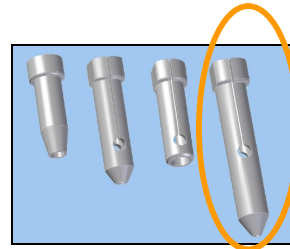
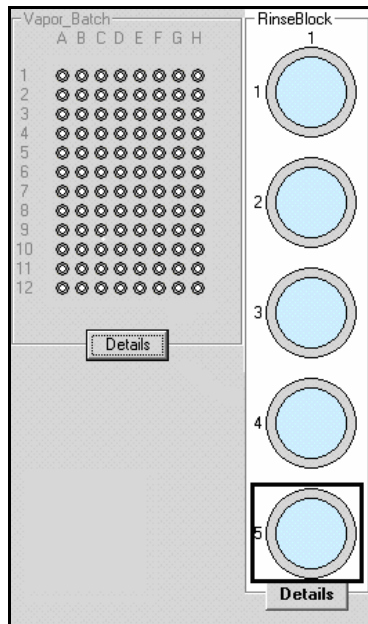
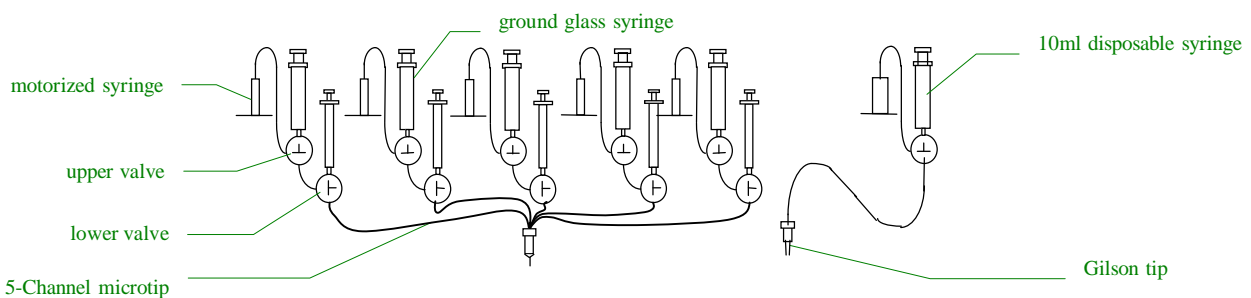


Hardware Preparation

1. Clean a Douglas Vapor Batch [VB] plate with compressed air, and place it on the top left corner of the Plate Loader as shown below.
2. Place the Rinse Block to the right of the VB plate with a bottle in well five. Fill this bottle 95% full with paraffin oil.



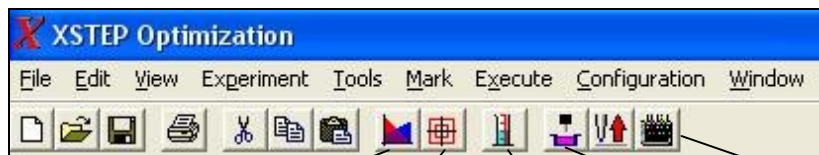
3. Connect a 5-channel Microtip to the 5 channels of Oryx. Place the tip in the 5-channel "collet" (holder) on the left-hand Z-arm of the Plate Loader.
4. Place a disposable Gilson pipette in the right-hand V-arm of the plate loader. Flush it with air if there is any oil or solution in it.
5. Fill the ground glass syringes of the upper valves with degassed pure water and replace them.



Creating an Experiment File

6. Switch on the computer and the MCC control unit. Click on the Start button, Programs, Douglas Instruments, then XSTEP Optimization.
7. If you want to create a new file, select File, then New Project. Now select File again, then Save As and provide a name for the new file. Alternatively, to work with an existing file, select Files, Open Project.
8. If you have created a new file, or if you want to modify the names or concentrations of the stock solutions of an old file, double click in the ingredients shown on the left of the spreadsheet. Now modify the concentrations and names of the ingredients displayed. Fill in the viscosities of PEG solutions etc. As a guide, the viscosity of 40% PEG 8K is 40, while the viscosity of water is 1.
9. Click on Experiment, Dispensing Parameters. Specify the Volume of the Droplets and the Number of Stirs. Ensure that the Laying On and Oil Dropping options are selected. If viscous solutions such as PEG are to be dispensed, increase the Pause to 4 seconds.

10. Return to the spreadsheet. Each group of numbers shows the concentrations of the 4 ingredients in a well (other than water). Double click on a well to edit its values. To interpolate values between two wells highlight the appropriate block and select **Tools, Interpolate**. To optimize around a well, click on it and select **Tools, Autodesign**. Wells marked for execution are colored (by default) blue. When the experiment is ready, click on the save button to save it to disk.

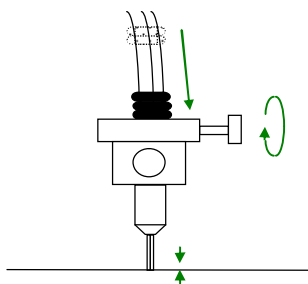


- Interpolate Selected Block Auto Design around Selected Well Display Volume Usage Execute Experiment Go to Front Panel

Debubbling and Setting the Height of the Microtip

11. At the beginning of each day the system will require debubbling. Place a small bottle or vial under the Microtip in the Z-arm, select from the menu: **Execute, Debubble**, and follow instructions. Any air bubbles that were present at the top of the motorized syringes should have passed out into the connecting tubing.
12. Now follow instructions and remove the air bubbles from the connecting tubing:
- Remove the PTFE tubing from the needles of debubbled motorized syringes.
 - Expel water and air bubbles from the tubing using the ground glass syringe.
 - Reconnect the tubing carefully, ensuring no air bubbles re-enter.
13. Occasionally there may be bubbles between the upper and lower valves. Turn the top valves to the **flush position** (⊥) and flush the bubbles out through the microtip with the ground glass syringes.
14. If you suspect that the 5-channel Microtip is not set to the correct height select from the Menu: **Execute, Install Tip**. The arm(s) will move to its lowest position. Follow the instructions on the screen:
- Move one o-ring towards the tip and the other two away from the tip
 - Adjust the height of the Microtip until it is just touching the table by pushing through the lower o-ring
 - Mark the height by moving the top two o-rings down to the top of the collet.

Execute	Configuration	Window	Help
Zero Motors			Ctrl+Z
Debubble			Ctrl+D
Prepare Syringes			
Flush Channels			Ctrl+F
Install Tip			Ctrl+I
Test Tip and Plate Position			Ctrl+T
Home PlateLoader			Ctrl+H
Load Protein			
Set Protein			
Recover Protein			
Execute Experiment			Ctrl+X
Execute Selected Wells			Ctrl+W



Running the Experiment

15. Selecting **Execute, Execute Experiment** executes the experiment automatically. This will take about 5 minutes for 24 wells when viscous solutions are used. On completion, you may wish to print the experiment - select **Files, Print**. Alternatively you can select a well or block of wells and right click on them to **Execute selected wells**. 19. After dispensing, add 3 ml paraffin to the VB plate. Place the plate in an incubator at the desired temperature.
16. Quit **Front Panel** before turning off your computer so that the motor positions are stored for the next use of the equipment.
17. Turn off the MCC by pressing the power button on the front of the machine.