Immunofluorescence assay (IFA) and Calcafluor White (CW) staining

We will perform IFA using *Colpodella* sp. from a diprotist culture containing *Bodo caudatus*. The cells were fixed in 5 % formalin and spread onto glass slidesto air-dry. Cells were permeabilized in 0.1 % Tri-X 100 for 5 min at room temperature (RT) and blocked in 3 % BSA for 30 min also at RT. Slides for CW staining will be used without permeabilization or blocking.

Each group will receive 2 slides for IFA and 2 slides for Calcafour White staining.

Immunofluorescence assay (IFA)

Tuesday

1. Place slides for IFA on the template provided (odd numbered slides are for antibodies; even numbered slides are for the negative control normal rabbit or goat serum). Add 10 µl primary antibodies and 10 µl negative control normal rabbit or goat serum to the spots indicated on the template. Carefully place the the slide into the large container containing moist paper towels (moist chamber). Incubate slides in the 37 degreeS incubator for 1 hour. (Slides can also be incubated at RT for 1-2 h or incubated at 4 degrees overnight.

   <u>Note:</u> #1, 3, 5 will be incubated with Rat anti-IMC antibodies
   #7 and 9 will be incubated with Rabbit anti-RhopH3 antibodies (686)
   All groups will place NRS and GS on the control slides

2. Use a squeezer to apply PBS to the surface of the slide to wash off the antibodies into a beaker. Do not contaminate the negative control slides with the antibodies from the slides. Place PBS in a coplin jar and using forceps place the slides in the PBS. Gently shake the coplin jar to wash the slides. Pour out the PBS and repeat two times. Using the forceps, remove the slides from the coplin jar and lean them against the sides of the jar or against the test tube rack for about 1 min. Shake off excess PBS.

3. Place the slides on the template and add the secondary antibody to the same spots that you applied primary antibodies. Rat primary antibodies will need Goat anti-rat secondary antibodies and rabbit primary antibodies will need Goat anti-rabbit secondary antibodies.

4. Carefully place the slides in the large container with moist paper towels (moist chamber) and incubate chamber at 37 degrees for 1 h. After 1h, was slides as in #2. After the last PBS wash, add distilled water to the coplin jar and wash the slides a fourth time.

5. Lean the slides against the coplin jar or test tube rack for about 1 min. Place a piece of foil over the slide.

6. Add 2 drops of fluoroshield mounting medium to the slides and gently place a cover slip on the drop. Using the needles/pins provided, carefully push down on the cover slip. Once the mounting solution has spread evenly over the smear, turn the slides upside

down over paper towel so that the excess mounting solution can be absorbed. Place a piece of foil over the slide.

7. Turn the slide right side up and apply clear nail polish around the edges of the coverslip.Leave the slides on the bench with a piece of foil over the slides. Allow the nail polish to dry.

8. Place the slides in the slide box provided.

Calcaflour White staining

1. Place the slide on the template for CW to ensure that the smear fits the shaded area of the template.

2. Add 2 drops of 10% KOH/glycerin on the smear. Use a pipet tip to gently mix the solution over the smear without contacting the smear on the glass slide. **Do not scratch the smear**.

3. Add 2 drops of Bactidrop Calcafluor White to the KOH on the slide and mix gently with the pipet tip

4. Stain for 5 min at RT.

5. Wash the slides twice with distilled water in a coplin jar.

6. Lean the slides against a coplin jar or test tube rack for about 1 min, shake the excess water off the slides and lay the slide face up on paper towel.

7. Add 2 drops of fluoroshield mounting medium to the slides and gently place a cover slip on the drop. Using the needles/pins provided, carefully push down on the cover slip. Once the mounting solution has spread evenly over the smear, turn the slides upside down over paper towel so that the excess mounting solution can be absorbed. Place a piece of foil over the slide.

8. Turn the slide right side up and apply clear nail polish around the edges of the coverslip. Leave the slides on the bench with a piece of foil over the slides. Allow the nail polish to dry.

9. Place the slides in the slide box provided.