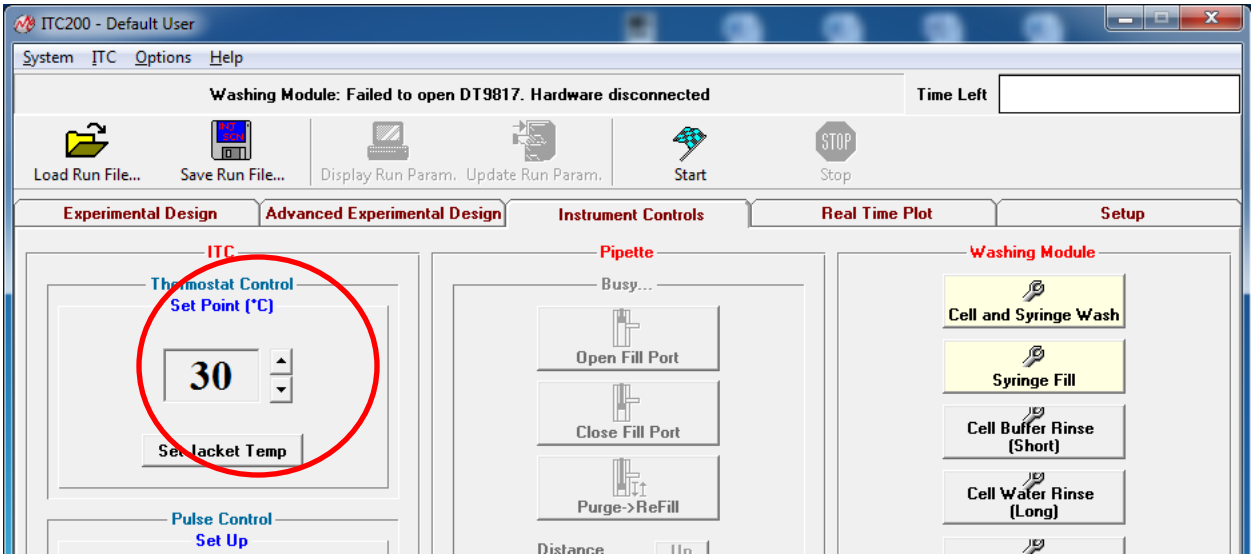
Water-Water Titration for the MicroCal ITC200

Goal:

Water-Water titrations are a good way to check for possible problems, especially the cleanliness of the ITC and your skills in cell filling. If a water-water titration works well the instrument is in good working order and your cell filling procedure is correct.

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. You can change the temperature setting right away to 25°C. This will save you time later.
3. Under the Instrument Controls tab (see figure below) change the Set Point temperature to 25°C and click "Set Jacket Temp".



4. Fill 5 to 10 ml fresh MiliQ water into a clean 15 ml conical tube.

5. If you are using the water-water titration for trouble shooting you should replace the water in the water wash bottle and the contense of the buffer wash bottle with fresh MiliQ water and refreshen the Methanol in the Methanol wash bottle.

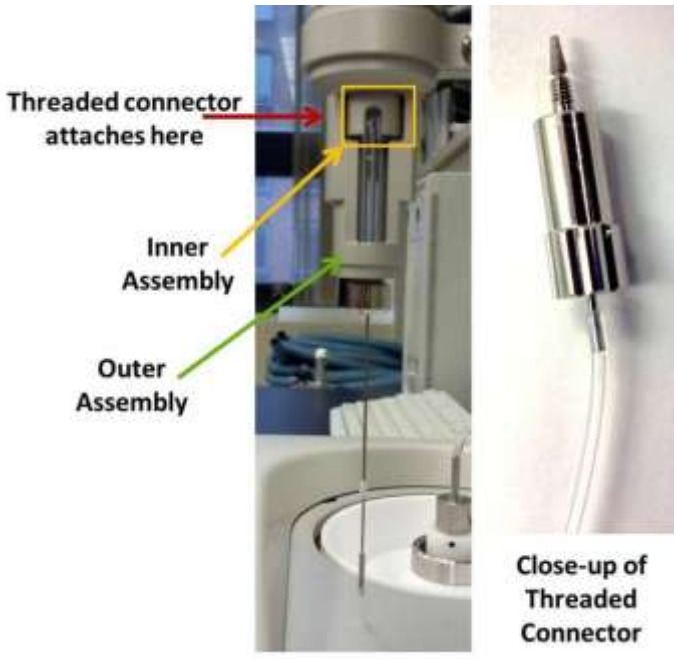
Loading Sample Cell and Syringe:

