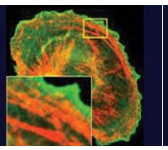
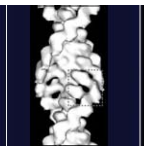
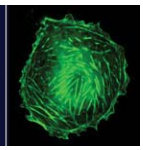

IMMUNOSTAINING & FIXATION OF CELLS - PBS METHOD

Stock Solutions: (make fresh each time)

A. *4% Paraformaldehyde fixative*
20 mls dH_2O - hot
2g paraformaldehyde
add 3 drops NaOH
25 mls dH_2O
5 mls 10x PBS

B. *1x PBS solution*
20 mls 10x PBS
190 mls dH_2O
20ul 0.1M NaN_3

C. *0.1% Igepal*
20 mls PBS/NaN3 solution
200 uL 1% Igepal

D. *1% BSA*
50 mls PBS solution
0.5g BSA

E. *Mounting solution*: 0.017g PPD +1.5 ml 7.5% Sodium Bicarbonate $NaHCO_3$
+ 0.3 ml 0.5M Sodium Carbonate Na_2CO_3
Add 120 uL above solution + 100 uL 10x PBS + 780 uL 50% glycerol (keep in dark)

Procedure:

1. Fix cells with 2 mls paraformaldehyde solution - 15 minutes

2. Wash 2x with PBS solution

3. Permeablize with 0.1% Igepal - 10 minutes

4. Wash 1x with PBS solution

5. Block with 1% BSA and add Rhodamine/Phalloidin (normally used at 1:1000)- 1ml per dish
   - prepare 1ml aliquots of solution and spin down 5 minutes in microfuge
   - avoid pellet, if any when adding to coverslip
   - incubate 30 minutes at room temperature

6. Add primary antibody + 1ml of BSA per dish. (antibodies normally used at 1 - 10µg/ml)
   - prepare 1ml aliquots and spin down 5 minutes, avoiding pellet
   - incubate 1 hour at 37° C

7. Wash 3 times with PBS solution for 5 minutes each time

8. Add secondary antibody + 1ml of BSA per dish.
   - prepare 1ml aliquots and spin down 5 minutes, avoiding pellet
   - incubate 30 minutes at 37° C

9. Rinse 1x and wash twice for 5 minutes each time

10. Mount slide: add 10 uL mounting solution to slide, remove solution from dish, carefully blot coverslip and quickly place down on slide. Blot gently, let dry, then seal.