secondary antibodies bind to one primary antibody. The Fluophore accumulates.



Direct staining: DNA - blue

Indirect staining: Microtubuli - red