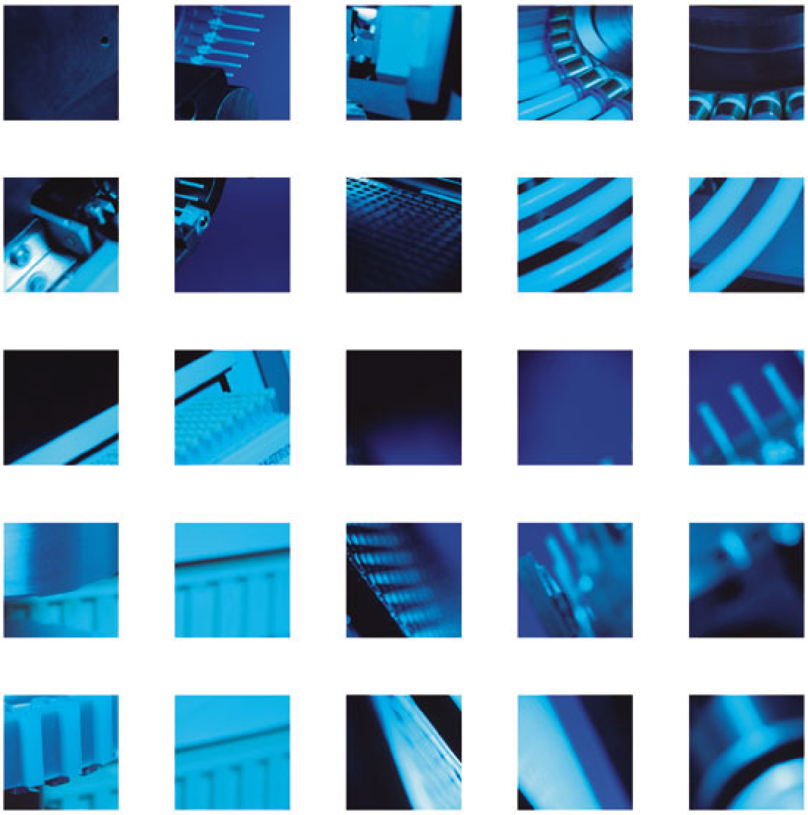
TTP LABTECH LTD

mosquito Advanced Operator & Training Manual

Version 3.2.1



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Author	Simon Carter

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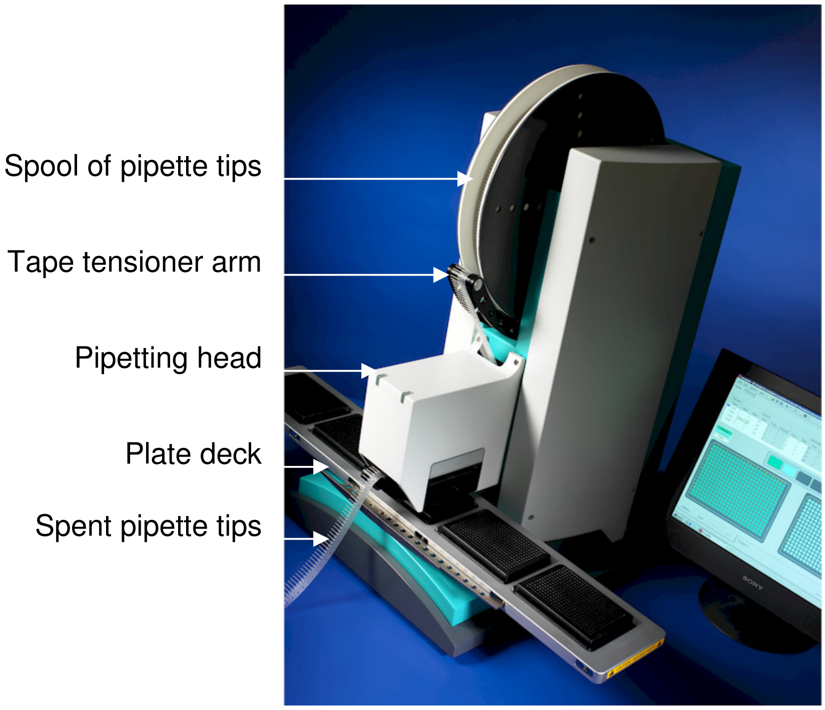
1 MOSQUITO OVERVIEW

mosquito is the first of a range of instruments to offer a general purpose dispensing platform capable of precision liquid handling in a compact, bench-top instrument.

The instrument comes with easy-to-use Windows application software that makes process development easy and quick. Links for interfacing to a variety of plate-stacking robots are available via a hardware interface.

At the core of mosquito is the bandolier spool of miniature syringe-like micropipettes that provides a continuous feed of disposable tips to the pipetting head.



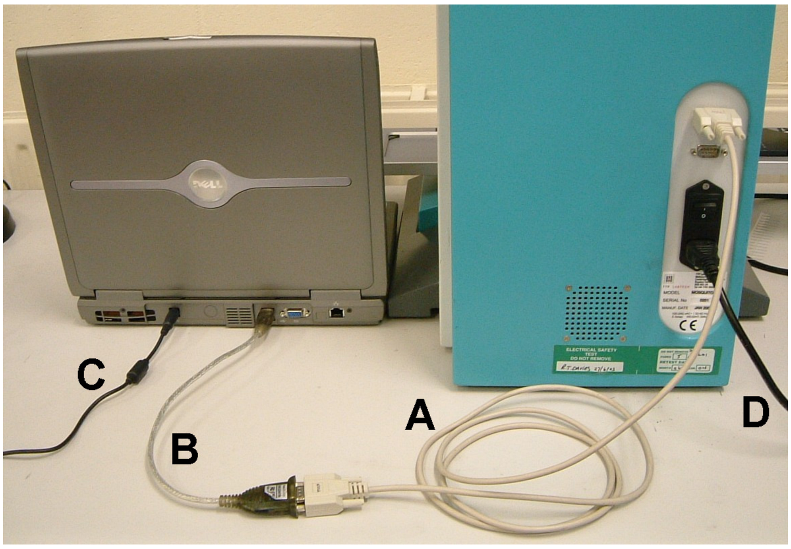
1.1 Setting Up Mosquito

1.1.1 mosquito System Connections

Mosquito system connections are made as shown in Figure 1 below.

The mosquito instrument and the laptop are joined using network cables A and B. Note that if mosquito has been supplied with a desktop PC then there will be no cable B.

Both the mosquito instrument and the PC must be plugged in to a mains power supply (cables C and D).



Connection cables:

A = RS232 Null modem (crossover) cable

B = USB to RS232 converter (connects laptop to cable A)

C = laptop or PC power supply (connects to mains)

D = mosquito power supply (connects to mains).

Figure 1: Rear view of mosquito instrumentation showing cable connections

1.2 Starting up the mosquito System

To start up mosquito:

- □ Turn on mosquito using the switch at the back of the instrument (next to the power lead D).
- □ Power up the computer as normal, then start the mosquito application software by double-clicking on the application icon: .
  Note: the instrument must be turned on approximately 30 seconds before the software is started.
- □ When the software has started it will ask whether to initialise the instrument – click **Yes** to do so.

Both the blue and green LED's on the top left of the instrument light up when it is ready to run.

1.3 Shutting Down the mosquito System

To close down the mosquito software, select **File|Exit** from the main menu or click the **Close** button at the top right corner of the application window.

When the software application is closed, the instrument may be turned off using the power switch at the back (next to the power lead D).

2 INSTALLING THE MOSQUITO SOFTWARE

mosquito is supplied with a PC with the mosquito application software already installed. Do not attempt to reinstall the mosquito software on the control PC without first contacting TTP LabTech.

However, the mosquito software may be installed on a PC which is not connected to an instrument for stand-alone demonstrations and training, and for off-line protocol development (see Section 3.5 for details of Simulation Mode).

See Section 2.3.9 for information on upgrading an existing installation of mosquito.

2.1 Requirements

- 1 gigahertz Intel or AMD processor
- 512Mb of RAM
- at least 2 GB of free disk space
- Windows XP SP2 or greater (Vista not currently supported)
- Serial port (we have successfully used USB to serial converters in the past, but ran into issues with several manufacturers. Please contact Mosquito support if you are considering this option)
- Careful configuration of virus scanner & automatic updates. We prefer the mosquito machine to run stand-alone, disconnected from a network which allows us to remove the need for a virus scanner. We have in the past had issues which were traced back to virus scanners interfering with the Mosquito software.

2.2 Installing

To install mosquito insert the supplied CD-ROM into your CD drive. The installation software should start automatically. If it does not, use Windows Explorer to navigate to your CD drive and double-click setup.exe.

Click **Next >** to proceed to the next screen and specify and installation directory.

Click **Next>** twice to start the installation, which may take a couple of minutes.

