

*Douglas Instruments Ltd.*

# Oryx Nano

*Hardware Manual*



Description and Configuration

Revision 5.3, August 2006

Compiled by Patrick Shaw Stewart, Peter Baldock and James Smith

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Douglas Instruments Limited

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<http://www.douglasinstruments.com>

## Warnings



**Always turn off the MCC and disconnect the power cord from the mains supply** before disassembling any parts of the machine or unplugging the leads for the Chassis or Plateloader

For **indoor use only** within the temperature range of 4-30°C 0-75%RH (Non-condensing)



Oryx Protein Crystallization machines are designed for use with **non-hazardous materials only**. Should the user choose to process hazardous materials it would be the user's responsibility to observe any special handling procedures.



The **Red stop button** on the MCC will terminate any movement of the plateloader, arms and syringes. The purpose being to avoid spillage or other loss of oil, protein or reagents. The machine complies with all Essential Health and Safety Requirements of the EU Machinery Directive for CE Marking

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## Specifications

Power: 100-240 Vac Universal Input; 5A: 50Hz ~ 60 Hz

Continuous sound pressure level in normal operation at the operator's workstation: below 70dB(A) – ear protection need not be worn.

Manufactured by: Douglas Instruments Ltd.  
Douglas House, RG17 7HD, UK  
<http://www.douglas.co.uk>



Type: ORYX CRYSTALLIZATION MACHINE

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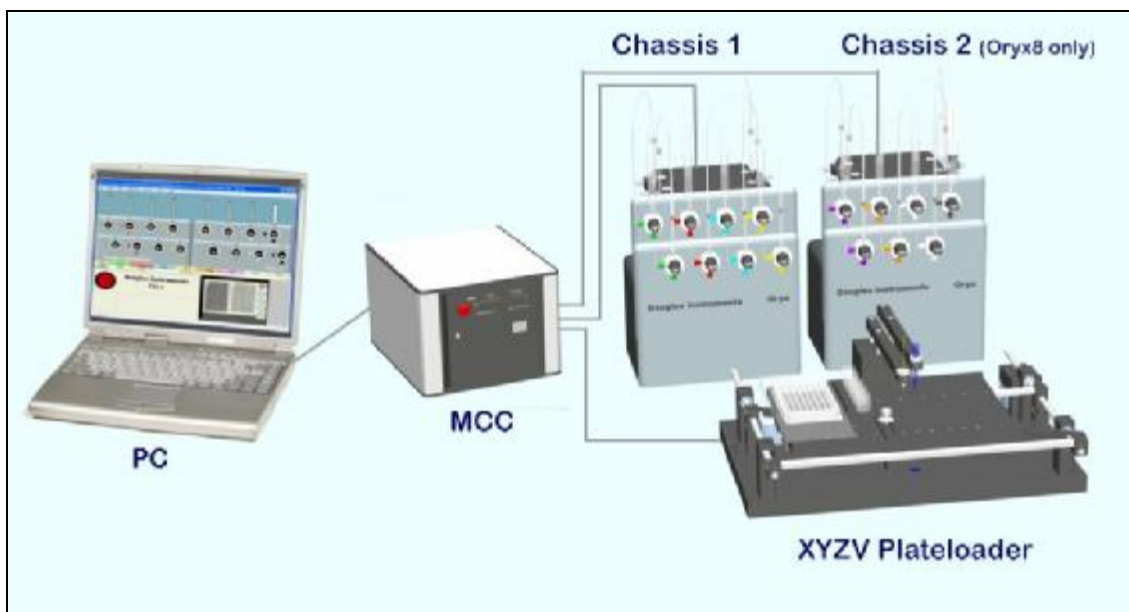
## SETTING UP THE SYSTEM

### Software Installation

Follow the instructions in the document on the CD called Installation Instructions (Long).rtf entitled "**Installation of Douglas Instruments Crystallization Software**" to install the crystallization software.

### Layout of the Hardware

The layout of OryxNano is shown in the figure below (ignore Chassis 2). The system is driven by a PC, which is connected to the MCC (Motion Control Center). The motorized syringes and the XYZN Plateloader are in turn driven by the MCC using stepper motors. The MCC is a computer that generates the steps for the stepper motors, logs their positions etc. It drives the motors using 2 DB6 stepper motor driver cards.



Oryx Hardware layout

## Recording the Positions of Motors

When the system is first set up, the motors must be rezeroed using the *Front Panel* program, as described below.

After this, the software will automatically log the positions of all of the motors in the system and it will save these positions to disk at the end of each run. This means that it is not necessary to rezero the motors each time the computer is switched on. Only rezero if you have some reason to believe that the system may be incorrectly positioned. However, you must not switch off the computer without quitting all crystallization software. If you do this, you will be forced to rezero the next time that you use the system.

## Unpacking

Take the Chassis, MCC Motion Control Center, and the XYZN Plateloader out of their packaging. Unwrap the Z- arm. Take the motorized syringes and ground-glass syringes out of their packaging.

## Electrical Connection

1. All cables are individually labeled. Use the **long 9-way D-type cable** to connect the port labeled "serial port" on the MCC to the serial port (COM1) of the computer.
2. Follow the labeling to connect MCC to the Chassis and Plate Loader using the two **37-way D-type cables**

The MCC also possesses outputs for a screen and keyboard. These can be used to diagnose malfunctions.

## Installing the Z Arm

Now install the Z-arm as follows:

1. Unwrap the Z-arm.
2. Remove the cardboard or plastic protective sleeving from around the Z lead-screw.
3. Lower the Z-arm into position and allow the leadscrew to settle on the centre of the motor inside the Z-box. The arm will automatically be pulled in to the correct position on the first rezero of the table.

