Fixation of Tissue Culture Cells for Fluorescence Microscopy:
(unless otherwise specified, all solutions are prepared in PBS. **Preparation of solutions in water will result in osmotic lysis of cells**).

1. Seed cells at desired density on glass cover slides
2. Following day (or in 2 days), wash cells 1X with PBS
3. Fix for 15-20 min. in 4% paraformaldehyde'
   - wash 2X PBS
4. Quench with 50 mM NH_4Cl (to get rid of background fluorescence from free aldehyde groups) 10 min.
   - Wash 2X PBS
5. Permeabilize 15' in 30 μg/mL digitonin
6. Wash 2X PBS
7. Block 30' RT (or O/N 4 ºC) in 0.1% Teleost Fish gelatin/0.5% Triton X-100

- Cells are ready to be treated with antibody

Rationale: digitonin is a gentle detergent, 0.5% triton X-100 will maintain premeabilization created by digitonin without generating new permeablizations
Gelatin vs. BSA – gelatin should bind more nonspecifically.