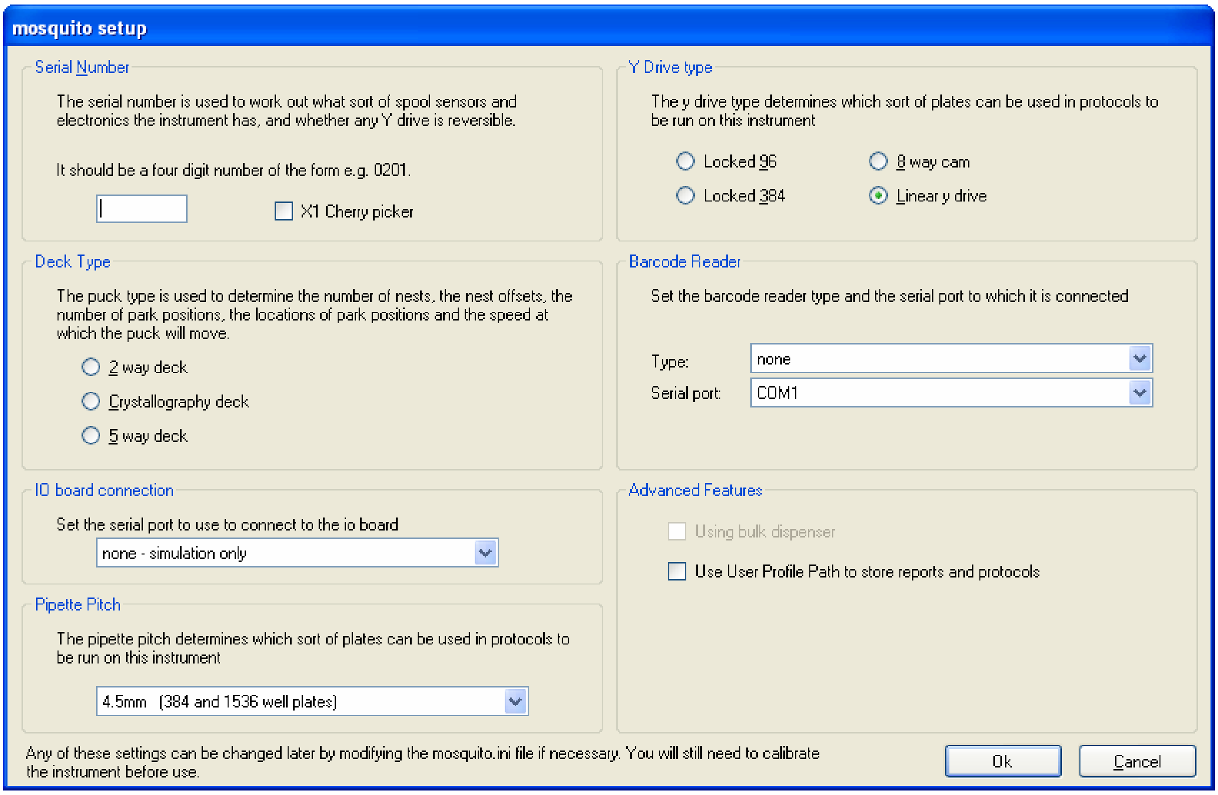
[**NOTE**: A black command prompt window (or 'DOS' screen) will appear during installation. This is normal, and is due to the installation procedure optimising the Mosquito software.]

The installer will place shortcuts to mosquito on your desktop and the Start Menu.

2.3 Configuring mosquito

The first time you run mosquito you will be prompted to enter some information about your mosquito installation. This information should match the type of instrument which you have. If you are unsure, please contact TTPLabtech.

The following screen is displayed:



mosquito setup

Serial Number

The serial number is used to work out what sort of spool sensors and electronics the instrument has, and whether any Y drive is reversible.

It should be a four digit number of the form e.g. 0201.

□ X1 Cherry picker

Y Drive type

The y drive type determines which sort of plates can be used in protocols to be run on this instrument

○ Locked 96　○ 8 way cam

○ Locked 384　● Linear y drive

Deck Type

The puck type is used to determine the number of nests, the nest offsets, the number of park positions, the locations of park positions and the speed at which the puck will move.

○ 2 way deck

○ Crystallography deck

○ 5 way deck

Barcode Reader

Set the barcode reader type and the serial port to which it is connected

Type: none

Serial port: COM1

IO board connection

Set the serial port to use to connect to the io board

none - simulation only

Advanced Features

□ Using bulk dispenser

□ Use User Profile Path to store reports and protocols

Pipette Pitch

The pipette pitch determines which sort of plates can be used in protocols to be run on this instrument

4.5mm (384 and 1536 well plates)

Any of these settings can be changed later by modifying the mosquito.ini file if necessary. You will still need to calibrate the instrument before use.

The various settings are described below:

2.3.1 Serial Number

Enter the serial number as found on the identification plate on the rear of the mosquito instrument. For a simulation only setup enter the same serial number as that of the instrument you are simulating, or if none is available enter 0299.

2.3.2 X1 Cherry picker

Tick this box if the instrument is a single-tip ‘cherry picker’

2.3.3 Deck Type

Select the Deck type which you have or are simulating.

2.3.4 IO Board Connection

Select ‘None - simulation only installation’ if you are installing the mosquito software on a stand-alone pc.

2.3.5 Pipette Pitch

Select the pitch of the tape you are using.

2.3.6 Y Drive Type

Select the Y drive type you are using.

- If you have a linear y drive select **linear** – this is the default
- If you have a cam-driven y drive select **8 way cam**
- If you do not have a Y drive and are using only 96 well plates select **Locked 96**
- If you are using only 384 well plates without a y drive select **Locked 384**.

2.3.7 Barcode Reader

If you do not have a barcode reader attached to your Mosquito, choose 'none' as the model. Otherwise select the model you have (likely to be a TC1100).

The barcode reader connects to the controlling computer via a separate serial port. Select which one you are using in the 'Serial Port' box.

2.3.8 Bulk Dispenser

The mosquito bulk dispenser setting should be enabled if a bulk dispenser is to be attached.

2.3.9 Directories

The default directories for the storage of protocols and report files is under the mosquito installation directory. If you want to use Documents and Settings\<username> for this data so each user's work is stored in their own directory then tick this box. This setting can also be changed via the **Options | Application Preferences** dialog.

2.4 Installing with an Existing mosquito Installation

If you already have an installation of mosquito, it is possible to upgrade the existing installation or to install an addition copy of mosquito which does not affect the current installation – see Section 2.4.2 for information on how to do this.

2.4.1 Upgrading an Existing Installation

NOTE: Please review the release notes to see how to upgrade an existing installation. Upgrading from any version prior to 3.0.0 to version 3.0.0 or above should be performed by or in conjunction with a service engineer.

To upgrade an existing installation, where that version is 3.0.0 or above, proceed as in Section 2.2 but select the directory where the current mosquito installation is. The current mosquito.ini, protocol files will not be updated. Plate files will only be updated if they have remained unmodified since the previous installation.

There may be slight changes in behaviour between the two versions. This may mean you will need to alter some ini settings by hand. Consult the release notes supplied with the newer version of the software for this information.

For additional security, you may wish to make a backup copy of the existing mosquito.ini, plate and protocol files to another location before beginning the upgrade process.

2.4.2 Installing a Second Copy of Mosquito

The installation of a second version is no longer supported.

While mosquito 3.2.1 will read plate and protocol files created with previous versions of the software the reverse is not true; once a file has been saved from within software version 3.2.1, older versions will be unable to read the file.

2.5 Mosquito Application Security

The mosquito software includes facilities to prevent unauthorised users from performing calibration and seeing the engineering panel.

In versions prior to 3.0.0, this security was implemented via Microsoft Windows users and groups, and managed via the **Users and Groups** tool in the control panel. However, this security is now inbuilt into mosquito.

2.5.1 Enabling Calibration Mode

By using the File | Calibration Mode menu it is possible to change the user mode of the mosquito software.

To enable Calibration Mode

- from within the mosquito software, select **File | Calibration Mode** and enter the username and password. (If you do not know the username and password, please contact Mosquito support).

In this way the current user mode level can be boosted from within the software to perform calibration and diagnostic functions.

To revert back to Normal User Mode, select **File | Calibration Mode** again – which should be ticked. This toggles Calibration Mode back to off.

2.5.2 Automatically Reverting Back to Normal User Mode

After a period of inactivity where the machine is not used, mosquito will switch back to Normal User Mode if it was in Calibration Mode. The period can be modified if required.

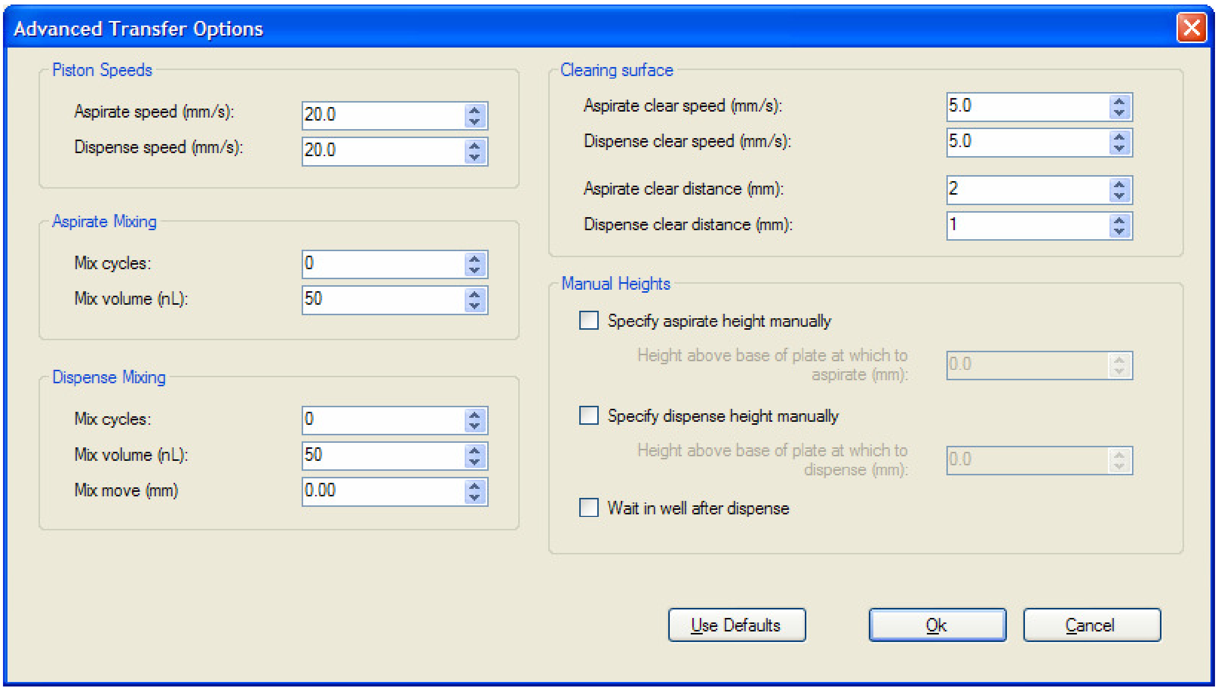
3 ADVANCED PROTOCOL OPTIONS

3.1 Advanced Transfer Options

The advanced column of the transfer table exposes a number of options for use in optimising pipetting characteristics. These are:

- Mixing the well contents
- Setting the aspirate or dispense heights manually
- Specifying the distance by which to clear the surface of the liquid and the speed at which to do it.
- Waiting in well after dispense.