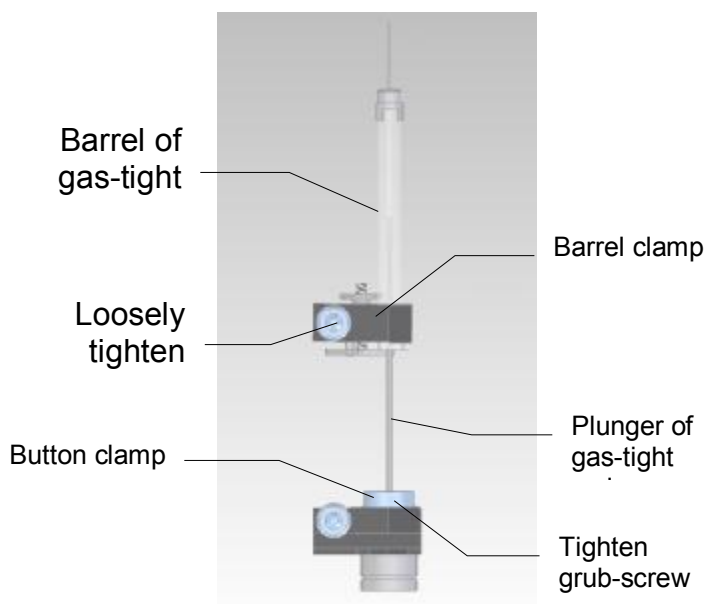
## Rezeroing Motorized Syringe Drivers

1. Switch on the MCC. Wait for around 20s to allow it to fully boot up (All red and green lights should go out). Switch on the host PC, and allow it to boot up.
2. On the PC, click on the start button, then *Program Files | Douglas Instruments | Front Panel*. Allow *Front Panel* to load.
3. Click on *Actions | Rezero*.
4. Make sure that the check boxes are checked for all of the syringes, and click on *continue*.
5. If the 100  $\mu$ l syringes are already installed, turn the top row of valves as indicated. Click *Rezero*
6. Wait for around 30 seconds while the syringe drivers move to their lowest positions.

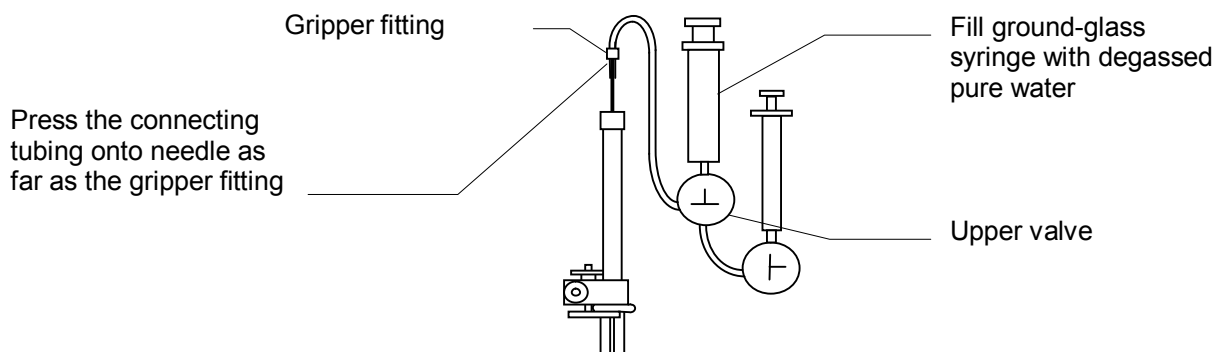
The motorized syringe drivers should now be in their **lowest** positions. When they reach the end of their travel, the syringe drives will miss steps and make a rattling noise. This will not harm the syringe drivers in any way. When you are sure that **all** of the syringe drives have reached their lowest positions, you can save time by clicking on the large red *stop button* to end the procedure.

## Mounting 100 ml Gas-Tight Syringes on Motorized Syringe Drivers, and Filling them with Water

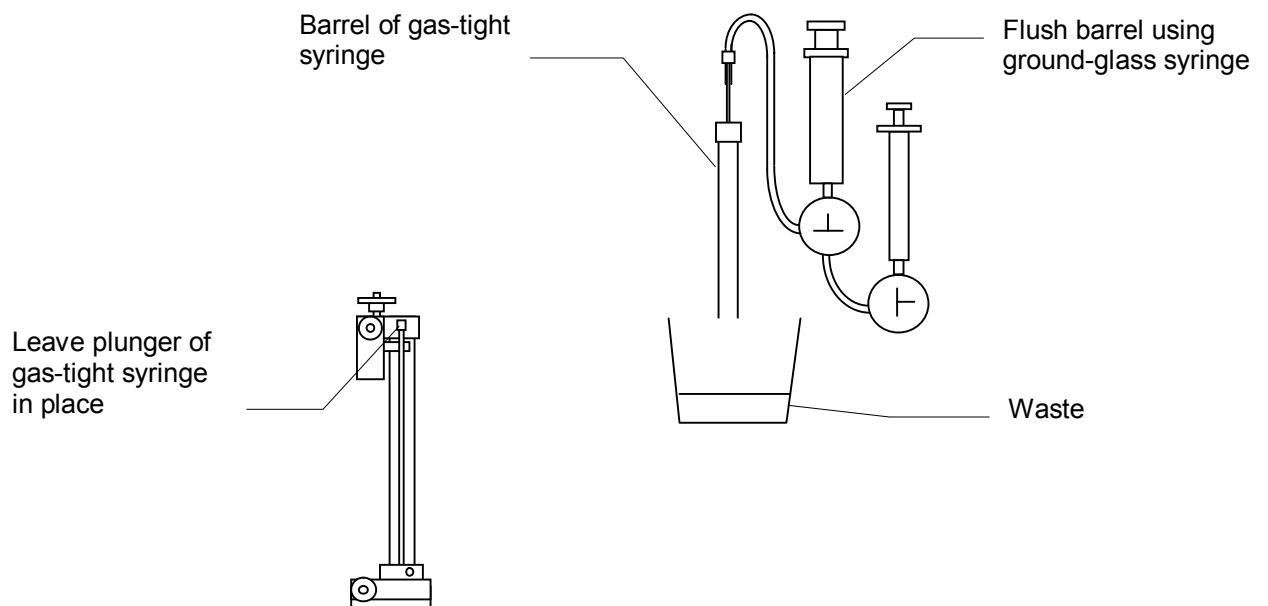
1. Prepare about 250 ml of degassed pure water. This will prevent bubbles forming, and is essential for accurate dispensing.
2. Rezero syringe drives as described above.
3. Place a Hamilton Gas-Tight syringe in position on the first motorized syringe driver. Loosely clamp the barrel with the **barrel clamp**.
4. Place the **plunger** of the gas-tight syringe in the **button clamp** and tighten the grub-screw to fix the plunger.



