COST Action: How to fixate cells for AFM experiments

Hans Oberleithner, Institute of Physiology II, University of Münster, Germany

1. Culture cells (kidney, gut, lungs, brain, etc) in an incubator on solid supports (plastic, glass, coated glass, filter membranes, etc, whatever your cells like).

2. Get glutaraldehyde as a fixative (sold by SERVA; 25% stock solution; Catalogue No:23114) and predilute it with your respective culture medium to 5%. You can also predilute it with HEPES buffer (in mM: NaCl 140, KCl 5, MgCl2 1, CaCl2 1, HEPES 10 mM; pH 7.4).

3. Transfer cells from the incubator to the hood and pipet an adequate volume of your 5% glutaraldehyde solution (fixative solution) into the medium of the culture dish where the cells are. Achieve a final concentration of 0.5% glutaraldehyde in your dish. This means, e.g., that you have to add 300 µl of the fixative solution to 3 ml of culture medium that is already in the culture dish. Give the dish a 2 times "rotating swing" so that the fixative distributes well.

4. Let it sit for about 1 hour.

5. Remove the medium and add Hepes buffer. Be careful that the cells are always covered by some fluid.

6. Seal the dish with parafilm and store the sample at 4 - 8°C.

7. In my experience, you can use this sample immediately but also store it for at least 2 weeks (frig).

8. Perform AFM experiments in fluid (e.g. in HEPES buffer; see above).

9. In case you want to work on an air-dried sample, remove the fluid after fixation (after point 4), wash the sample with H_2O and let it sit in air. Store (dust-free) in air at room temperature.

You can do AFM imaging, cell volume, cell surface area, mean roughness and possibly also nanomechanics on these cells. Their morphology is nicely preserved, they are "caught in action" right at the moment you add the fixative. As attachments, please find 5 papers, one on methods (Methods in Molecular Medicine) and 4 showing results in fixed cells.

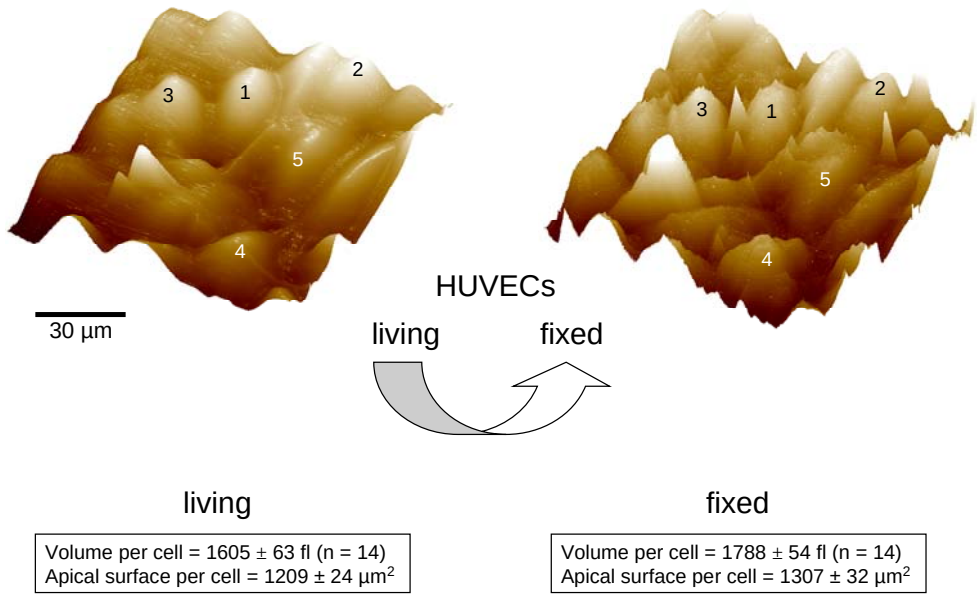

Image taken from Oberleithner et al J Cell Science 2006