To access these options, click the ... button in the Advanced column of the transfer to display the Advanced Transfer Options dialog box:



Advanced Transfer Options

Piston Speeds
Aspirate speed (mm/s): 20.0
Dispense speed (mm/s): 20.0

Aspirate Mixing
Mix cycles: 0
Mix volume (nL): 50

Dispense Mixing
Mix cycles: 0
Mix volume (nL): 50
Mix move (mm) 0.00

Clearing surface
Aspirate clear speed (mm/s): 5.0
Dispense clear speed (mm/s): 5.0
Aspirate clear distance (mm): 2
Dispense clear distance (mm): 1

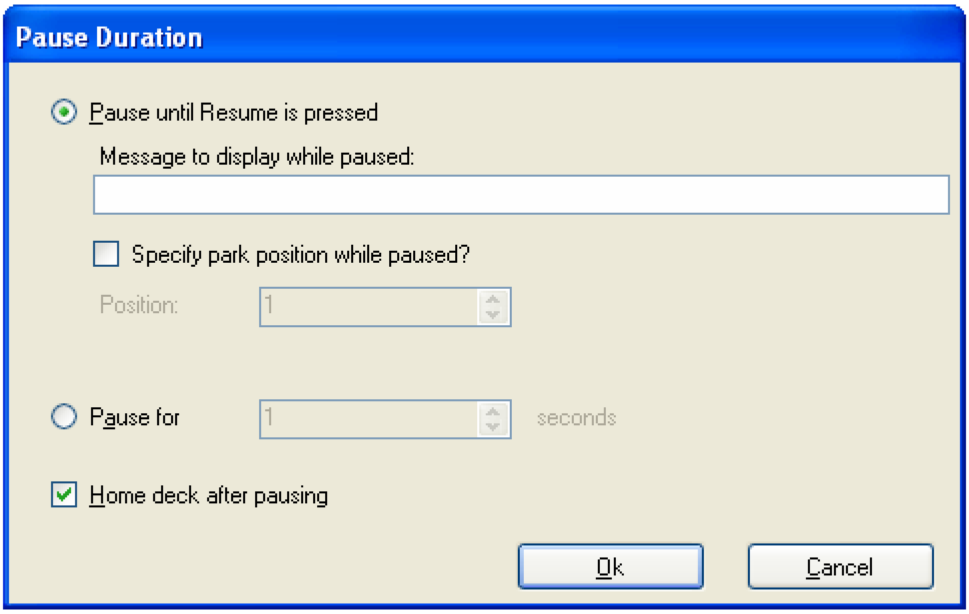
Manual Heights
□ Specify aspirate height manually
Height above base of plate at which to aspirate (mm): 0.0
□ Specify dispense height manually
Height above base of plate at which to dispense (mm): 0.0
□ Wait in well after dispense

To restore the default values to all of these settings press the **Use Defaults** button.

3.1.1 The Pause Transfer Type

The Pause transfer type is used to pre-programme a pause in operations during a protocol. This pause can be indefinite – until the user presses the **Resume** button – or for a specified time.

To set a pause during a protocol, create a Pause transfer type and then click on the Advanced column to access the Pause Duration dialog box.



Pause Duration

● Pause until Resume is pressed

Message to display while paused:

□ Specify park position while paused?

Position: 1

○ Pause for 1 seconds

☑ Home deck after pausing

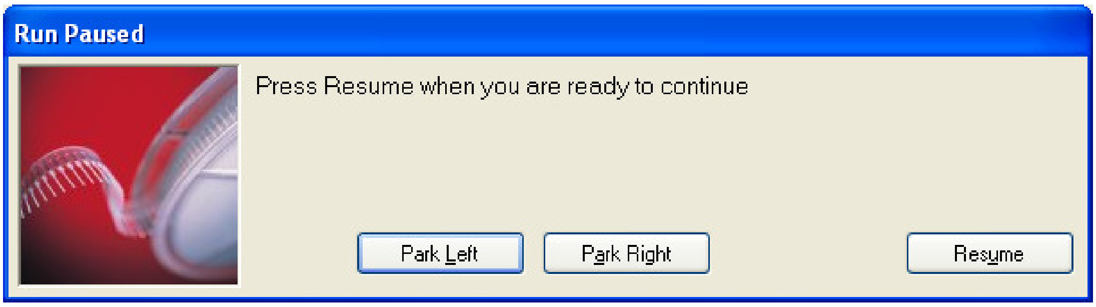
The user can select ‘Pause until Resume is pressed’ or specify a number of seconds to pause for. The user can also press **Resume** at any point during a timed pause.

The **Park Left** and **Park Right** buttons for moving the deck are enabled while a protocol is paused, so it is possible to perform manual interventions at a particular point in a protocol.

Since a Pause transfer may be used to perform a manual intervention it may be desirable to home the deck before resuming operation in case it has been knocked out of position. This can be disabled by unchecking the ‘Home deck after pausing’ option.

3.1.1.i Indefinite Pauses

If a Pause transfer is indefinite, i.e. ‘Pause until Resume is Pressed’ then a message box like the following:



Run Paused

Press Resume when you are ready to continue

is displayed until the user presses Resume. It is possible to specify the exact message displayed in the pause options dialog box above.

Furthermore, it is possible to specify the location where the deck will park during an indefinite pause and checking the ‘Specify park position while paused?’ box and selecting the park position number. If this is not specified then the deck will pause at the current position.

3.1.2 Mixing Well Contents

Destination well contents may be mixed either while aspirating or while dispensing by sucking up and dispensing repeatedly within the same well.

To mix well contents, click on the Advanced column to access the Advanced Transfer Options dialog box.

The user can specify, for both aspirating and dispensing:

- The number of mix cycles. One mix cycle consists of one aspirate from and dispense back into the same well.
- The mix volume. This value specifies how much liquid is drawn up into the pipette for the mix cycle, to a maximum of 1200 nL.

When dispensing, the user can specify:

- The Mix move, in mm. This causes the tip to move up and then down this distance in each mix cycle.

The Dispense Mixing operation may only be performed if the Tip Changing column is set to 'Always' to prevent cross-contamination. The software will automatically reset the Tip Changing column value, if Dispense Mixing is selected.

3.1.3 Manual Aspirate Height

In normal operation mosquito calculates the height at which to aspirate liquid using the base height of the plate type being used and the well volume and well area of the plate from which it aspirates. However, it is possible to override this calculation and set the aspirate height manually.

To do this, click on the Advanced column to access the Advanced Transfer Options dialog box, then select the 'Specify aspirate height manually' check box and enter the height at which you wish to aspirate in the corresponding edit box. This height may be negative if required.

3.1.4 Manual Dispense Height

The normal dispense height calculations can be overridden in the same manner as the aspirate height.

3.1.5 Clearing the Liquid Surface

As mosquito finishes aspirating or dispensing it will slowly withdraw the pipettes from the liquid to a predefined distance above the liquid before moving more quickly to get clear of the plate. The speed at which this withdrawal takes place can be specified individually for aspirate and dispense operations, as can the distance to which the pipettes are withdrawn.

These options may be useful in improving pipetting characteristics with particularly viscous liquids.

3.1.6 Wait in Well After Dispense

If enabled, this option causes the tips to remain in the well between the dispense and the next aspirate. However, this only happens if it's possible – and for it to be possible, the next aspirate must be from the same position, and the tips mustn't be set to change between the transfers.

The test button can be used to see if the protocol will perform the 'Wait in Well' or not. 'Wait in Well' will only by listed if it will actually occur.

3.2 Multi-Aspirate Transfer Type

The *Multi-Aspirate* transfer type makes it is possible to aspirate more than once before dispensing.

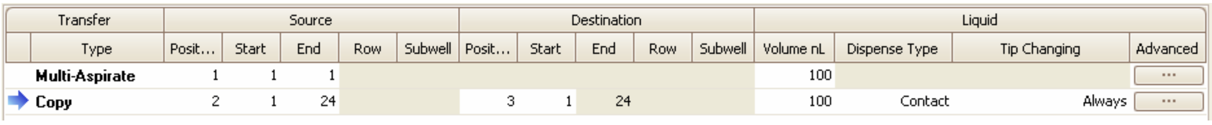
A multi-aspirate transfer in the table will modify the succeeding Copy, Aliquot, Pool or Mirror transfer. If the succeeding transfer is part of an optimised group of transfers with the *Multi-Dispense* or *Between Transfers* tip change option, then all of the transfers in the group will be modified by the multi-aspirate transfer.

A multi-aspirate transfer must have the same number of source columns as the succeeding Copy (etc), or be from just one column.

It is possible to have more than one successive multi-aspirate transfer; all such transfers will modify the succeeding Copy operation. It is also possible to use the advanced transfer options such as mixing in conjunction with multi-aspirate operations.