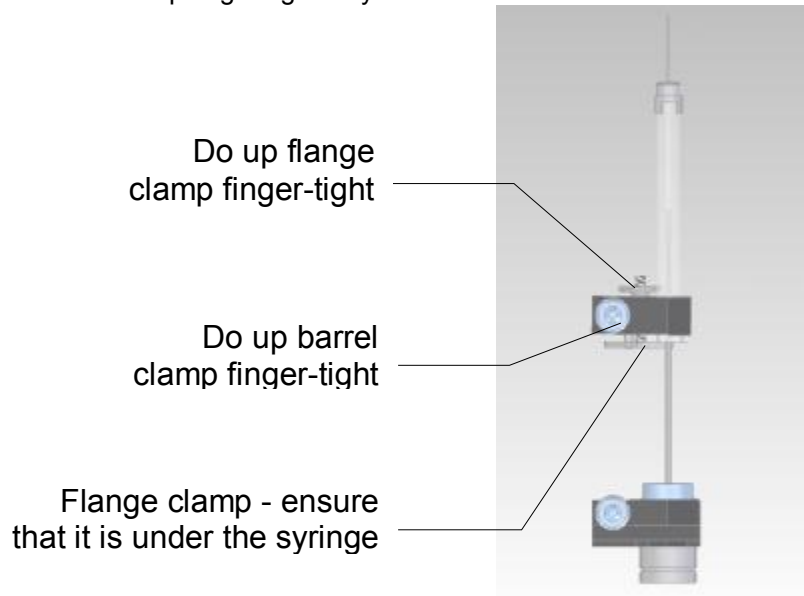
5. Connect the needle of the gas-tight syringe to the upper valve by pressing the connecting tubing onto it as far as the gripper fitting.
6. Fill a ground-glass syringe with degassed pure water and place it in the female Luer connection on the upper valve.



7. Release the barrel clamp, and remove the barrel of the gas-tight syringe, leaving the plunger in position.
8. Place the barrel over a waste container, and flush all air and bubbles out of the connecting tubing and gas-tight syringe. To ensure that no bubbles are left in the upper valve, tap the plunger of the ground glass syringe a few times. Flush away any bubbles that appear into the waste container.



9. Mount the gas-tight syringe onto the motorized syringe driver as follows:
  - a. Slacken the flange clamp – labeled below
  - b. Feed the plunger back into the syringe barrel
  - c. Place the barrel into the V on the top of the syringe driver nose, *ensuring that the flange of the syringe barrel is between the nose and the flange clamp*
  - d. Tighten the barrel clamp
  - e. Tighten the flange clamp. The flange clamp and the barrel clamp should be done up finger-tight only.



10. Turn the upper valve to the flush position (┤), and the lower valve to the dispense position (┴). Place a waste beaker under the lower valve. Flush air and bubbles out of the connecting tubing between the upper and lower valves.
11. Repeat steps 1-8 for the remaining low volume channels.

## Testing the Performance of the Plateloader

1. Start *Front Panel*
2. Click *Options | Advanced Menu Items* - to turn on Advanced Menu Items
3. Click *Options | Administrator Mode* – to turn on Administrator mode
4. Click *Plate Loader | Diagnostics | Test Axis*
5. Set *Axis* to X, *Speed factor* to 1.3, *Amplitude* (mm) to 10, *Oscillations* to 10, *Places to test* to 5, and *Starting at* to 0.0. Click *Test*.
6. If the Plate Loader misses steps, inform Douglas Instruments of the problem. Then reduce the *Speed factor* to 1.2 and repeat step 4 (again, try five times). If this fails, continue reducing the speed.

7. Follow a similar procedure for the Y, and Z axes. If the Plate Loader misses steps, reduce the speed.
8. Click *Options | Administrator Mode* – to turn off Administrator mode
9. Click *Options | Advanced Menu Items* - to turn off Advanced Menu Items

## Realigning the Plateloader

1. Follow steps 1-3 above to turn on Administrator mode
2. Click *Plate Loader | Clear Tip Offsets* - this clears any adjustment made to the tip position.
3. Install the 'mounted needle' into the Z-arm (you should find this in the red plastic box)
4. Click *Plate Loader | Diagnostics | Align Table and Arm ...*
5. First run the 'Find Index Positions' option. – This will jiggle the table many times while it finds the optimum offsets for the home position.
6. Now do 'Align Pointer with Table Dowel' you can adjust the table left/right. forward/back to get the needle in the centre of the dowel in the table This is a stainless pin near the 4 screws in the table, it should be obvious when it goes there as it should not be out by more than about 1.5 mm. If it is out by a lot, the Plateloader may have had a heave knock during shipping and the z-box may have shifted.
7. Do not adjust the arm up and down at this point (you may use the 'Lower' / 'Raise' buttons, but don't try to make the needle touch the dowel using the arrow buttons)
8. When the needle is centered, and with the arm in the 'lowered' position, click 'Save'. Add a comment to the end of the message displayed if you wish.
9. Your table should now be aligned properly.
10. Click *Options | Administrator Mode* – to turn off Administrator mode
11. Click *Options | Advanced Menu Items* - to turn off Advanced Menu Items