Preparing the ITC 200 for sample loading

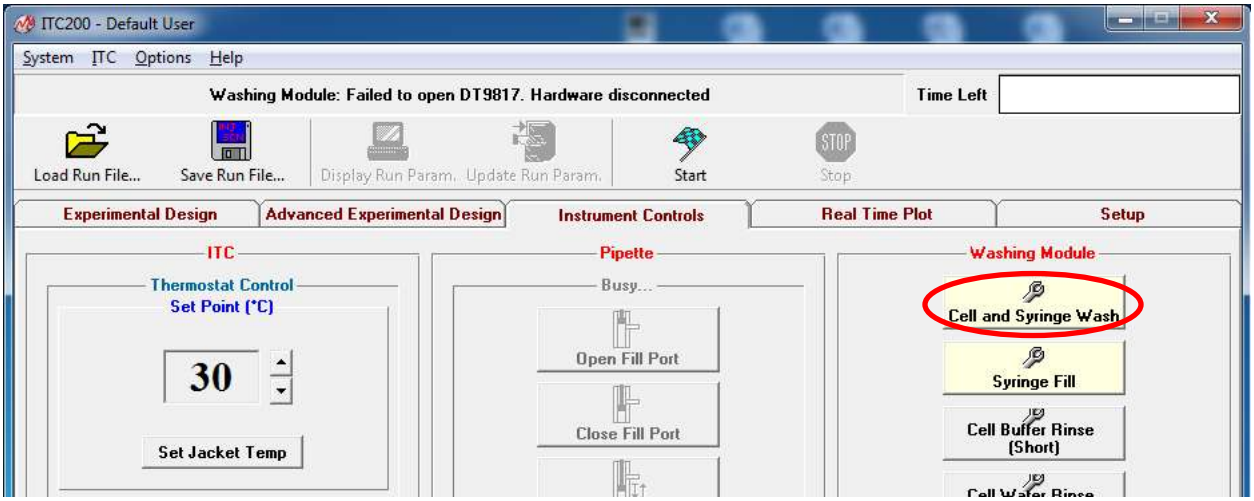
1. Remove the white cap from the cell and insert the cleaning apparatus into the sample cell. Make certain that the cleaning apparatus is pushed firmly into the sample cell.


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 drying of the syringe might not work correctly leaving Methanol in the syringe and the sample will not be drawn into the syringe right. **If you overtighten it you will break the syringe!**

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



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/empty the cell. It has a protective sleeve over the needle to avoid scratching the sample cell. **Do not use any other syringe!!!!**
7. Insert the empty loading syringe into to the cell until it **gently** touches the bottom. Pull the syringe up a millimeter so that it is no longer pressing against the bottom. Remove any remaining liquid from the cell.
8. Wash the loading syringe 3 times with fresh, clean MiliQ water.
9. Fill the sample cell with fresh MiliQ water from the 15 ml conical.
    - To fill the cell, slowly draw up ~300µl water 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 water 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 water 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 water for 2 minutes.

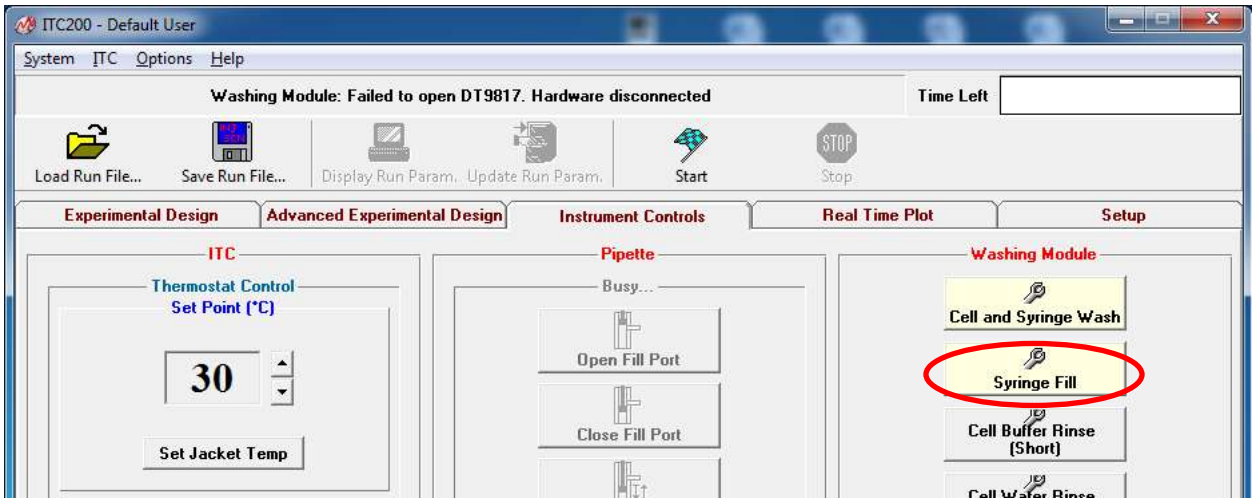
10. Remove the water from the sample cell using the loading syringe and discard the solution.

