For Staining and fixing suspension cells:

Stain on ibidi dish

Adapted from Kari's old protocols + https://www.future-science.com/doi/10.2144/000114610 to adjust for suspension cells

Requires:

- Ibidi dish https://ibidi.com/chambered-coverslips/251--slide-8-well-high.html
- Ibidi mounting media https://ibidi.com/cell-culture-microscopy/105-ibidi-mounting-medium.html

Protocol

1. Pellet cells (250 × *g* for 5 min)
2. Resuspend cells in PBS at ~1×10^6 cells/mL.
3. Pipet 300uL into 1 well of ibidi dish
4. Let sit for 30 min for gravity sedimentation
5. Gently remove PBS (hand aspirate or reduce flow in vacuum aspirator by stacking with pipet tips) then cells with warm (37 °C) 4% PFA for 10 min*
    a. *May need to change fixation based on antibodies being used.
    b. We have been finding the 2.5% PFA for 20min is better for many suspension cell types to stop blebbing because it help keep the cell pressurized while fixing!
6. Wash 2 x 5 min with PBS
7. Permeabilize and block for 0.5-1hr (2%FBS, 0.1%Triton X in PBS-this is what I used, if you use BSA it should be fine as well)
8. Primary antibody staining (in general times can vary per antibody):
    a. Add antibody and incubate at 37 °C) for 1 hr
    b. Remove antibody and wash 3x5min with PBS
    c. Repeat with other primaries
9. Secondary staining
    a. Using Alexa fluor antibodies at 1:500 in PBS (if multiple are being used can mix in same tube)
    b. Incubate at RT for 1hr
10. Add DAPI (use 1:1,000 of 1mg/mL DAPI in PBS) and incubate for 5min
11. Wash 3x with PBS
12. Remove PBS and replace with ibidi mounting media w/o DAPI (https://ibidi.com/cell-culture-microscopy/105-ibidi-mounting-medium.html)