# Calcium-EDTA Tutorial for the MicroCal ITC200

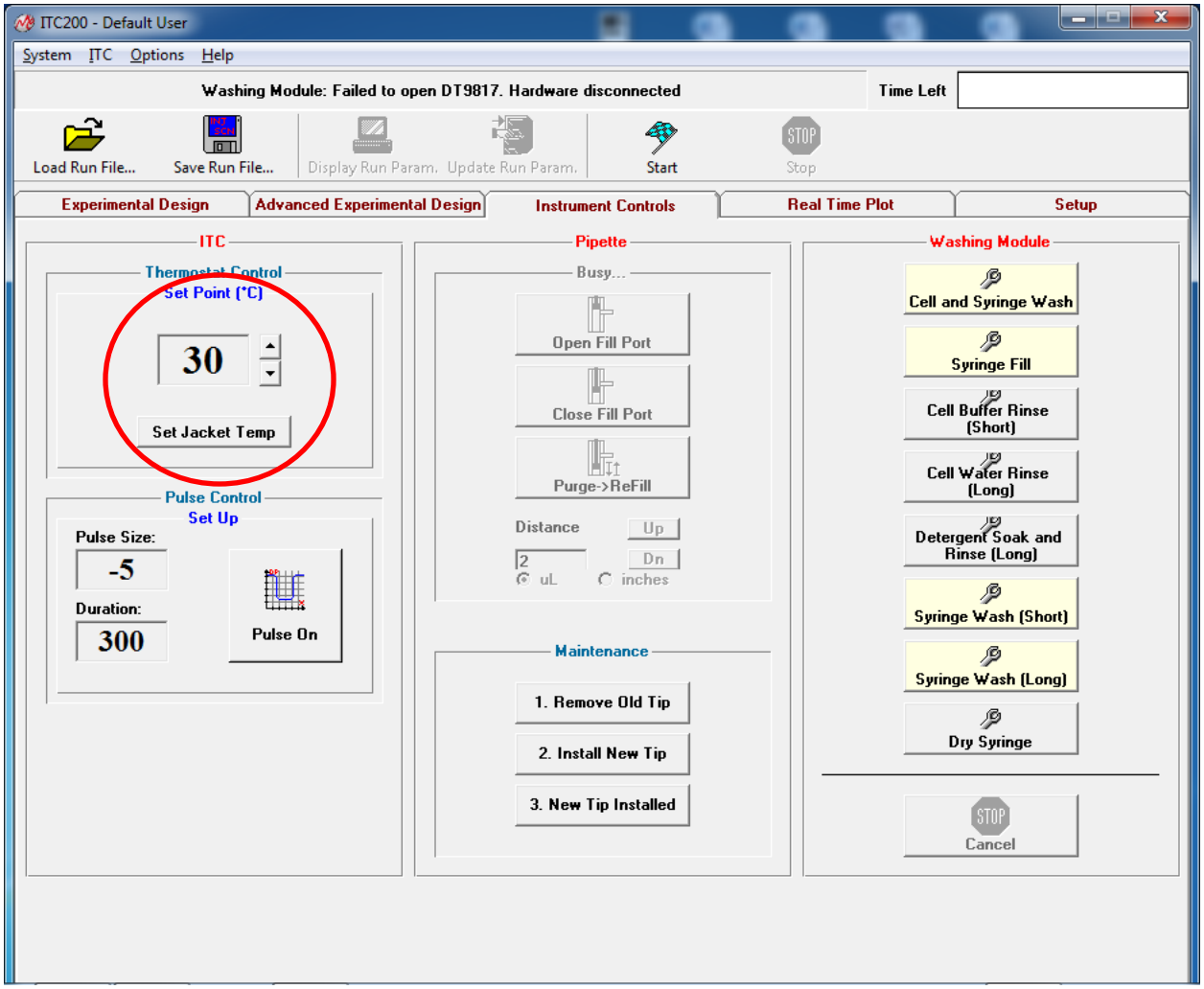
## Getting Started

1. The computer and the ITC200 are normally left on. If they have been switched off for any reason makes sure that you boot them in the order shown below. If you don't, you may encounter communication problems between computer and ITC.
   - Turn on the ITC 200 computer.
   - Turn on the Micro-Cal ITC200.
   - Launch the ITC 200 with Origin software.
2. The software will establish a connection with the instrument, and the instrument will then heat the cell to 30°C.
   - If you are planning on using a different temperature, change the temperature setting right away. This will save you time later.
   - Under the Instrument Controls tab (see figure below) change the Set Point temperature and click "Set Jacket Temp".