### Please note:

When you align the tip on the destination plate in an experiment these offsets are used on all other plates. If the tip is bent and the end of the tip is far away from the centre of the collet then it may appear to be off centre when it loads protein. If you look closely you should see that the end of the tip is in the centre of the protein tube when it starts to go down, but the collet may be so far off centre that it hits the side of the tube.

It is best to try to straighten the tip by hand or to adjust the tip in the guide loop to be pointing straight down and in the centre of the collet before starting an experiment.

## Rezeroing all Motors of the Plate Loader

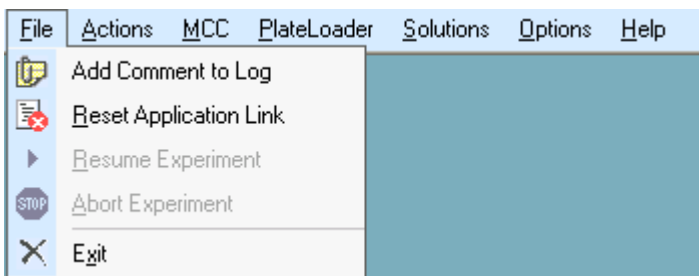
1. Switch on PC and MCC, and start the program *Front Panel* as described above.
2. *Actions | Rezero*. Follow instructions to rezero Syringe Drives, Z, Y and X motors.

The Z motor will make a loud rattling noise when it is rezeroed. This noise is caused by the motor missing steps. This will not harm the system in any way. When the loud noise starts it can be cut short by pressing the red Stop Button (or by clicking on the red Stop Button on the screen).

If this rezeroing routine is not correctly carried out, mechanical errors may occur (e.g. the microtip may be damaged, and the gas-tight syringes may be broken). However, it need only be carried out during the initial setup, or if there seems to be an error with the motor positions. Normally the motor positions are saved to disk after use of the system, and rezeroing is not necessary.

## FRONT PANEL MENU ITEMS

### File Menu



#### Add Comment to Log

Use this Command to add a comment to the current application log file.  
Mostly used for debugging problems.

#### Reset Application Link

Used to re-establish a link with another application.  
Mostly used for debugging.

#### Resume Experiment

Resume Current Experiment.

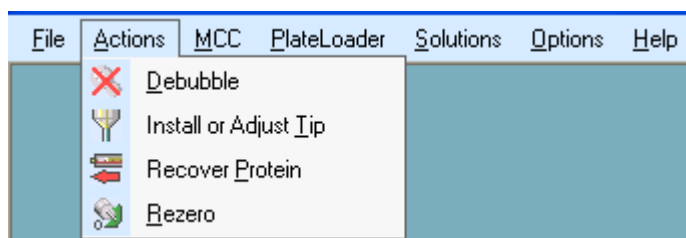
#### Abort Experiment

Abort Current Experiment.

#### Exit

Exit Front Panel.

### Actions Menu



All of these items can also be found in other menus but are grouped here for convenience and for inexperienced users: If 'Advanced Menu Items' is turned off in 'Options' menu then only the 'Actions Menu' will be displayed.

#### Debubble

Run the Debubble wizard to remove air from the tubing above the top valves.

#### Install or Adjust Tip

Runs the Install and Adjust tip wizard.

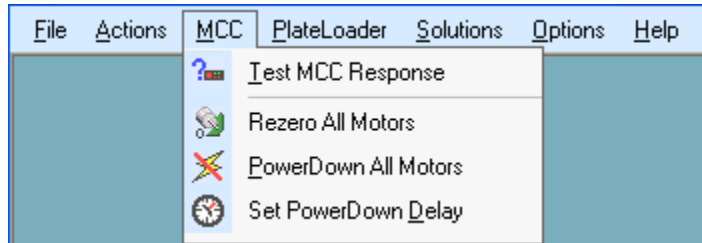
## Recover Protein

Runs a wizard to recover protein to a plate or tube.

## Rezero

Runs a wizard to rezero the syringes and / or table if they have been obstructed.

## MCC Menu



### Test MCC Response

Sends a test signal to the MCC which should return the MCC info message for diagnostics.

### Rezero All Motors

Forces a rezero of all motors to recalibrate start positions – use in case syringes or Plateloader was obstructed during use.

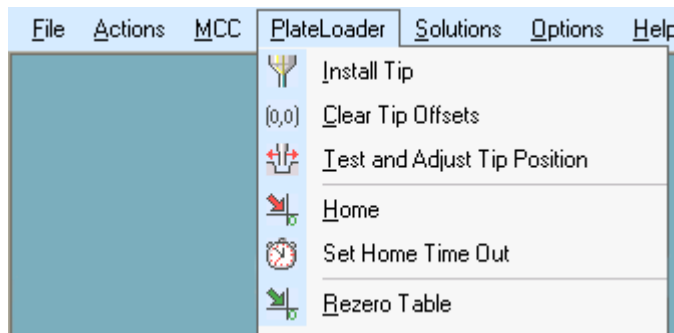
### Power Down all motors

Turns off power to all motors to allow manual positioning of the table or to stop the noise produced by the syringe drives. Table will automatically rezero before next move.

