Application of Live-Cell Florescent Dyes on USF Nanofilm Plates

Supplies needed:

- Cells of Interest
- USF Nanofilm Plate
- Hank's Balanced Salt Solution
- Live-Cell Imaging Solution
- Fluorescent Dyes (Suggested Cytoplasmic Dye: Calcein AM)

Protocol:

This protocol has been optimized for Calein AM (Invitrogen, C1430)* with cells plated 48hrs prior to loading dye.

Dye Loading:

1. Warm HBSS and Live Cell Imaging solution at 37°C. Allow Calcein AM to come to warm to room temperature (protected from light).
2. Prepare a 1uM working stock of Calcein AM in HBSS.
3. Aspirate media from wells intended for imaging. Add 300uL of Calcein AM working stock to wells with pre-plated cells and incubate for 45 minutes at 37°C protected from light.
    - Note: Avoid disturbing the nanofilm surface during pipetting; dispense media against the side of the well when possible.
    - Optional: Hoechst 33342 as a nuclear counter-stain
        - 0.5–1 µg/mL (add for final 5–10 min only)

Imaging:

4. After incubation, aspirate Dye-loading solution and replace with pre-warmed Live-Cell Imaging solution.
5. Incubate cells at 37°C for 15 minutes for enhanced fluorescent signal (AM ester removal)
6. Image cells.

Additional Notes:

- Keep nanofilms wet at all times. Drying may cause fiber clumping and alter scaffold behavior.
- *Alternative dyes can be used. Please contact us for a list of dyes that have been found to be most compatible.
- Concentrations and incubation times may need to be adjusted depending on cell type.
- It is recommended to wait at least 48 hours after plating cells to achieve optimal imaging conditions.