## Solutions Needed For This Experiment

| Name | Composition |
|---|---|
| EDTA solution | 0.4 mM EDTA, 10 mM MES pH 5.6 |
| Calcium solution | 5 mM Calcium Chloride, 10 mM MES pH 5.6 |
| MES buffer pH 5.6 | 10 mM MES pH 5.6 |

3. Please follow the separate instructions to make the solutions.
   - You will only get good data if your concentrations are precisely what you think they are and if all solutions have perfectly matched buffer.


ITC200 - Default User
System ITC Options Help
Washing Module: Failed to open DT9817. Hardware disconnected
Time Left
Load Run File...
Save Run File...
Display Run Param.
Update Run Param.
Start
Stop
Experimental Design
Advanced Experimental Design
Instrument Controls
Real Time Plot
Setup
ITC
Thermostat Control
Set Point (°C)
30
Set Jacket Temp
Pulse Control
Set Up
Pulse Size:
-5
Duration:
300
Pulse On
Pipette
Busy...
Open Fill Port
Close Fill Port
Purge->ReFill
Distance
Up
Dn
2
uL
inches
Maintenance
1. Remove Old Tip
2. Install New Tip
3. New Tip Installed
Washing Module
Cell and Syringe Wash
Syringe Fill
Cell Buffer Rinse (Short)
Cell Water Rinse (Long)
Detergent Soak and Rinse (Long)
Syringe Wash (Short)
Syringe Wash (Long)
Dry Syringe
STOP
Cancel

## Loading Sample Cell and Syringe

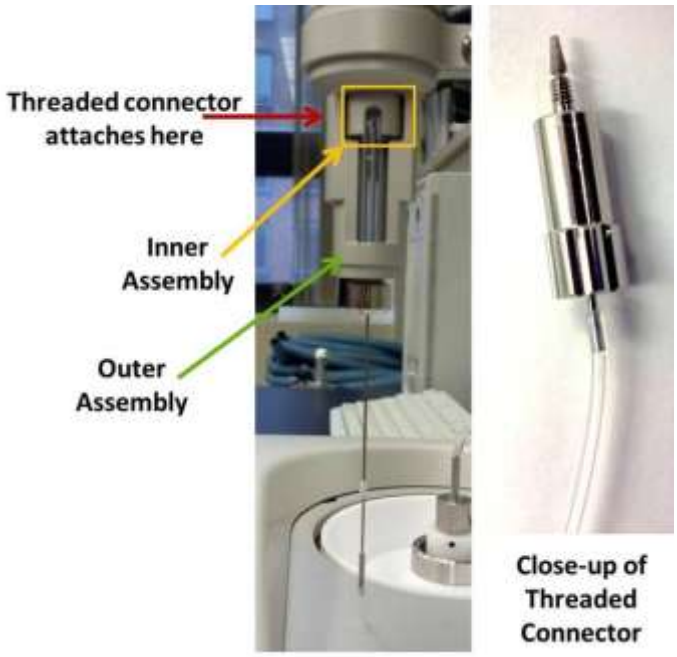
### Preparing the ITC 200 for sample loading

1. Remove the white cap from the cell and insert the cleaning apparatus into the sample cell. Make certain that the cleaning apparatus is pushed firmly into the sample cell.