### Set PowerDown Delay

Sets the delay before the power is automatically turned off when the table is in the Home position.

## Plateloader Menu



### Install Tip

Starts a wizard to install a different tip including the initial height setting for a new tip

### Clear Tip Offsets

Sets the X, Y and Z offsets used in 'Test and Adjust Tip Position' back to all Zeros.

### Test and Adjust Tip Position

Starts a wizard to align the tip to a well on the target plate.

### Home

Moves the table to the Home position.

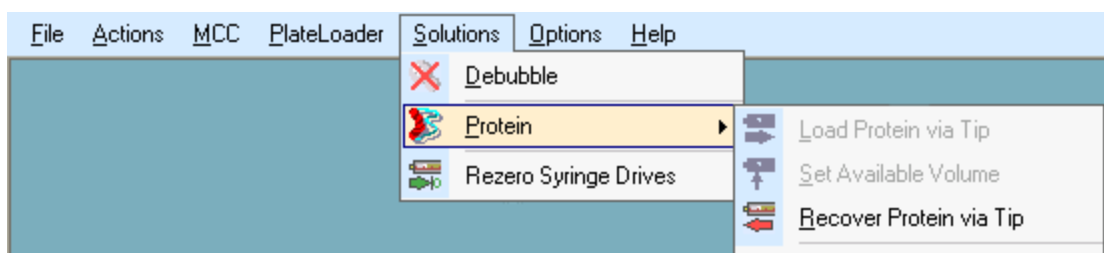
### Set Home Time Out

Sets the delay before the table automatically goes to the Home position and the Powerdown Timer starts.

### Rezero Table

Rezeros the Plateloader table

## Solutions Menu



### Debubble

Run the Debubble wizard to remove air from the tubing above the top valves.

### Protein

#### Load Protein via Tip

Manually load protein

#### Set Available Volume

Override current available volume – for advanced use

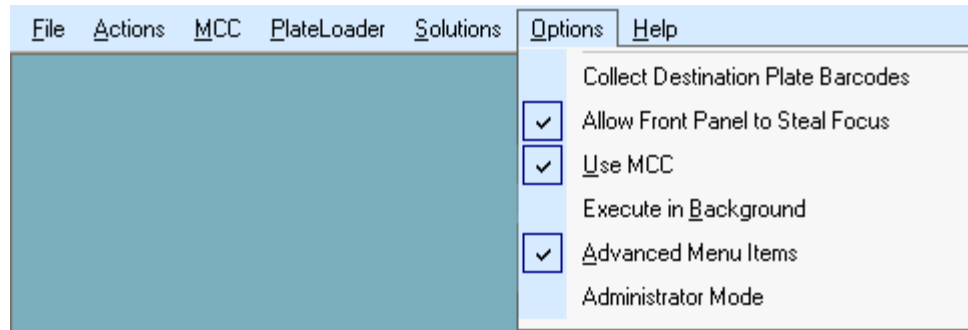
#### Recover Protein via Tip

Runs a wizard to recover protein to a plate or tube.

### Rezero Syringe Drives

Force a rezero of the Syringe Drives if it is believed that they are out of position or have been obstructed during use.

## Options Menu



### Collect Destination Plate Barcodes

On/Off - Require barcodes for new plates. Default = off

### Allow Front Panel to Steal Focus

On/Off - Allows Front Panel to 'jump' to the front when called – overrides default Windows behavior of flashing the taskbar to get attention. Default = On

### Use MCC

On/Off - Lets Front Panel communicate with the MCC. Default = On. Used for diagnostics

### Execute in Background

Allows Front Panel to execute in the background while using other applications. Default = Off

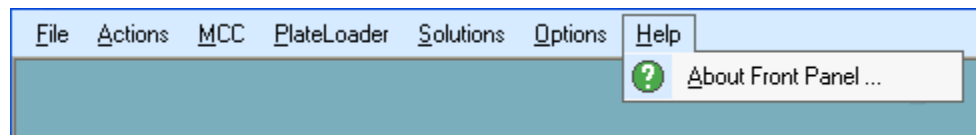
### Advanced Menu Items

On/Off - Enables Menu Items for advanced users. Default = On

### Administrator Mode

On/Off - Enables Advanced and diagnostic menus for Administrator use only. These menu items are not described in this manual and are generally only used by installers to set up and calibrate the system. Default = Off

## Help Menu



### About Front Panel

Displays Front Panel Version Information and Douglas Instruments contact information.

## USE AND MAINTENANCE OF THE HARDWARE

### Installing Microtips

Simply screw the colored end-fittings into the ports on the bottom of the lower valves, following the color-coding. The 2-bore Microtips are generally used for screening experiments, while the 3 and 4 bore can be used for seeding or multiple protein experiments.

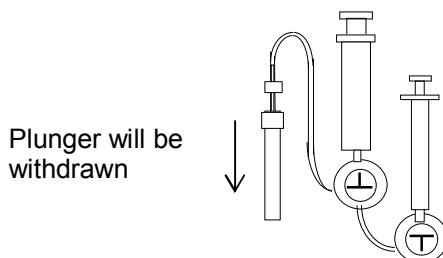
Take a little trouble when installing Microtips. You will find that each tube has two “natural” positions (the tube runs back and then to the side, or, alternatively, to the side and then back). One of these natural positions allows the tube to clear the Plate Loader, whereas the other position causes the tube to collide with the Plate Loader. Choose the former.

### Use of Liquid Handling System Including Valve Positions

#### Refilling 100μl Gas-Tight Syringes with Water

The 100μl syringes contain only degassed pure water. This means that there is no need to flush them when the stock solutions are changed. Degassing is very helpful in reducing bubbles.