3.2.1 Multi-Aspirate Transfer Example 1 – Aspirate from a single column

Consider a protocol so:



Transfer		Source					Destination					Liquid			
	Type	Posit…	Start	End	Row	Subwell	Posit…	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
	Multi-Aspirate	1	1	1								100			…
➡	**Copy**	2	1	24			3	1	24			100	Contact	Always	…

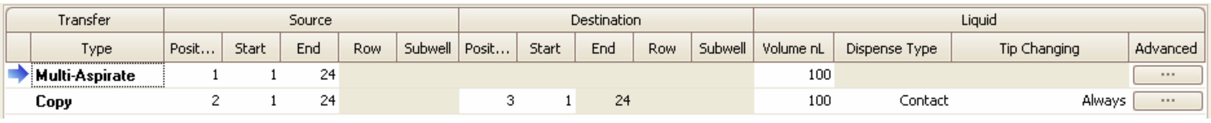
The exact sequence of operations will be as follows:

```
Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C1)
Dispense 200 nL  to  (P3, C1)
Change pipettes
```

```
Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C2)
Dispense 200 nL  to  (P3, C2)
Change pipettes

Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C3)
Dispense 200 nL  to  (P3, C3)
Change pipettes

Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C4)
Dispense 200 nL  to  (P3, C4)
Change pipettes

etc
```

3.2.2 Multi-Aspirate Transfer Example 2 – Aspirate from multiple columns

If the multi-aspirate transfer is changed so as to be from multiple columns so:



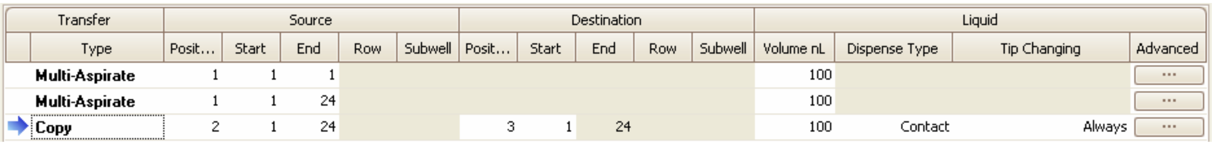
Transfer	Source					Destination					Liquid			
Type	Posit…	Start	End	Row	Subwell	Posit…	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
Multi-Aspirate	1	1	24								100			···
Copy	2	1	24			3	1	24			100	Contact	Always	···

Then the exact sequence of operations will be as follows:

```
Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C1)
Dispense 200 nL  to  (P3, C1)
Change pipettes

Aspirate 100 nL from (P1, C2)
Aspirate 100 nL from (P2, C2)
Dispense 200 nL  to  (P3, C2)
Change pipettes

Aspirate 100 nL from (P1, C3)
Aspirate 100 nL from (P2, C3)
Dispense 200 nL  to  (P3, C3)
Change pipettes

Aspirate 100 nL from (P1, C4)
Aspirate 100 nL from (P2, C4)
Dispense 200 nL  to  (P3, C4)
Change pipettes

etc
```

3.2.3 Multi-Aspirate Transfer Example 3 – Two Multi-Aspirate steps

If we add a second multi-aspirate step so that the source of the first is a single column and the source of the second a whole plate so:



Transfer		Source					Destination					Liquid			
	Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
	Multi-Aspirate	1	1	1								100			...
	Multi-Aspirate	1	1	24								100			...
➡	Copy	2	1	24			3	1	24			100	Contact	Always	...

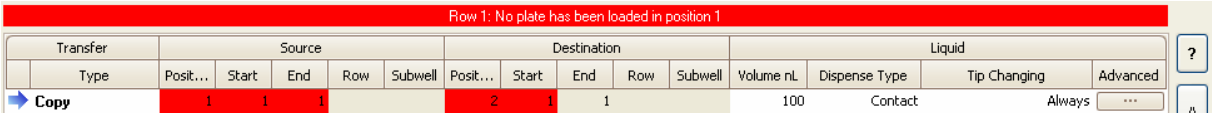
Then we get the following behaviour:

```
Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C1)
Aspirate 100 nL from (P3, C1)
Dispense 300 nL  to  (P4, C1)
Change pipettes

Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C2)
Aspirate 100 nL from (P3, C2)
Dispense 300 nL  to  (P4, C2)
Change pipettes

Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C3)
Aspirate 100 nL from (P3, C3)
Dispense 300 nL  to  (P4, C3)
Change pipettes

Aspirate 100 nL from (P1, C1)
Aspirate 100 nL from (P2, C4)
Aspirate 100 nL from (P3, C4)
Dispense 300 nL  to  (P4, C4)
Change pipettes

etc
```

3.3 Validation of Transfer Parameters

mosquito performs some automatic checking on the values entered in the transfer table. The software highlights any invalid parameters entered by shading the cell red.

To get extra information about why a cell has turned red, click on the ? button on the right of the transfer table, or hover with the mouse over that cell and a message will appear above the grid like so:



Row 1: No plate has been loaded in position 1

Transfer		Source					Destination					Liquid			
	Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
➡	Copy	1	1	1			2	1	1			100	Contact	Always	...

3.4 Specifying Row Offsets (Quadranting)

Entering an offset value in the Row column of the transfer table changes the rows of the well plate addressed by mosquito. Setting offsets for rows allows pipettes to address all wells in plates which have well pitches smaller than the fixed pitch of the pipette tape. mosquito does this by moving the plate along the axis of the line of pipettes (i.e. front to back). If the mosquito instrument supplied has no Y-axis jog function then only one possible offset value is available.

When the pitch of the pipette tape is the same as the vertical pitch of the wells in a plate the offset value entered in the Row column of the transfer table is 1. For example, 96 pitch (9 mm) tape used on a 96 well plate has a Row value of 1.

If the pitch of the pipette tape is larger than the vertical pitch of the wells in a plate, then a range of offsets is available. The offset controls which of the rows in a given column is visited.

For example, when using 96 pitch (9 mm) tape with a 384-well plate, which has a vertical well pitch of 4.5 mm, there are two possible offsets for the Row value, 1 and 2.

- Row 1 visits the top row (A) of the column, the third row (C) and so on.
- Row 2 means visit the second row (B) of the column, the fourth row (D) and so on.

A	1
B	2
C	1
D	2
E	1
F	2

Similar behaviour results when using 384 pitch (4.5 mm) tape with a 1536 well plate, which has a vertical well pitch of 2.25 mm.

When using 96 pitch (9 mm) tape with a 1536 well plate (2.25 mm pitch), the available offsets for Row values are 1, 2, 3 or 4.

The following table shows the available offsets for each combination of tape pitch and plate format:

Tape Pitch vs. Plate Format	**96**	**384**	**1536**
384 pitch (4.5 mm)	n/a	1	1,2

96 pitch (9 mm)	1	1,2	1,2,3,4

Some specialist plates use custom-set offsets.

3.4.1 Copying Blocks of Wells Using Row Offsets

When a range of offsets is available (that is: when the pitch of the tape is larger than the vertical pitch of the wells in a plate) it is not possible for mosquito to visit every row of a column in a single operation.

For consistency, mosquito extends this behaviour horizontally across the plate as well as vertically down it. Thus, if mosquito cannot visit every row in the column in a single operation, it will not visit every column in a block transfer either. Instead, columns are addressed in the same pattern as rows are addressed.

For 9 mm (96 pitch) tape copying a 384 well plate, setting a destination of Start column 1 and Row 1 in the transfer table copies sample to the following wells:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	▨		▨		▨		▨		▨		▨		▨		▨
B															
C	▨		▨		▨		▨		▨		▨		▨		▨
D															
E	▨		▨		▨		▨		▨		▨		▨		▨
F															
G	▨		▨		▨		▨		▨		▨		▨		▨
H															

For 9 mm (96 pitch) tape copying a 384 well plate, setting a destination of Start column 1 and Row 2 in the transfer table copies sample to the following wells:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A															
B	▨		▨		▨		▨		▨		▨		▨		▨
C															
D	▨		▨		▨		▨		▨		▨		▨		▨
E															
F	▨		▨		▨		▨		▨		▨		▨		▨
G															
H	▨		▨		▨		▨		▨		▨		▨		▨

For 9 mm (96 pitch) tape copying a 384 well plate, setting a destination of Start column 2 and Row 1 in the transfer table copies sample to the following wells:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A		▨		▨		▨		▨		▨		▨		▨	
B															
C		▨		▨		▨		▨		▨		▨		▨	
D															
E		▨		▨		▨		▨		▨		▨		▨	
F															
G		▨		▨		▨		▨		▨		▨		▨	
H															

For 9 mm (96 pitch) tape copying a 384 well plate, setting a destination of Start column 2 and Row 2 in the transfer table copies sample to the following wells:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A															
B		▨		▨		▨		▨		▨		▨		▨	
C															
D		▨		▨		▨		▨		▨		▨		▨	
E															
F		▨		▨		▨		▨		▨		▨		▨	
G															
H		▨		▨		▨		▨		▨		▨		▨	

In this way, quadranting can be set up for reformatting plates to different well layouts.

3.5 Simulation Mode

Mosquito can simulate the running of a protocol without an instrument being attached, showing which wells are visited and so on, without the need to attach an instrument. This is intended to simplify off-line protocol development, demonstrations and training.

Create a protocol as normal and engage Simulation Mode by selecting **Options | Simulation Mode.** The status bar will show whether Simulation mode is on or off. Run the protocol by pressing the Run button as normal; mosquito will show the progress of the protocol as if it were being run.

Note that if no instrument is attached or the instrument is turned off then mosquito will display a warning message and automatically run in simulation-only mode. It is possible to configure mosquito at installation time to only run in simulation mode, in which case this warning message will not be displayed.

It is possible to combine single-step and simulation modes and run with both turned on.

3.6 Report Files

As a protocol runs, mosquito generates a report file which shows which aspirate, dispense and tape-indexing steps have been performed. If an error occurs, the file indicates which step the error occurred at.