11. Refill the sample cell with fresh water from the 15 ml conical in the same way as described above.

Note: If you do the water-water titration for trouble shooting you should refill the reference cell with fresh MiliQ water as well.

Loading the titration syringe:

12. Fill a 200 µl PCR tube with 80 µl water out of the same 15 ml conical. 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).
13. To fill the syringe, go to the Instrument Controls tab and click Syringe Fill.



14. 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.

15. Disconnect the fill adapter from the syringe.
16. Insert the syringe pipette into the cell.

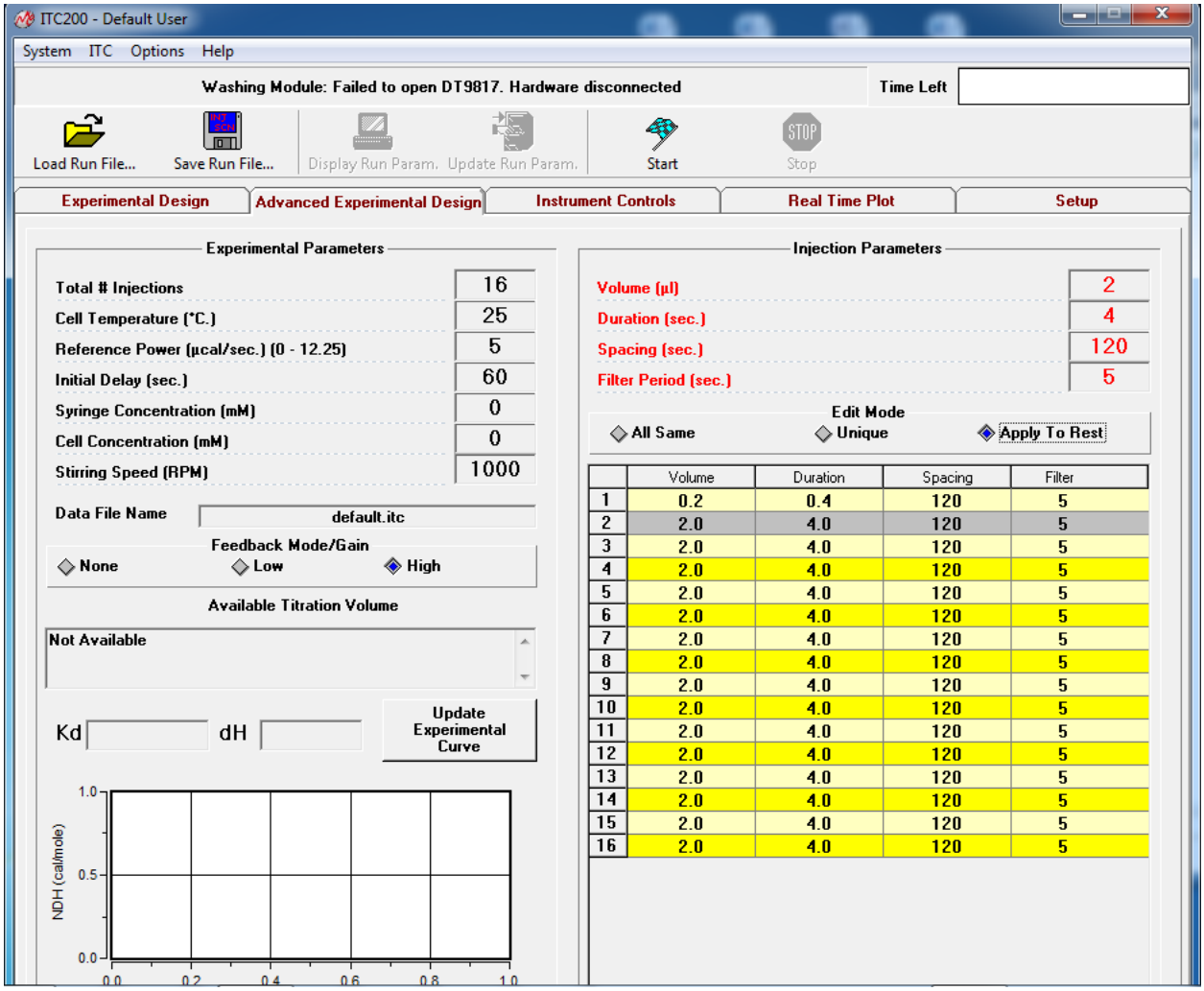
Rest position

PCR tube with titrant

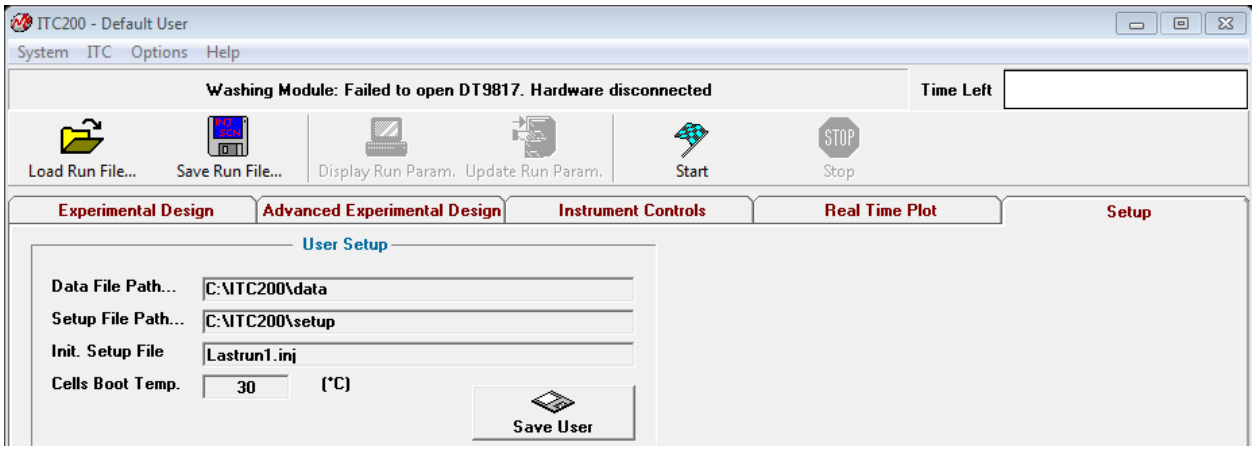
Load position

Setting Run Parameters

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



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



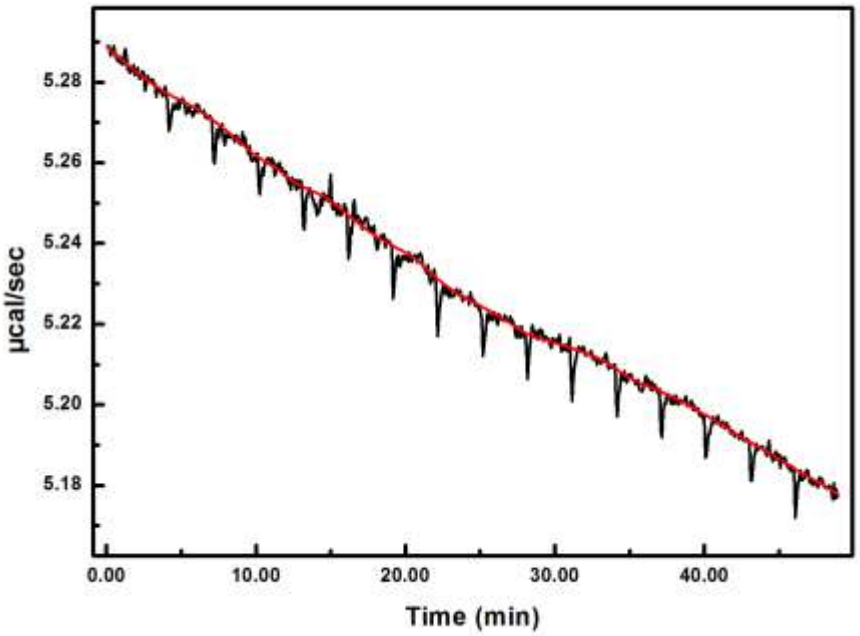
Starting the Run:

19. Switch off the overhead light if you used it.
20. Double check that the fill adapter is disconnected and press the start button.
21. The system will adjust to the chosen temperature.
22. Once the temperature is reached, the inner assembly of the titration syringe will begin to spin.
23. The system will adjust the reference power.
24. 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.

25. Check that you switched off the overhead light (it will heat the cell from above, making it harder to stabilize the reference power).
26. 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.

Result:
The plot below shows a typical result for a normal water-water titration.



Clean up after the run:

27. Remove the syringe pipette from the cell and insert firmly into the wash position.
28. Attach the fill adapter to the syringe pipette as described above.
29. Remove the solution from the cell using the loading syringe. You can keep the solution to double check the concentration.
30. Insert the long needle of the cell cleaning apparatus into the sample cell and push down carefully until the O-ring has sealed.
31. Go to the instrument control tab and preform a "cell and syringe wash".
32. Leave the instrument in this configuration.

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

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