Immunofluorescence Microscopy, Cell Wall Stain Only

Qingwei Luo and Dan Haeusser,
modified from the Immunofluorescence Microscopy protocol from "Microscopy Techniques for Bacterial Cell Biology"

1. Prepare 15- or 8-well slides. Put 10 ul of 1% poly-L-Lysine solution into each well. Let stand 5 minutes and aspirate off. Wash once with sterile deionized water, add another 10 ul of H2O to keep it wet and suck off H2O during next step. (It is important not to touch the wells with the pipette tip during all washes.) Store slides in empty Petri dishes to protect them from dust, etc. Use slides within 1 hour of poly-L-Lysine treatment for best results.
2. Cut parafilm strip. Place 10 ul of fixed cells on it. Add 10 ul of lysozyme [2mg/ml (1:10 dilution from 20 mg/ml lysozyme stock)]. Allow incubation on parafilm for 1-2 minutes. (5 minutes for IFM) Suck off H2O from slide, add cells, and leave on slide for 2 minutes to allow adhesion.
3. Aspirate off liquid, wash once with 1X PBS.
4. Then make lectin or wheat germ agglutinin solution [1 ul in 200 ul of 2% BSA (in PBS) solution], add 10 ul of mixture on each well, and incubate for 30 minutes to 1 hour at room temperature.
5. Wash wells 10 times with PBS. Apply one drop (10ul) of Slow Fade equilibration buffer from Slow Fade kit (Molecular Probes) to each well and let stand 5 minutes. [1 ul/ml DAPI (stock solution is 1 mg/ml) in Slow Fade equilibration buffer can be added at this point to stain nucleoids.]
6. Aspirate off and apply one drop of Slow Fade (5 ul) in glycerol to each well in the uppermost row. Carefully put on cover slip, angling it so that it covers first the top wells and then the middle and bottom wells. Make sure not to get any Slow Fade on top of the cover slip and avoid creating bubbles in the wells below. Aspirate off any extra Slow Fade, as it will make the slide hazy when viewed through an oil objective. Cover slip can be replaced with a fresh one if necessary. (push down with 1000 ul pipette tip, aspirate extra solution.)
7. The slide is now ready to be examined. Store slides in foil wrapped Petri dishes at -20C.