A typical report file has the following format:

```
Mosquito report file
```

```
Serial number:    0203
Software version: 2.5
User:             src
2 position deck
Position:
1: Corning 1536 square
2: Corning 1536 square
05-Mar-2004 10:40:23    Ok: Aspirate 100 nL from (N1, C1, R1, S1), well volume 0 µL
05-Mar-2004 10:40:24    Ok: Dispense 100 nL  to  (N2, C1, R1, S1), well volume 0 µL
05-Mar-2004 10:40:26    Ok: Index
```

A run which has been aborted may look like the following:

```
Mosquito report file

Serial number:    0203
Software version: 2.5
User:             src
2 position deck
Position:
1: Corning 1536 square
2: Corning 1536 square
05-Mar-2004 10:40:23    Ok: Aspirate 100 nL from (   N1, C1, R1, S1), well volume 0 µL
05-Mar-2004 10:40:24    Ok: Dispense 100 nL  to  (N2, C1, R1, S1), well volume 0 µL
05-Mar-2004 10:40:26    Ok: Index
05-Mar-2004 10:40:27    Ok: Aspirate 100 nL from (N1, C5, R1, S1), well volume 0 µL
05-Mar-2004 10:40:28    Failed: Dispense 100 nL  to  (N2, C5, R1, S1), well volume 0 µL
Aborted
```

The aspirate and dispense steps in the report file correspond with those which are displayed when pressing the **Test** button.

The report file is created in the C:\Program Files\TTPLabtech\Mosquito\Reports\ directory and has a name constructed from the protocol file name with a number appended to generate a unique filename, e.g. “1536 Copy_1.Report”. Alternatively, report files may be created in the current user’s profile directory, as described in Section 4.1.4 below.

The creation of report files may be disabled using the **Options|Application Preferences** menu, see Section 4.1.3.

3.6.1 CSV-format report files

Report files may also be created in comma-separated variable format, so that they can easily be read by 3rd party applications such as Microsoft Excel(tm), as detailed in Section 4.1.5 below. The files will then have the extension .csv. A typical csv-format report file is as follows:

```
Mosquito report file
Serial number, 0201
Software version, 2.7.51
User, src
Deck, 5 position
1, Greiner 384 (round),[no id]
2, Greiner 384 (round),[no id]
3, [no plate], [no id]
4, [no plate], [no id]
5, [no plate], [no id]
Status, Date, Time, Operation, Volume, Position, Column, Row, Subwell, Well volume
Home tape and change pipettes
Ok, 29-Sep-2005, 14:28:56, Aspirate, 100, 1, 1, 1, 1, 0
Ok, 29-Sep-2005, 14:28:57, Dispense, 100, 2, 1, 1, 1, 0
```

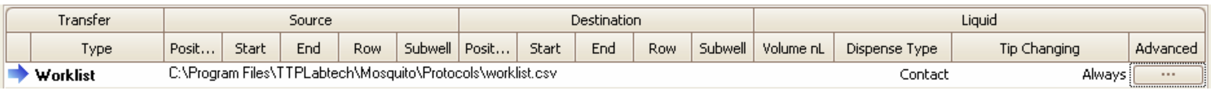
3.7 Worklist transfers

Instead of using the protocol grid to specify the exact sequence of aspirate and dispense operations, the user can specify the operations in a worklist file. It is possible to create a protocol which uses more than one worklist file, or to mix worklist and non-worklist transfers in the same protocol.

Worklist files are comma-separated variable files; they can easily be exported from Microsoft Excel(tm) or similar packages. A sample Excel file and the worklist it generates are installed in the \Mosquito\TTPLabtech\protocols directory.

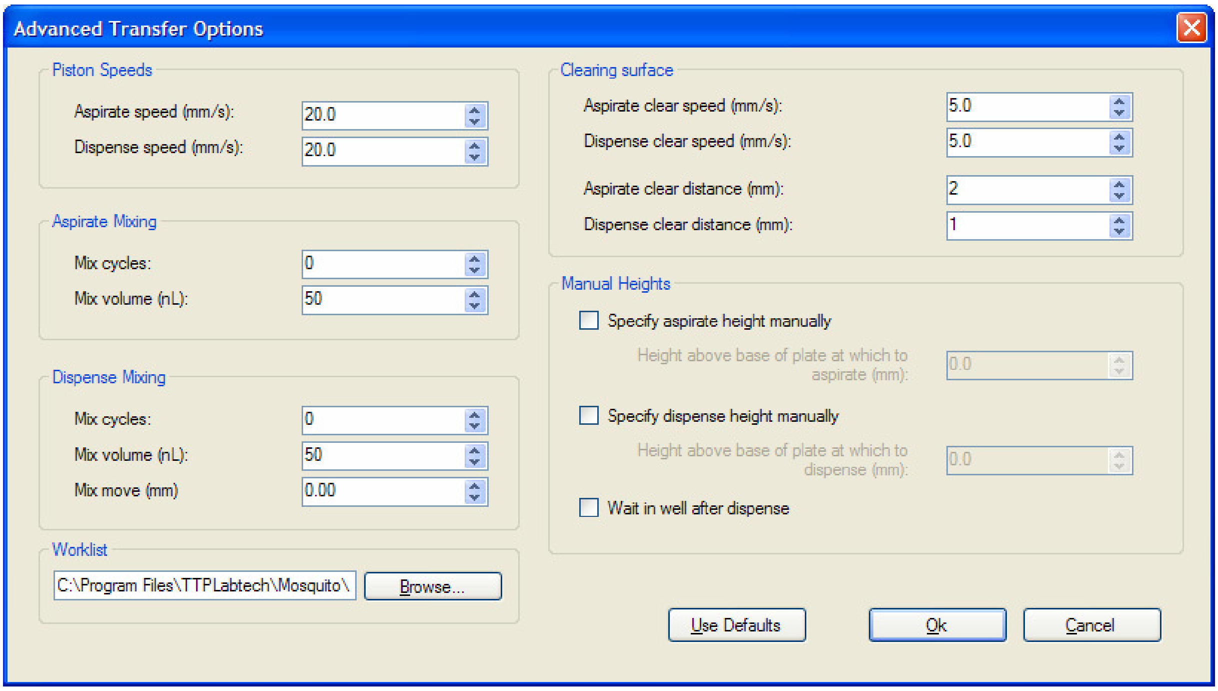
3.7.1 Specifying a worklist file

To do this, select the worklist transfer type so:



Transfer	Source					Destination					Liquid			
Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
Worklist	C:\Program Files\TTPLabtech\Mosquito\Protocols\worklist.csv											Contact	Always	...

Press the ... button to specify the worklist file. The advanced options dialog appears with and extra section for specifying the worklist file:



Advanced Transfer Options

Piston Speeds
Aspirate speed (mm/s): 20.0
Dispense speed (mm/s): 20.0

Aspirate Mixing
Mix cycles: 0
Mix volume (nL): 50

Dispense Mixing
Mix cycles: 0
Mix volume (nL): 50
Mix move (mm) 0.00

Worklist
C:\Program Files\TTPLabtech\Mosquito\

Clearing surface
Aspirate clear speed (mm/s): 5.0
Dispense clear speed (mm/s): 5.0
Aspirate clear distance (mm): 2
Dispense clear distance (mm): 1

Manual Heights
□ Specify aspirate height manually
Height above base of plate at which to aspirate (mm): 0.0
□ Specify dispense height manually
Height above base of plate at which to dispense (mm): 0.0
□ Wait in well after dispense

Either type in a filename or – if the file already exists press the Browse... button to navigate to the file.

3.7.2 Worklist file format

The format of the worklist file must adhere to certain standards. The word 'Worklist' must appear on its own on a line prior to the worklist data. It must be followed by either a carriage return or a comma.

All numeric data in the file will then be interpreted as pipetting commands; all other data will be ignored.

Each line of the file specifies a single copy operation so:

Source `Position`, Column, Row, Subwell, Destination Position, Column, Row, Subwell, Transfer Volume

Thus

```
1,1,1,2,2,1,1,3,100
```

Represents a copy of 100nL from position 1, column 1, row 1 and subwell 2 to position 2, column 2, row 1, subwell 3.

Note that the subwell parameters are optional; if they are omitted then subwell 1 is assumed. However, if one (source or destination) subwell is omitted then the other must be too. Thus the following is valid:

```
1,1,1,2,1,1,100
```

and represents a copy of 100nL from position 1, column 1, row 1 and subwell 1 to position 2, column 2, row 1, subwell 1.

3.7.3 Specifying a tip-changing strategy

An optional final argument can be appended to specify a tip-changing strategy for each line of the worklist. This can be one of:

```
always
multi-dispense
between-transfers
never
```

These have exactly the same meaning as if you were specifying a strategy for a transfer in the protocol tab. If no tip-changing strategy is specified, the tip-changing strategy will be that of the worklist transfer in the protocol.

For example, if you created a worklist transfer with a tip changing strategy of `never` and specified a file containing the following:

```
Worklist
1,1,1,2,1,1,100
1,1,1,3,1,1,100
1,1,1,2,1,1,100,always
1,1,1,3,1,1,100,always
```

You would not get tip changes between the first two transfers of the worklist (they inherit the worklist's `never` strategy). The last two transfers however, would be guaranteed fresh sets of pipettes.

3.7.4 Specifying plates and advanced options in worklists

The plates to be used must still be specified via the mosquito user interface.

The advanced options for worklist transfers may be specified by pressing the ... button as normal; those options will apply to all of the transfers in the worklist.