When a motorized syringe is almost empty, the software will detect that it is necessary to refill the syringe with water. Follow instructions, including turning the upper valve to the refill position (⊥) as indicated.



The refill valve position (⊥) is also used for rezeroing motors and debubbling.

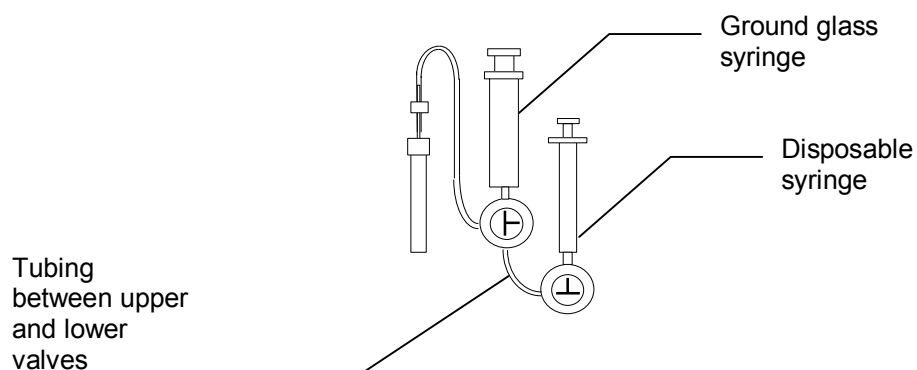
#### Debubbling

It is essential that all tubing on channels 1–4 is completely filled with water, and that there are no air bubbles. Any air bubbles will cause significant inaccuracy in dispensing. The motorized syringes contain only degassed pure water. (If you do not use degassed water you may have to debubble up to twice a day.)

To debubble, select the *debubble* option (under either *syringes* or *execute* on the main menu) and follow instructions.

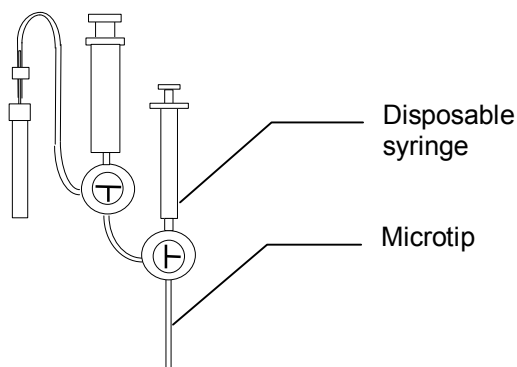
## Flushing Tubing between Valves

It will occasionally be necessary to remove bubbles or to flush debris out of the short length of tubing between the upper and lower valves. Turn the upper valve to the flush position ( $\vdash$ ) and the lower valve to the fill position ( $\perp$ ). Flush water from the ground glass syringe to the disposable syringe. This prevents debris from being flushed out of the microtip, which could cause blockages.



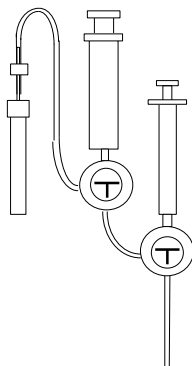
## Filling Microtip with Solution

Software will tell you when to fill the Microtip with stock solution or water. Follow instructions, including turning the lower valve to the flush position ( $\vdash$ ).



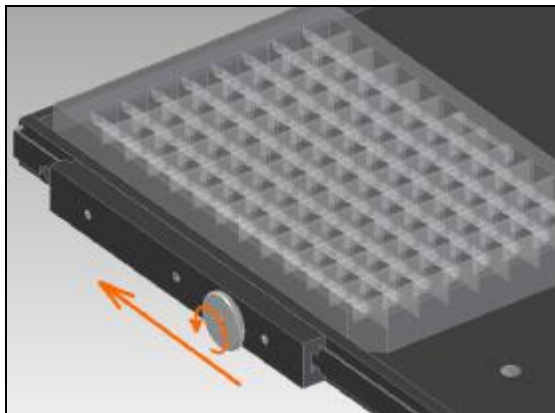
## Dispensing Experiments

Follow instructions and turn all valves to the dispense position ( $\top$ ) as shown:

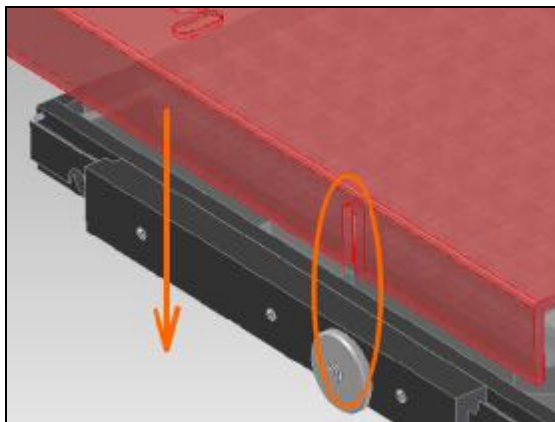


## Attach the Evaporation shield for Vapor Diffusion Experiments

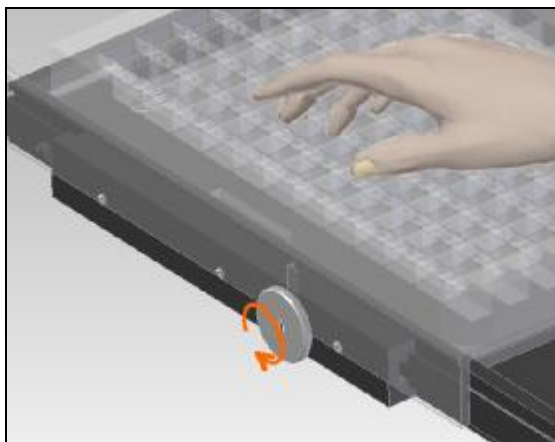
1. Move slider to be next to the plate and undo the clamp knob to create a gap for the shield:



2. Place the shield onto the plate with the groove on the side sliding over the screw in the slider:



3. Put light pressure on the shield above the plate with one hand and tighten the knob with the other:



4. Now leave the shield with the large slot somewhere over the plate and the machine will find it in the next step.

## Flushing Microtip after Use

The Microtip should be thoroughly flushed after each session. Place a 1ml syringe containing distilled water in each valve in the lower row, turn the valve, and press in the plunger firmly. Repeat this three times. Finish by passing air through the Microtip and disconnecting. Store it coiled up and flat to avoid bending of the tip.



