



How to fixate cells for AFM experiments

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Abstract

The method can be applied to any cells in culture. It is fast, cheap and reliable. You can do AFM imaging, cell volume, cell surface area, mean roughness and even relevant nanomechanics on these cells. Their morphology is nicely preserved, they are "caught in action" right at the moment you add the fixative.

Step-by-step description of procedure

1. Culture cells (heart, 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 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 μ l 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 fluid.
6. Seal the dish with parafilm and store the sample at 4 - 8°C.
7. You can use this sample immediately but you can also store it for at least 2 weeks.
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 H₂O and let it dry in air. Store (dust-free) at room temperature.

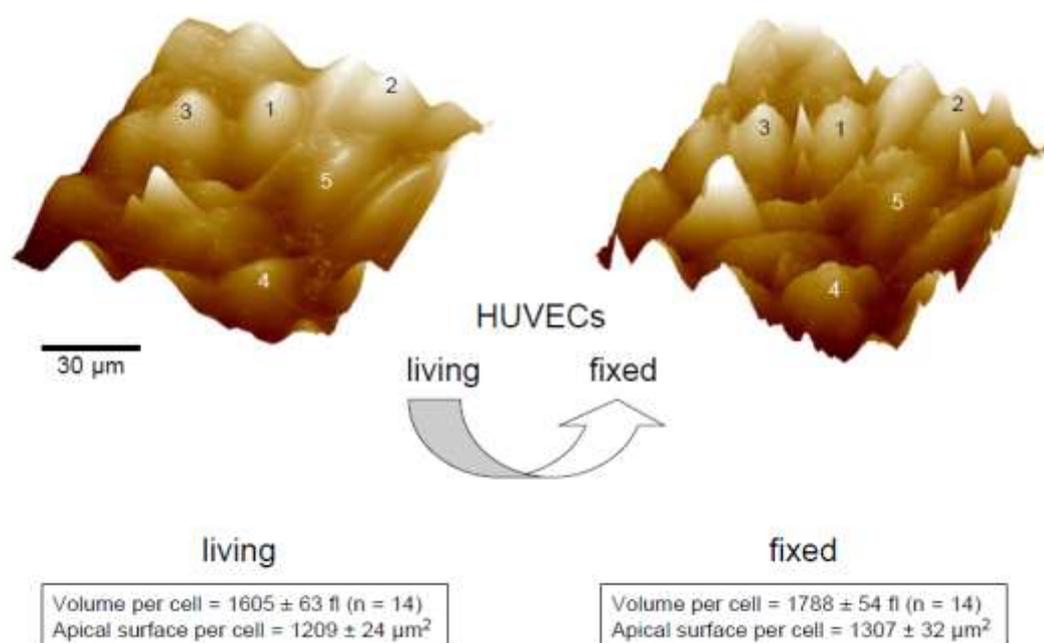


Image taken from Oberleithner et al J Cell Science 2006

Special comments

Good luck

Materials/chemicals/devices required

Glutaraldehyde is sold among the others by SERVA, 25% stock solution; catalogue No:23114.

References

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