2. Attach the fill adapter to the injection syringe.
   - To do this the syringe should be firmly seated in the wash position.
   - Align the inner and outer ring so that you can see the hole the connector is going to be screwed into. Stabilize the aligned assemblies with your fingers and screw the connector in. It should fit snugly. If it is too loose the syringe dry might not work correctly leaving Methanol in the syringe and the sample will not be drawn into the syringe right.
   - **If you over tighten it, you will break the syringe!**

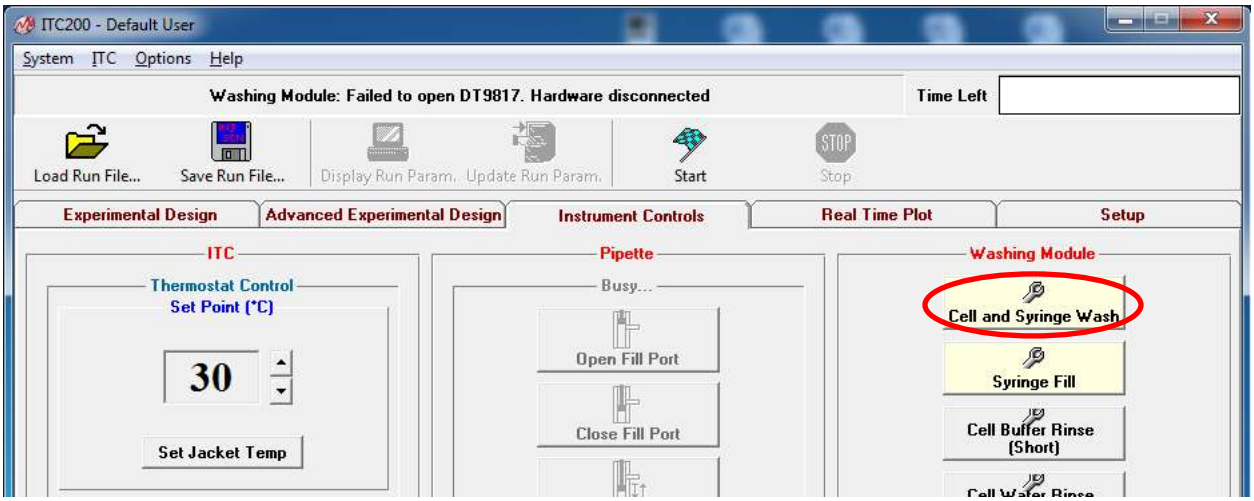
Note:

This is how the instrument should have been left by the previous user)




Close-up of Threaded Connector

3. Click on the Instrument Controls tab. (See Figure above)
4. Under the Instrument Controls → Washing Module, click on the "Cell and Syringe Wash" button. The "Wash Module" will first flush the cell with water. Once it is done with the cell it will sound a small beep. After that it will flush the syringe with water and methanol and dry it with air.




## Loading the Sample Cell:

5. After the beep, while the syringe wash is going on you can start filling the cell.
6. Use the dedicated loading syringe to fill the cell. It has a protective sleeve over the needle to avoid scratching the sample cell. **Do not use any other syringe!!!!**
7. Fill the sample cell with the EDTA solution.
   - To fill the cell slowly, draw up ~300µl of EDTA solution into the loading syringe. Avoid air bubbles. If you inject air bubbles into the sample cell your experiment will not work!
   - Slowly insert the syringe into the cell until the tip **gently** touches the bottom of the cell. Pull the syringe up slightly, so that it is no longer pressing against the bottom.
   - Begin to inject the EDTA solution into the cell.
   - Once you have injected ~150 µl into the cell, inject the rest of solution in short pulses to dislodge any bubbles that may be clinging to the walls of the cell. Continue in this manner until you see the EDTA solution well up around the syringe.

