Preparation of paraformaldehyde-fixed cells

Growing the cells

Adherent cells

- put coverslips in the wells of 24-well plates
- seed adherent cells 1 day before the experiment

Suspension cells

- lymphocytes generally stick to the glass if seeded in 0.5% FCS for 10-20 min

→ the attachment of cells can be improved by pretreating the glass with 1 M HCl for a few minutes, followed by thorough washing with water or PBS (this increased the positive charge of the glass responsible for attachment)

Fixation with paraformaldehyde:

Preparation of fixative

→ for preparation of paraformaldehyde, see separate protocol **"Method to prepare Paraformaldehyde"**

→ formaldehyde (the product of the paraformaldehyde preparation protocol) is not stable, but if aliquoted and frozen, it can be used for a few weeks

→ to minimise inhalation of hazardous paraformaldehyde, use the granular rather than the powder form

Fixation

- replace the growth medium by 0.5 ml paraformaldehyde fixative
- fix for 5-10 min *(duration depends on the protein = lysine content and its localisation, e.g. in big complexes or membranes)*
- wash 1x with PBS + 0.1M ammoniumchloride (stops fixation)
- wash 2x with PBS
- if required, permeabilise and stain at this step

Mounting

- rinse coverslips 1x by dipping carefully in distilled H_2O
- air-dry coverslips for a few minutes
- add 10 µl of mounting medium to a slide *(e.g. ProLong Mounting Medium (Molecular Probes #P7481)*
- remove the last drop of water from the coverslips with some soft tissue *(be careful, coverslips easily stick to the tissue)*

- put coverslips on drop mounting medium *(upside down, of course)*
- let the mounting medium harden / dry at least overnight at room temperature / dark *(with Prolong, they must completely dry out)*
- store in the fridge until imaging
- after imaging, they can be stored in the freezer for long-term storage (with good mounting medium they should last 1-2 years)