Paraformadehyde fixation of cells on USF Nanofilm Plates

Supplies needed:

- Cells of Interest
- USF Nanofilm Plate
- 4% Paraformadehyde
- PBS (1X, Sterile, pH7.4)

Protocol:

Fixation:

1. Aspirate media carefully without touching or scraping nanofilm surface
   - Note: Perform wash steps quickly. Nanofibers should not be allowed to dry out; drying may cause fiber clumping and alter scaffold behavior.
2. Add 0.5 mL of PBS to each well.
   - Note: Avoid disturbing the nanofilm surface during pipetting; dispense media against the side of the well when possible.
3. Repeat wash two additional times (total of three washes).
4. Add 300 – 500 uL of 4% Paraformaldehyde to well and incubate at 25°C for 10 minutes.
5. Remove Paraformaldehyde solution carefully.
6. Add 0.5 mL of sterile PBS to each well.
7. Repeat PBS wash two additional times (total of three washes).
8. Proceed to downstream workflow (permeabilization/blocking) or store for later use
   - Storage: Seal the plate with Parafilm to minimize evaporation and store at 4 °C, protected from light. Keep wells filled with PBS.

Additional Notes:

- Keep nanofilms wet at all times. Drying may cause fiber clumping and alter scaffold behavior.
- It is recommended to wait at least 48 hours after plating cells to achieve optimal imaging conditions.
- Concentrations and incubation times may need to be adjusted depending on cell type.
- Perform PFA steps in a fume hood with PPE. Collect PFA waste in designated formaldehyde containers per your IBC/chemical safety protocols.