4 APPLICATION PREFERENCES

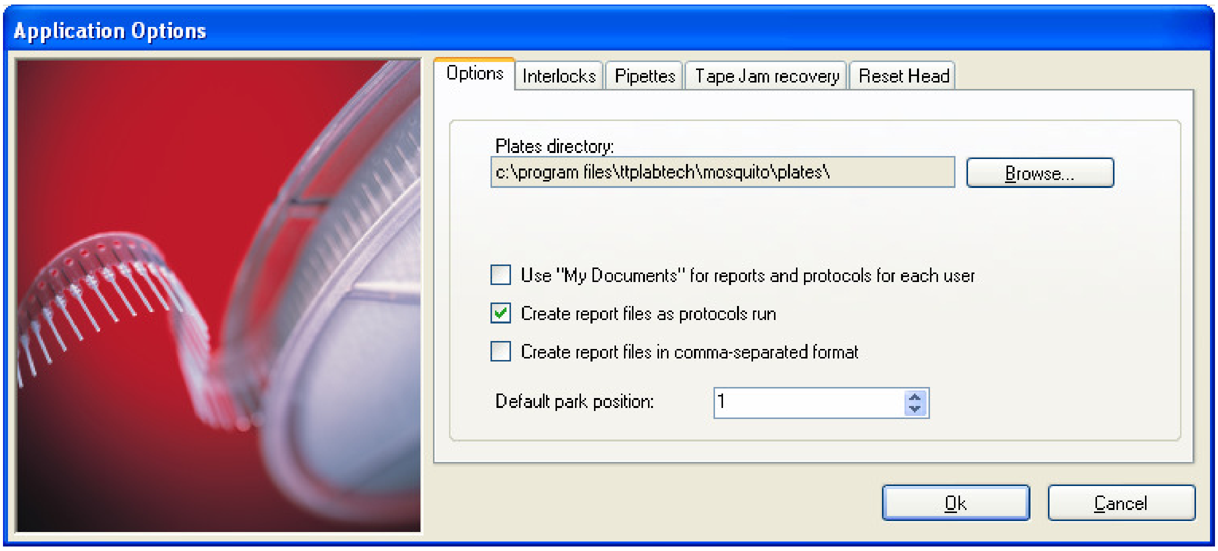
A number of application-wide preferences may be set by the user. These are:

- Specifying the location of the Plates directory
- Specifying where protocols and report files are to be stored
- Setting the default park position of the plate deck
- Specifying whether or not report files are created
- Specifying the format of report files
- Specifying whether or not to home drives at the start of a protocol
- Specifying whether to load clean pipettes at the end of a protocol
- Specifying the pitch of the pipette tape
- Overriding certain interlocks

These preferences are set using the Application Options dialog box, reached from the **Options|Application Preferences** menu.

There are a number of separate tabs in this dialog. Interlocks, Tape Jam recovery and Reset Head are only available when in Calibration Mode. The Tape Jam Recovery tab is discussed in Section 9.3.6

4.1 Options



Plates directory: c:\program files\ttplabtech\mosquito\plates\

☐ Use "My Documents" for reports and protocols for each user

☑ Create report files as protocols run

☐ Create report files in comma-separated format

Default park position: 1

4.1.1 Specifying the Plates Directory Location

When the mosquito software is started it loads all of the available plate files in the Plates directory. If the user wishes to load these files from a central server, the location of these plate files will need to be specified.

To specify a different location for plate files, select **Options|Application Preferences** to access the Application Options dialog box. Click on the **Browse** button next to the Plates Directory box, and navigate to the directory to be used. Click the **OK** button, and the directory path will be changed to the new directory.

The mosquito software must be closed and restarted for this change to take effect.

4.1.2 Setting the Default Park Position

The default park position to which the plate deck returns when homing or after finishing a run can be set by the user.

To specify a park position, select **Options|Application Preferences** to access the Application Options dialog box, then add the position number.

The park position is in the range 0 to a maximum of 4, depending on the exact specification of the instrument. The park position 0 is always with the leftmost plate position underneath the instrument head (so the deck is extended to the right of the user as they face the instrument).

This may be especially useful in configuring mosquito to work with a robot arm in an integrated application.

4.1.3 Generating Protocol Report Files

To disable report file generation, select **Options|Application Preferences** to access the Application Options dialog box, then uncheck the 'Create report files as protocols run' check box.

4.1.4 Specifying the reports and protocols directory

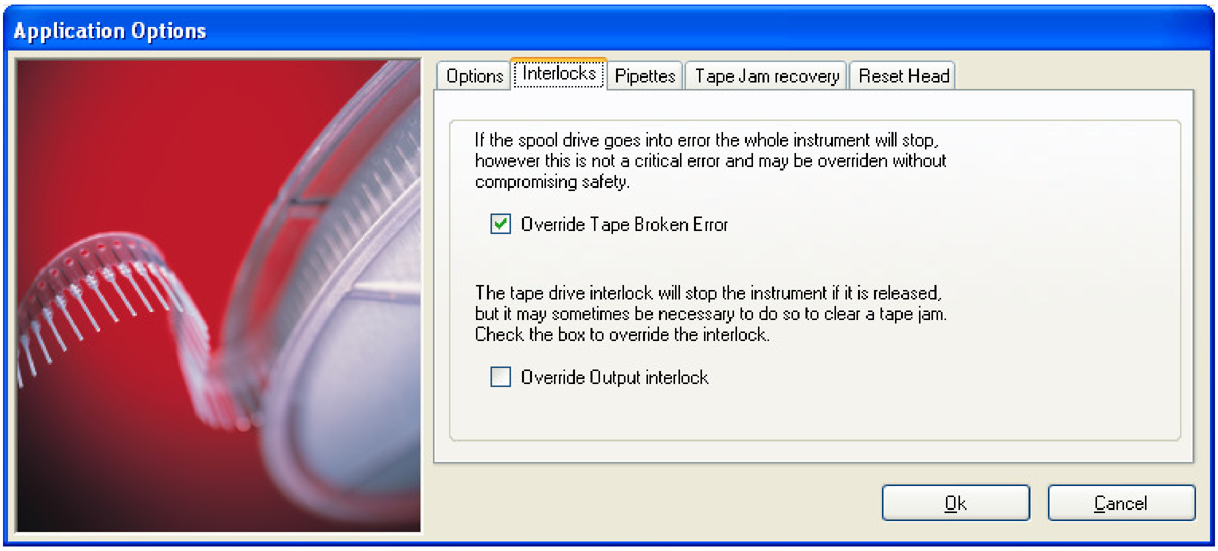
If there are many users of the mosquito instrument it may be desirable to have each user store their work in their own directory. Check the "Use 'My Documents' for reports and protocols for each user" to have their protocols and report files stored under:
\\Documents and Settings\<username>\Application Data\Mosquito\Protocols and
\\Documents and Settings\<username>\Application Data\Mosquito\Reports.

4.1.5 Specifying the report file format

It is possible to generate report files in comma-separated variable format so that they can be more easily read by 3rd party applications such as Microsoft Excel(tm)

4.2 Interlocks

A number of mosquito interlocks can be overridden. These interlocks are present for the safe and accurate running of the instrument but there are occasions when it may be desirable to disable them by checking them in the dialog show below:



If the spool drive goes into error the whole instrument will stop, however this is not a critical error and may be overriden without compromising safety.

☑ Override Tape Broken Error

The tape drive interlock will stop the instrument if it is released, but it may sometimes be necessary to do so to clear a tape jam. Check the box to override the interlock.

☐ Override Output interlock

4.2.1 Override Tape Broken Error

It is advised that the Override Tape Broken Error interlock checkbox is left unchecked. This will warn the user if the pipette tape breaks or spools incorrectly. However, when pipette tape is changed frequently, such as when developing assays, it may be useful to override the interlock by checking the Override Tape Broken Error box.

4.2.2 Override Output Interlock

This option disables the interlock which is used to detect the correct position of the device which guides pipette tape out of the head. Do not override this interlock unless directed to do so by mosquito technical support. It may be necessary to override this interlock when clearing a tape jam in the head

5 CALIBRATION

The **Calibration** tab allows recalibration of the user-calibratable parts of the mosquito instrument.

The **Calibration** tab is displayed by selecting **Options|Calibrate** from the main menu. The application must be in Calibration Mode in order to be able to access the Calibration tab.

Note: Recalibrating may alter the instrument behaviour and volumetric performance.

If in doubt, please contact TTP LabTech (see Section 9.1) before changing any of the settings on this tab.

Note further that additional calibration controls exist to assist service engineers if required, but these should not be used without the assistance of TTP LabTech.

During calibration:

- You can press **Cancel** at any time to cancel the calibration process without saving any parameters. The instrument will re-home when you do this.

- If you do not want to calibrate a certain step you may skip it by pressing **Skip >>.** If you have not made any changes in a particular step, pressing **Next >>** will have the same effect.

- When you press **Start >>** on the first screen of the wizard a backup settings file will be created. The original settings can be retrieved from this if necessary.

