Cellevate

Mounting nanofiber scaffolds for microscopy*

When imaging cells in the nanofiber scaffold at higher magnifications (>10x) the scaffolds may have to be extracted from each individual well and mounted on glass slides or coverslips. Typically, standard mounting protocols are applicable without any alterations. For optimal imaging of cells using i.e. confocal microscopy we recomend mounting using a mounting media that can be adjusted to match the refractive index of the nanofiber scaffold, e.g. 2,2'-Thiodiethanol (TDE) from Sigma-Aldrich. Below follows a suggestion on how to mount scaffolds using this medium:

1. Fixate cells. (See *"Immunocytochemistry of cells in nanofiber scaffolds"* for suggested procedure).

2. Immerse samples for 5 - 10 minutes in each of the TDE/PBS/water solutions according to the following dilution series:

    a) 10 % TDE (100 µL TDE, 50 µL PBS, 850 µL water).
    b) 25 % TDE (250 µL TDE, 50 µL PBS, 700 µL water).
    c) 50 % TDE (500 µL TDE, 50 µL PBS, 450 µL water).
    d) 97 % TDE (970 µL TDE, 30 µL PBS) – repeat this step three times.

3. Cut scaffold to appropriate size and place on glass slide or coverslip.
   Note: Do not let the samples dry out during the mounting procedure.

4. Add a drop of 97 % TDE to the sample before covering the sample with a coverslip, make sure not to trap any bubbles.

5. Seal coverslip, e.g. using nail polish.

*Suggested procedure, please adjust according to your experimental needs.