**Always wear protective goggles when handling caustic materials**

## Protein Coatings of Microtip

Certain proteins may have a tendency to coat the inside of a microtip. This may cause the air bubble (that is used to separate the protein sample from the water in the microtip) to become stuck or to break up. Tips that are coated with protein can be cleaned by flushing first with 1 M NaOH, then with buffer solution to get rid of the alkali. If this procedure does not work, try conc. HCl mixed with an equal volume of methanol (again followed with buffer to remove the acid).

## Blockages

Keep syringes in all valves when not in use to avoid the ingress of dust. Never allow precipitant to come into contact with tubing that has previously contained protein unless it has been cleaned using alkali or acid – see above. Filter all solutions using a 0.45  $\mu\text{m}$  filter or equivalent, and refilter any solutions that become cloudy or contain debris.

## Unblocking Microtips

1. First try forcing the debris out of the tip with high pressure. This method is only possible when the Microtip is full of liquid. Fill a 100  $\mu\text{l}$  gas-tight syringe with water. Attach a pointed 0.7 mm needle to it (this is the type of needle that spare syringes come with). Push this into the blocked bore of the Microtip at the End-Fitting. Press in the plunger of the syringe. Up to 100 atmospheres can be generated by this method.

If this does not work we recommend that the tip is replaced with a new tip

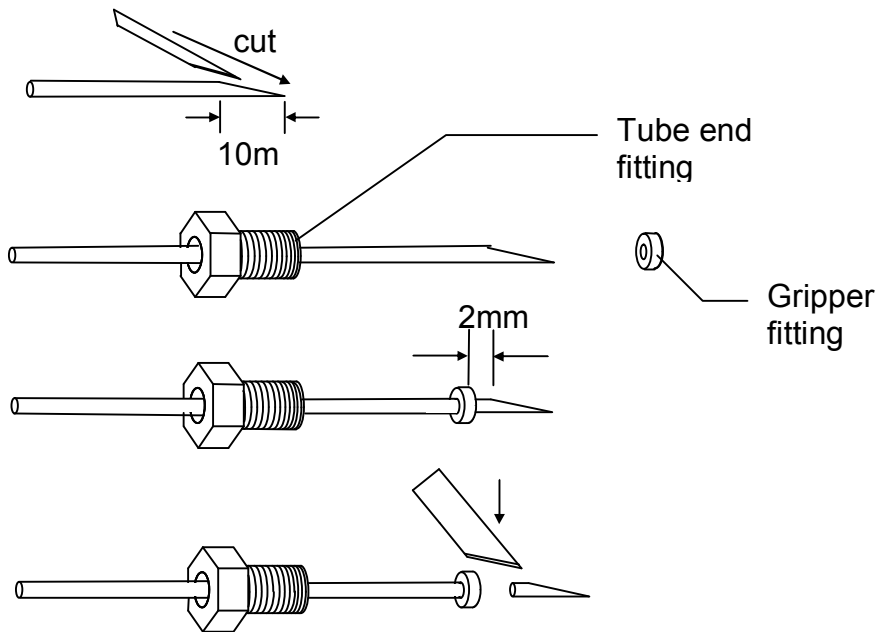
## Chemical Inertness

All surfaces that come into contact with solutions are chemically inert fluorocarbon polymers including FEP and PTFE. Only water comes into contact with the stainless steel needles of the gas-tight syringes.

## Tubing Connections

The needles of the gas-tight syringes are connected to the FEP tubing by enlarging the bore at the end of the tubing and pressing onto the needle. If any syringes need to be replaced retain the special needles.

All other connections are made using gripper fittings. These are fitted as follows:



1. Taper the tubing with a scalpel by cutting it at an acute angle.
2. Pass the end through the tube end fitting and feed into the stainless steel side of the gripper fitting.
3. Grip the end of the tube with a pair of pliers and pull the tube through the gripper fitting beyond the tapered portion of the tube onto its full diameter, and rotate twice to grip the tube.
4. Pull the tube end fitting down to meet the gripper.
5. Trim the tube flush to the Teflon face of the gripper with a scalpel.

## Lubrication of Syringe Drivers and Plate Loader.

### Lubrication of Shafts

No lubrication will be required in the first year. Thereafter, inspect the shafts of the Plate Loader and the Syringe Drivers once a year. If they appear to be dry, lubricate them with one or two drops of hypoid gear oil - SAE 80W-90 or EP 80W-90 or similar. (This oil is used in e.g. car differential gears.) This will contribute to the smooth running of moving parts, and protect from air-bourn corrosive materials such as are found in laboratories. (All parts are stainless steel or anodized aluminum, which will not generally corrode in the presence of moisture alone.)

### Lead-screws

**Lead-screws must not be lubricated with oil, as this may clog up the motors.** Lead-screws are sparingly lubricated by Douglas Instruments with special compound for lubricating plastic. Please contact Douglas Instruments if you feel that your lead-screws need to be lubricated or if they become corroded.

