Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)

L. Immunocytochemistry of cells on nanofibers

1. Rinse the cells or spheroids on nanofibers using prewarmed PBS (with 1mM $CaCl_2$ and 1 mM $MgCl_2$)
2. Fix the cells with freshly prepared 100 mM phosphate buffered saline (pH 7.4) containing 4% paraformaldehyde and 0.1% glutaraldehyde to enhance cell fixation. Fix cultures for 10 min at room temperature. Avoid using organic solvents that can warp (methanol) or dissolve (acetone) the nanofibers
3. Rinse the cultures 3x5 minutes with PBS
4. Permeabilize the cells and block non-specific binding for 1h at room temperature using 100 mM PBS containing 0.3% v/v Triton X-100 and 5% w/v serum bovine albumin or other suitable blocking agent (consider increasing the concentration of blocking agent to prevent antibody adsorption to the nanofibers)
5. Perform ICC in the same conditions as for cells cultured on conventional culture ware
6. For low-magnification microscopy (2X-10X) cells can be stained and imaged in their original nanofiber-coated plates. For high-magnification microscopy (>10X) the backing material from individual wells must be cut ("punched out" using a metal puncher or cork borer) and the cut- out nanofiber disks can be mounted on glass slides or large rectangular coverslips.