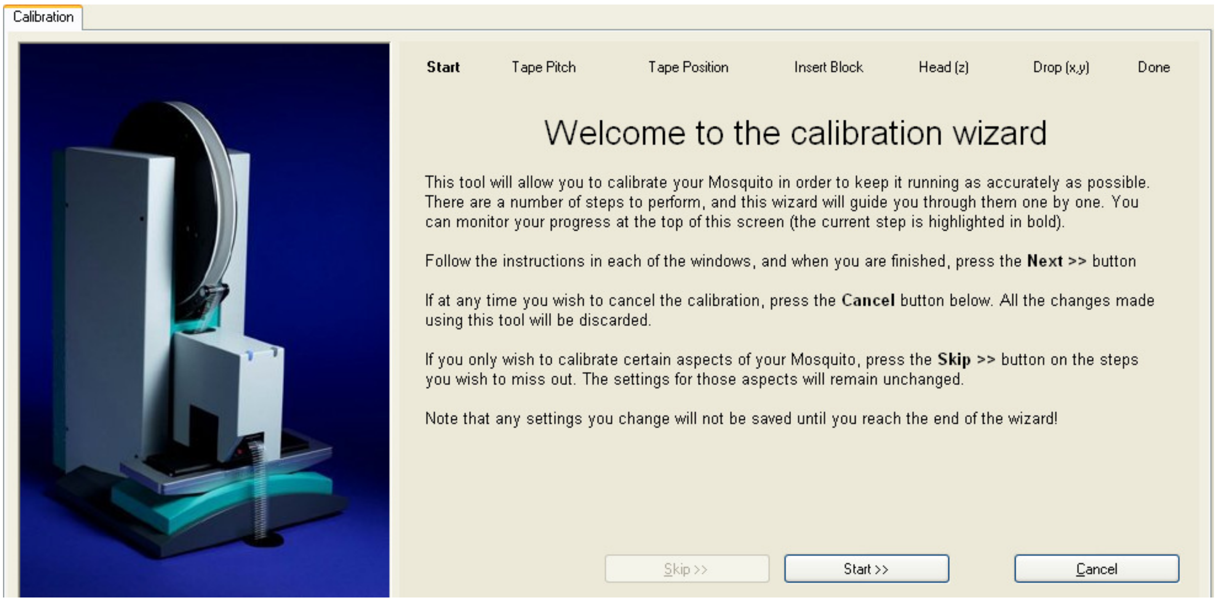
- Only those values which have changed will be saved. So if you move the deck but not the head drive, only the new deck position will be stored.

- The values are not saved until the wizard is completed – if you only wish to change settings in the earlier part of the wizard, simply skip the rest of the steps until you reach the end.

- If the status light on the tape position screen or on the head screen turns red – for example if the head has been raised too high – press the **Home** button to reset the tape/head drive as appropriate and continue calibrating.

6 EXERCISE 1: RECALIBRATING MOSQUITO

To recalibrate the mosquito instrument ensure that you have the calibration block supplied with the instrument to hand, then select **Options | Calibrate**. On screen instructions will be supplied throughout the calibration process, beginning with the following screen:



Welcome to the calibration wizard

This tool will allow you to calibrate your Mosquito in order to keep it running as accurately as possible. There are a number of steps to perform, and this wizard will guide you through them one by one. You can monitor your progress at the top of this screen (the current step is highlighted in bold).

Follow the instructions in each of the windows, and when you are finished, press the **Next >>** button

If at any time you wish to cancel the calibration, press the **Cancel** button below. All the changes made using this tool will be discarded.

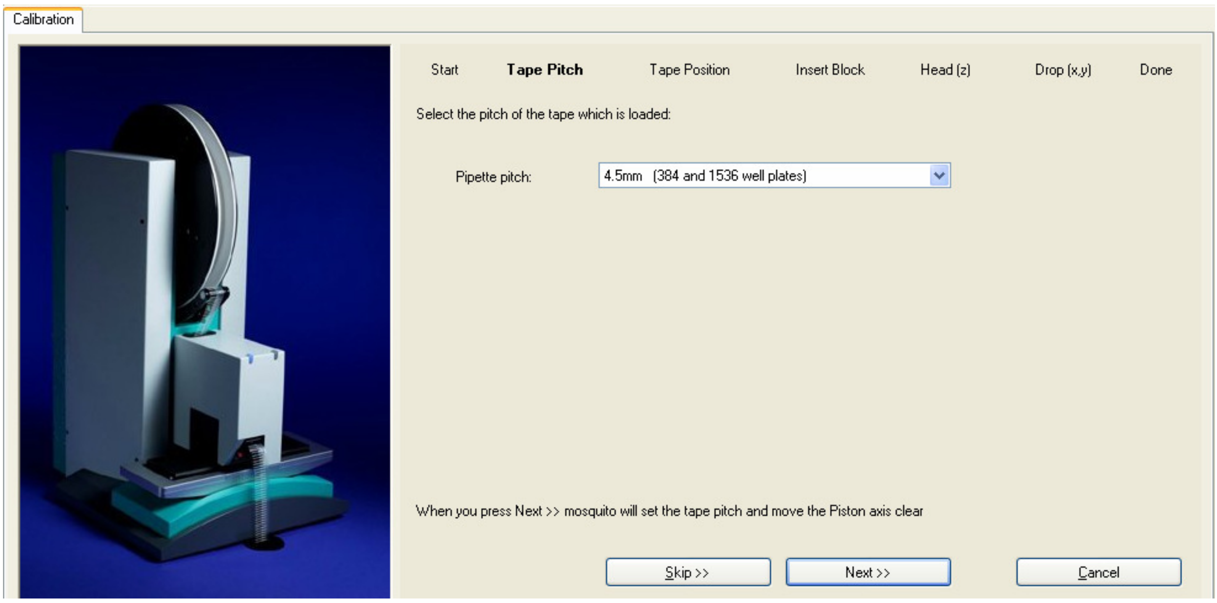
If you only wish to calibrate certain aspects of your Mosquito, press the **Skip >>** button on the steps you wish to miss out. The settings for those aspects will remain unchanged.

Note that any settings you change will not be saved until you reach the end of the wizard!

- Press **Start >>** to start calibrating mosquito.

6.1 Tape Pitch

The Tape Pitch screen requires you to select the pitch of the pipette tape which is loaded. It is *essential* that this is correct, as mosquito uses it to deduce the measurements for the calibration parameters.



Select the pitch of the tape which is loaded:

Pipette pitch: 4.5mm (384 and 1536 well plates)

When you press Next >> mosquito will set the tape pitch and move the Piston axis clear

- Press **Next >>** to save the tape pitch setting and go to the next screen.

6.2 Tape Position

The mosquito head is designed for use with various pitches of tape. The buttons available on this screen provide help with fine tuning the tape position.

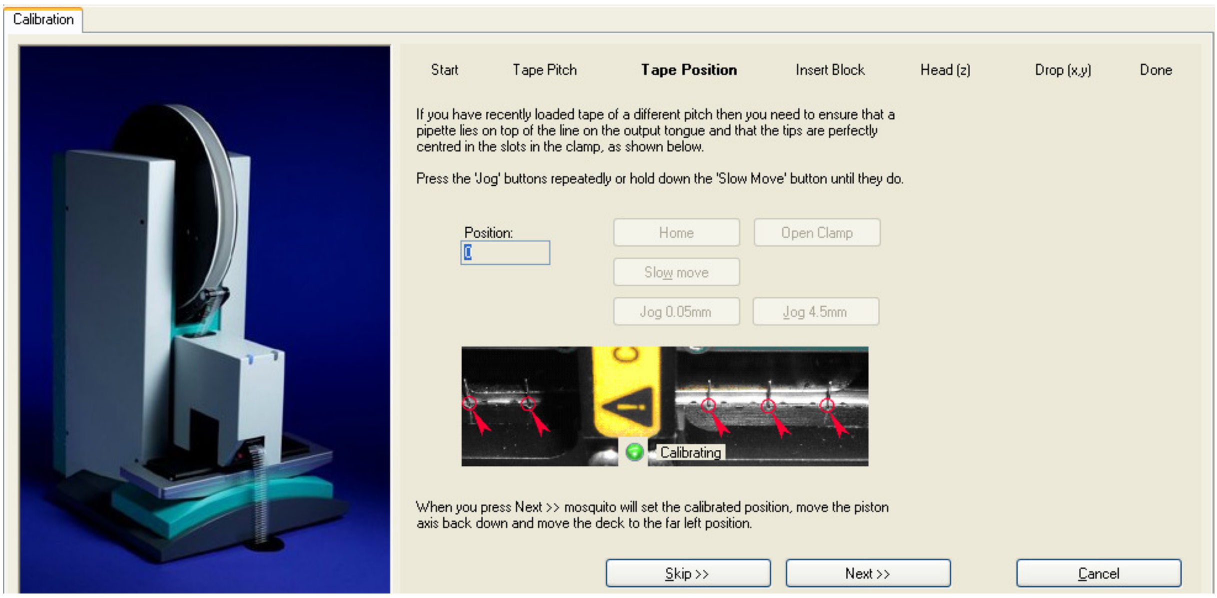
To prevent miscalibration of – and possible damage to – the mosquito instrument please contact TTP LabTech before following this procedure.

It is possible that, when loading tape with a pitch of 9mm or greater, it can line up with slots in the pipetting head which are only intended for use with 4.5mm tape. If this occurs then the pipettes will never line up with the calibration block crosshairs (horizontal lines) during calibration.

- If this is the case, press the **Jog 4.5mm** button until they do line up.

It is further possible that the pipettes line up only approximately with the slots in the pipetting head:

- To slowly move the tape forward hold down the **Slow Move** button.
- To slowly move the tape backward hold down the **Reverse** button.
- Release it to stop the tape movement and cause the clamp to close after a short pause.
- To move the tape a very small distance forward press the **Jog 0.05mm** button.
- You can use the clamp open/close button to help you check the alignment of the pipettes if necessary.



If you have recently loaded tape of a different pitch then you need to ensure that a pipette lies on top of the line on the output tongue and that the tips are perfectly centred in the slots in the clamp, as shown below.

Press the 'Jog' buttons repeatedly or hold down the 'Slow Move' button until they do.