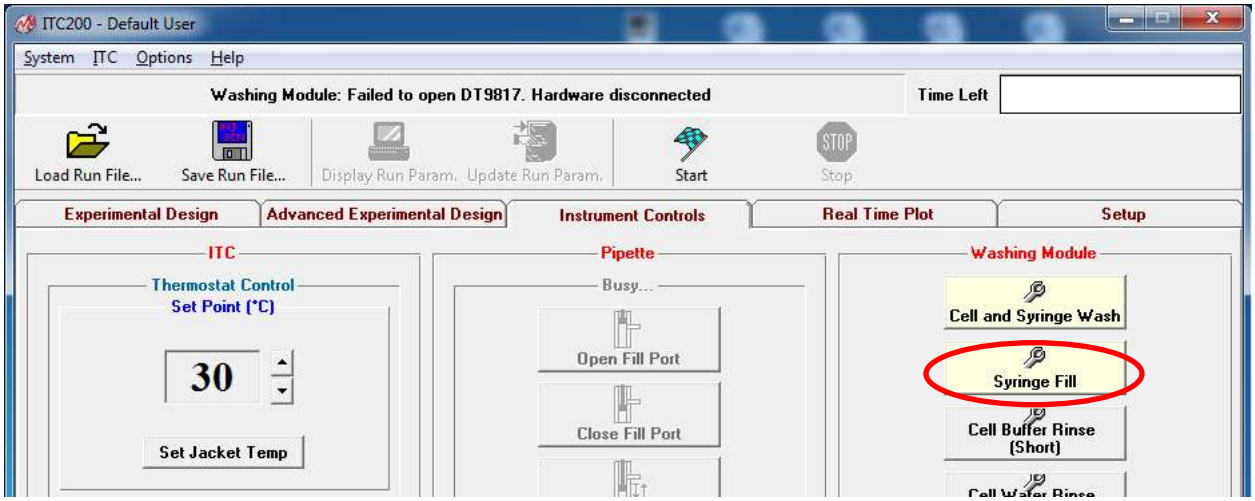
- Draw the syringe out of the cell and gently place it on the lip between the plastic collar and metal of the cell. Draw up any excess liquid.
- Soak the cell in the EDTA solution for 2 minutes. This allows any water remaining on the cell walls to diffuse into the EDTA solution.

**8.** Remove the EDTA solution from the sample cell using the loading syringe and discard the solution.

**9.** Refill the sample cell with fresh EDTA solution in the same way as described above.

## Loading the Titration Syringe

10. Fill a 200 µl PCR tube with 80 µl titrant (5 mM Ca solution). Do not close the lid. Insert the filled, open tube into the tube holder. Make sure it sits all the way down with the lid fitting into the slot provided (see photo below).
11. To fill the syringe, go to the Instrument Controls tab and click Syringe Fill.




12. Follow the prompts.
    - You will be asked to place the syringe pipette into the rest position. After that the plunger of the syringe will be moved down.
    - Next you will be asked to place the syringe into the loading position. Be careful when doing this and make sure that the needle does not get bent when inserted into the PCR tube with the titrant.

- Next, the washing module will fill the syringe. Observe the process carefully. If a large air bubble remains on the top of the solution you can restart the loading process but leave the syringe in the loading position for all steps. This will most likely eliminate the bubble.