### Spillages

If large amounts of liquids are spilt on any of the electric motors, the system should be turned off. Salt, acid or alkali will cause corrosion of motors and stainless parts, and must be washed off with water. Allow the system to dry out before reusing. The motors and their connections are electrically safe since they run at 12 V.

## XYZN Plate Loader

### Adjustment of XYZ Plate Loader

The XYZ Plate Loader is adjusted so that it accurately moves to the center of wells on plates. Douglas Instruments performs this adjustment before shipping. If the Plate Loader is subjected to shock or if screws are slackened, it may need to be readjusted. The adjustment can be made in either hardware or software.

If you believe that the Plate Loader is in need of adjustment, please contact Douglas Instruments. Please do not attempt to make adjustments without consulting the company, since there are hidden complications in this procedure.

It is possible, however, to adjust the alignment of an individual microtip using Front Panel, providing the misalignment is less than about 2 mm.

## HARDWARE CONFIGURATION AND CONTROL

### Using Different Syringes with the System

Changing syringes is not normally recommended because the screening and optimization software will both have to be changed. In special cases it may be worth considering. We recommend that you consult Douglas Instruments before embarking on such a project.

### Changing Plates

For a complete list of plates that can be used, consult the PLATES.DAT file in the directory *Global Data*. If the plate that you wish to use is not listed, then see the next section on adding new plate definitions.

#### WASP and WASPRUN

\*.XPT files for WASP directly specify the plates to be used on the Plate Loader table. In order to use different plate types, the appropriate PLATE statement in the \*.XPT file must be altered. This is simply a matter of using a text editor to modify the existing file.

The PLATE declaration statements are always near the top of the \*.XPT file, and refer to plates by type name - e.g. Nunc HLA refers to a standard 6x12 Nunc HLA tissue culture plate. For a complete list of plates that can be used, consult the PLATES.DAT file in the directory *Global Data*. If the plate that you wish to use is not listed, then see the next section on adding new plate definitions.

### Adding Further Plates to PLATES.DAT

See manual for Wasprun.exe

## Hardware Configuration Files

The hardware is defined by two files, which must match your particular system. These files are `HARDWARE.CFG` and `HARDWARE.FTH`. `HARDWARE.CFG` is found in the *global data* folder, while `HARDWARE.FTH` is found in the MCC folder, and it must be downloaded to the MCC. See the instructions for installing software.

Obviously, it is essential that the specifications match the hardware, and that these two files match each other. Please do not make any changes to either of these files. In exceptional circumstances Douglas Instruments may instruct you to make certain changes.

### HARDWARE.CFG

The typical contents of `HARDWARE.CFG` are as follows:

Serial No: "XYZN-08" "29/09/2008"

Drive 1 Channel 1 Pitch 314.9600 steps/mm Speed 4.00 mm/s Range 75.00 mm Length 92.00 mm  
Drive 2 Channel 2 Pitch 314.9600 steps/mm Speed 4.00 mm/s Range 75.00 mm Length 92.00 mm  
Drive 3 Channel 3 Pitch 314.9600 steps/mm Speed 4.00 mm/s Range 75.00 mm Length 92.00 mm  
Drive 4 Channel 4 Pitch 314.9600 steps/mm Speed 4.00 mm/s Range 75.00 mm Length 92.00 mm

Axis X Channel 9 Pitch -13.3477 steps/mm Speed 70.00 mm/s Range 350.00 mm Length 321.00 mm  
Axis Y Channel 10 Pitch 20.0369 steps/mm Speed 55.00 mm/s Range 155.00 mm Length 151.00 mm  
Axis Z Channel 11 Pitch 39.4541 steps/mm Speed 30.00 mm/s Range 55.00 mm Length 50.00 mm

END

### HARDWARE.FTH

The typical contents of `HARDWARE.FTH` are as follows:

string serialNumber "XYZN-08"  
string hardwareDate "29/09/2008"  
string userName "Hoffman-La Roche, Basel"

OryxNano DB6.6

( Hardware settings for ) userName count type cr

( Syringe currents, pitches and speeds )

1 0.533 SetCurrent 1 314.9600 SetPitch 1 92.000 SetLength 1 92.000 SetRange 1 4.00 SetSpeed 1 0.019 SetBacklash  
2 0.533 SetCurrent 2 314.9600 SetPitch 2 92.000 SetLength 2 92.000 SetRange 2 4.00 SetSpeed 2 0.009 SetBacklash  
3 0.533 SetCurrent 3 314.9600 SetPitch 3 92.000 SetLength 3 92.000 SetRange 3 4.00 SetSpeed 3 0.013 SetBacklash  
4 0.533 SetCurrent 4 314.9600 SetPitch 4 92.000 SetLength 4 92.000 SetRange 4 4.00 SetSpeed 4 0.016 SetBacklash

( PlateLoader currents, pitches and speeds )

9 0.450 SetCurrent 9 -39.6277 SetPitch 9 130.000 SetLength 9 132.000 SetRange 9 52.00 SetSpeed  
10 0.450 SetCurrent 10 39.4735 SetPitch 10 133.500 SetLength 10 135.000 SetRange 10 52.00 SetSpeed  
11 0.450 SetCurrent 11 39.3440 SetPitch 11 50.000 SetLength 11 55.000 SetRange 11 52.00 SetSpeed

: rezeroX 0 rezeroLS ;  
: rezeroY 1 rezeroLS ;  
: rezeroZ 2 rezeroLS ;

0.0 X NominalOptoPosition f!  
0.0 Y NominalOptoPosition f!  
0.0 Z NominalOptoPosition f!

1 constant BeltDrive?  
7 constant OptoAxes  
9 accelerationTime !  
600 initialPeriodFactor !

## Specifications

An up to date list of specifications for all Douglas Instruments' products can be found at  
<http://douglas.co.uk/specs.htm>

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