Immunofluorescence of Semi-Thin Sections

1) Cut semi-thin sections and place in cluster in center of coated slides.

2) Get rid of Sucrose! --- very high affinity for fluorescence.
   A. Outline Sucrose Droplet with Diamond Scraper.
   B. Place Slides in Humid Chamber.
   C. Cover Generously w/ plain PBS (over Sucrose droplet).
   D. Wash for ~15 min.

3) Make Ammonium Chloride (weak base) 0.05M in PBS, for quenching (blocking) free aldehyde groups (26.5 mg/10 ml).

4) Shake PBS off of slide --- into garbage. Cover sections w/ Ammonium Chloride 0.05M. Quench for 15 min.

5) Weigh out 100 mg BSA in 10 ml PBS (sonicate briefly) = 1% BSA solution. Filter w/ Whatman #1 filter. Then take a 50 ml metric cylinder and fill it w/ 45 ml PBS and 5 ml 1% BSA = 0.1% BSA/PBS.

6) Shake Ammonium Chloride off of slides and cover w/ drop of Wash Buffer (0.1% BSA/PBS). Let remain for 10 min. Repeat 2 more times (total wash = 30 min).

7) Dilute Antibodies in 1% BSA. Need at least 50 ul antibody dilution per slide.

8) Shake off Washing Buffer. Wipe everything dry on slide except area where sections are. Add antibody. Two hour incubation.

9) Rinse off w/ 0.1% BSA/PBS w/ pipet (not directly on sections). Shake off. Cover w/ 0.1% BSA/PBS. Leave for ten minutes. Repeat two more times. (Total washing step = 30 min.)

10) If bridge is needed it is added here, diluted in 0.1M PBS/BSA. Incubate for one hour.

11) Rinse the same as in step 9.

12) Dilute Fluorescence Reagents in 1% BSA/PBS. Spin dilution for 5 min at speed 13 in Beckman Microfuge. Shake off buffer. Wipe slides clean except for area where sections lie. Add fluorescence. One hour incubation in light protected box.

13) Rinse same as in step #8, but last wash--- third time, just PBS.

14) Add coverslip:
   A. Shake and Wipe off Ab (like in step 7).
   B. Add drop of glycerol/PBS/PPD.
   C. Add 22x40 Coverslips.
   D. Seal with Nailpolish.

13) Observe.