13. Disconnect the fill adapter from the syringe.
14. Insert the syringe pipette into the cell.

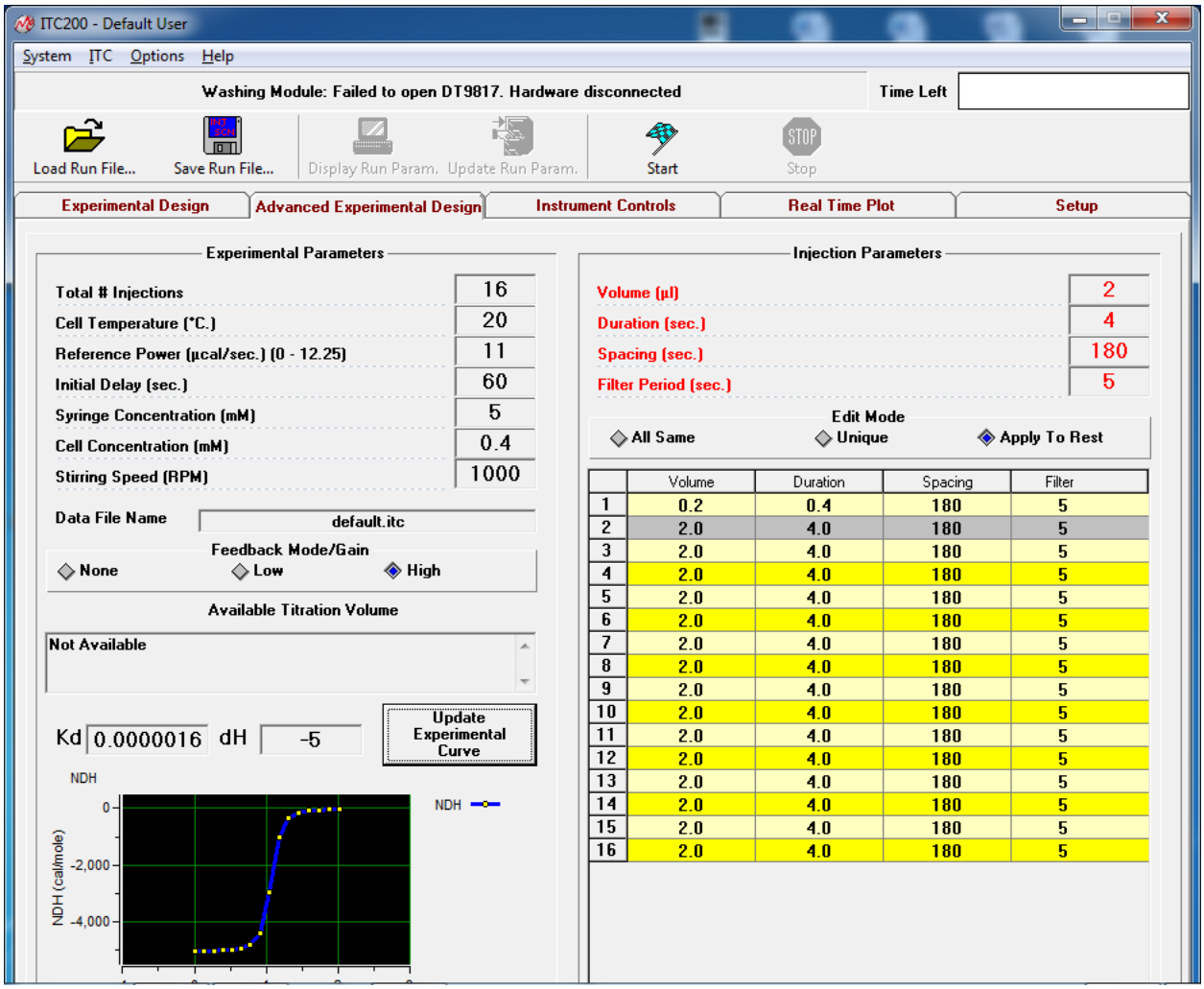
Rest position

PCR tube with titrant

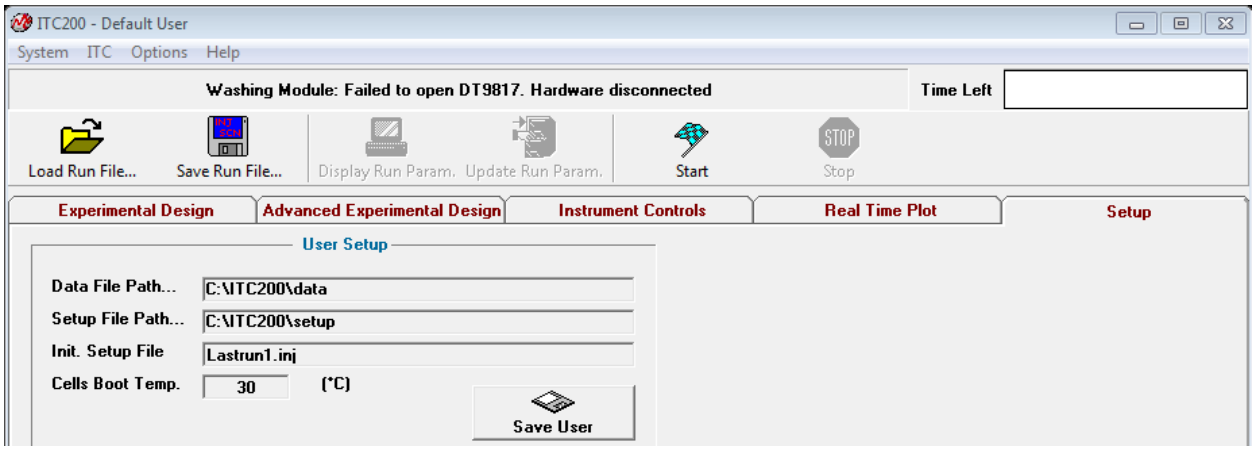
Load position

## Setting Run Parameters

15. Click on the Advanced Experimental Design tab and enter the parameters shown below in the indicated fields. Don't forget to change the filename.



16. Click the **Setup tab** and change the path for data storage to your folder.




Note:

If you want to save time you can set the temperature of the cell right after you loaded it to the desired start temperature. To do this click Instrument controle and set the jacket temperature to your desired value.

## Starting the Run:

17. Switch off the overhead light if you used it.
18. Double check that the fill adapter is disconnected and press the start button.
19. The system will adjust to the chosen temperature.
20. Once the temperature is reached, the inner assembly of the titration syringe will begin to spin.
21. The system will adjust the reference power.
22. Once the reference power is stabilized the run will begin.

Note:

If it takes much longer than 10 min to adjust the reference power, something might not be quite right.

23. Check that you switched off the overhead light (it will heat the cell from above, making it harder to stabilize the reference power).

24. You can consider reloading the cell, since air bubbles might affect the stabilization of the reference power. To do that, stop the run, return the syringe pipette into load position, and remove the sample from the cell using the sample loading syringe. Reload the cell, following the procedure above.

## Clean Up After the Run:

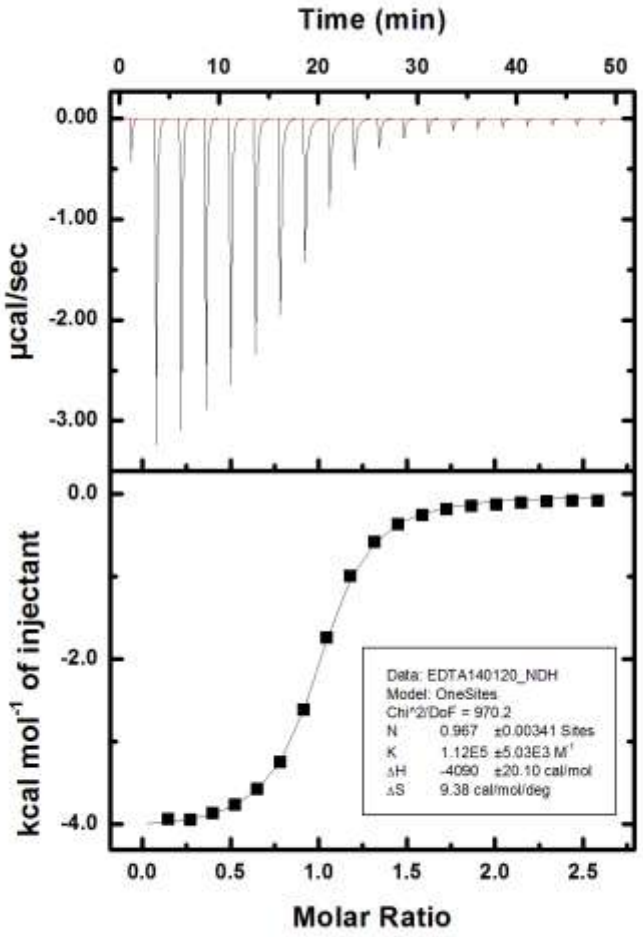
25. Remove the syringe pipette from the cell and insert firmly into the wash position.
26. Attach the fill adapter to the syringe pipette as described above.
27. Remove the solution from the cell using the loading syringe. You can keep the solution to double check the concentration.
28. Rinse the cell with 3 x300 uL water using the loading syringe.
29. Rinse the cell with 5% Contrat.
30. Rinse again with 3x water.
31. Insert the long needle of the cell cleaning apparatus into the sample cell and push down carefully until the O-ring has sealed.
32. Go to the instrument control tab and perform a "cell and syringe wash".

Note:

If you have very high protein concentration, liposomes, or you suspect that your sample could have precipitated please perform a detergent soak and rinse by click the "detergent soak and rinse" button and following the prompts.

- After the detergent soak and rinse is complete and the cell has cooled down, remove the detergent solution.
- Rinse the cell with 3 x300 uL water using the loading syringe.
- Insert the long needle of the cell cleaning apparatus into the sample cell and push down carefully until the O-ring has sealed.
- Go to the instrument control tab and perform a "cell and syringe wash".

33. Leave the instrument in this configuration.




**If you run into any problems or have questions about ITC let Dr.Erbse know.**

**Annette Erbse, Office C316, phone 2-0528, erbse@colorado.edu**