Position: 0

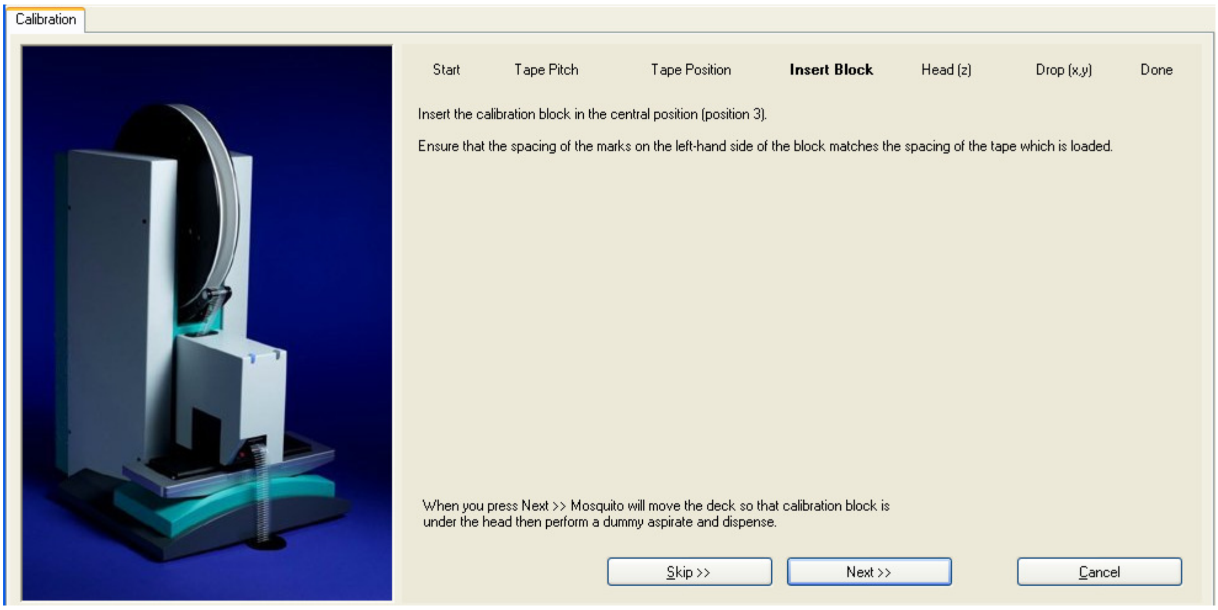
When you press Next >> mosquito will set the calibrated position, move the piston axis back down and move the deck to the far left position.

- When you are happy the tape is correctly positioned, press **Next >>** to save the calibrated position and go to the next screen.

Mosquito moves the deck to the far left position for you to insert the calibration block.

6.3 Insert Calibration Block

The next steps in the calibration wizard require the use of the calibration block. If you are using a 5-way instrument, insert the calibration block in the central deck position. Otherwise insert the block in the far left deck position. Ensure that the spacing of the marks on the left-hand side of the block matches the spacing of the pipette tape which is loaded. If you are using tape with a pitch other than 4.5mm or 9mm then ensure that the 4.5mm markings are at the left-hand side of the calibration block.



Insert the calibration block in the central position (position 3).

Ensure that the spacing of the marks on the left-hand side of the block matches the spacing of the tape which is loaded.

When you press Next >> Mosquito will move the deck so that calibration block is under the head then perform a dummy aspirate and dispense.

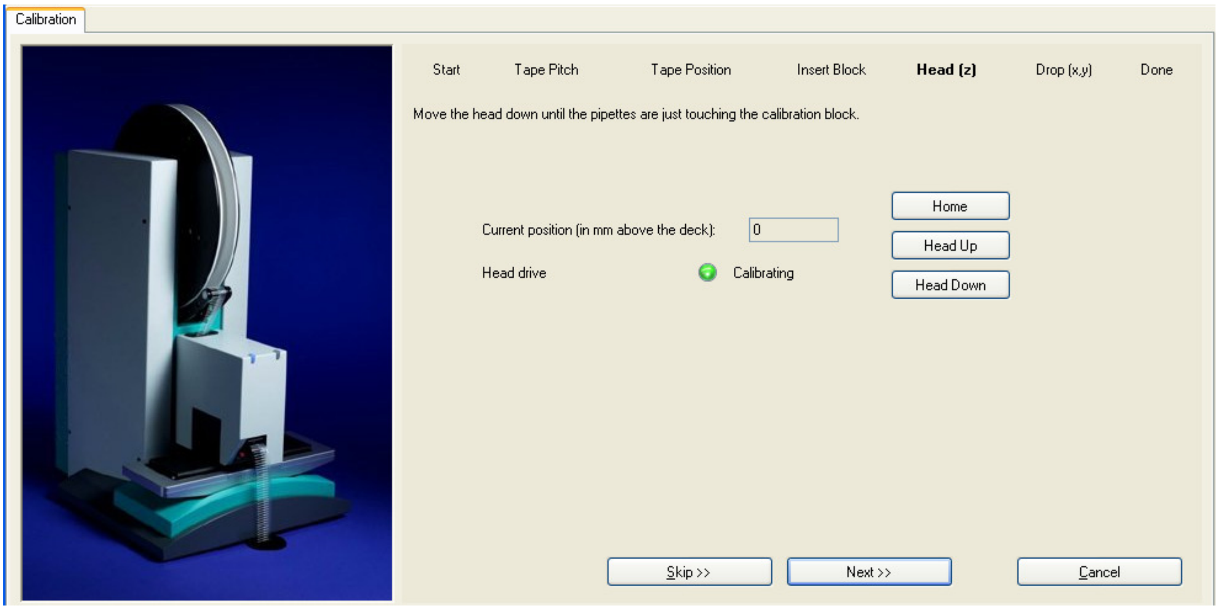
- Press **Next >>** to continue calibrating.

6.4 Head Position

Once the tape position has been set you can calibrate the vertical position of the pipetting head, to ensure Mosquito aspirates and dispenses at the correct height.

This screen provides buttons for you to adjust the positioning of the pipettes over the calibration block, and displays the current position of the head:

- Use the Head Down and Head Up buttons to move the pipetting head down (or up) until the pipette tips just touch the surface of the calibration block. Check this by looking along the line of tips under the head.



Move the head down until the pipettes are just touching the calibration block.

Current position (in mm above the deck): 0

Head drive ● Calibrating

When you are satisfied, press **Next >>** and Mosquito positions the calibration block correctly for calibrating the drop position.

6.5 Drop Position

Sometimes it may be necessary to adjust the position of drops within plate wells. This screen provides buttons for you to calibrate the instrument so that drops are positioned centrally within wells.

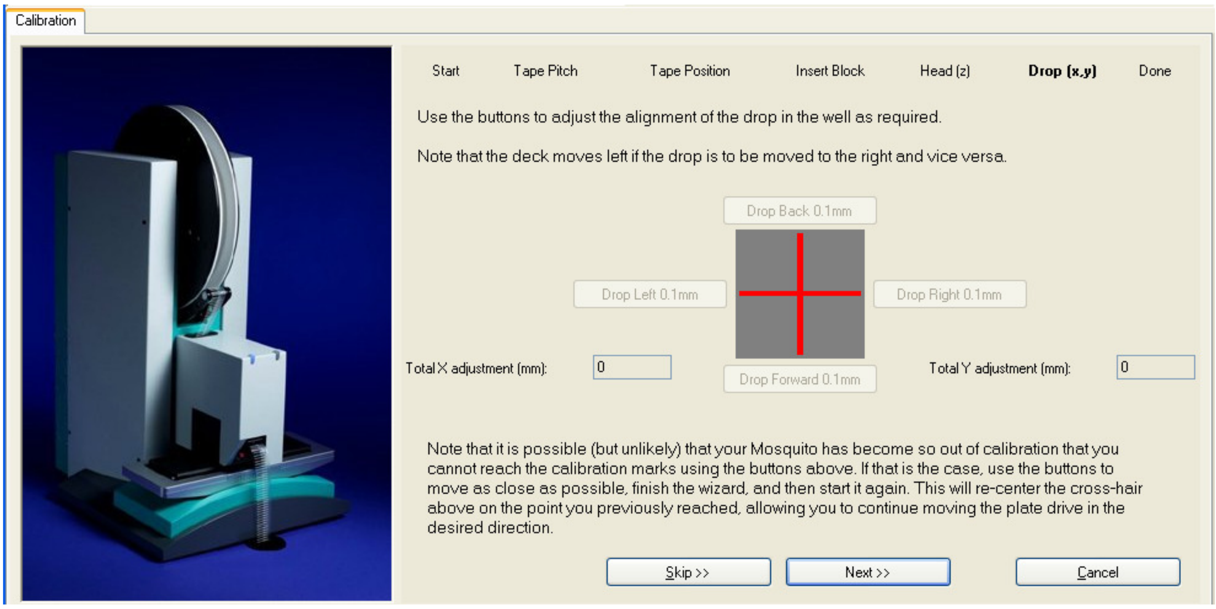
Use the buttons as described below to make the tips line up with each of the marks on the calibration block.

- If the drop is too far to the left, use the **Drop Right 0.2mm** button to adjust it as much as you need.
- If the drop is too far to the right, use the **Drop Left 0.2mm** button to adjust it as much as you need.

For instruments with a continuously variable Y drive, the position of the drop can also be adjusted forward and backward:

- If the drop is too far forward (away from the instrument), use the **Drop Back 0.2mm** button to adjust it as much as you need.
- If the drop is too far back (towards the instrument), use the **Drop Forward 0.2mm** button to adjust it as much as you need.

The total adjustment made in both directions is displayed. A positive X value indicates that the drop will be moved to the right, and vice versa; a positive Y value indicates that the drop will be moved backwards, and vice versa.



Use the buttons to adjust the alignment of the drop in the well as required.

Note that the deck moves left if the drop is to be moved to the right and vice versa.

Total X adjustment (mm): 0

Total Y adjustment (mm): 0

Note that it is possible (but unlikely) that your Mosquito has become so out of calibration that you cannot reach the calibration marks using the buttons above. If that is the case, use the buttons to move as close as possible, finish the wizard, and then start it again. This will re-center the cross-hair above on the point you previously reached, allowing you to continue moving the plate drive in the desired direction.

- When you are satisfied with the calibration, press **Next >>**.

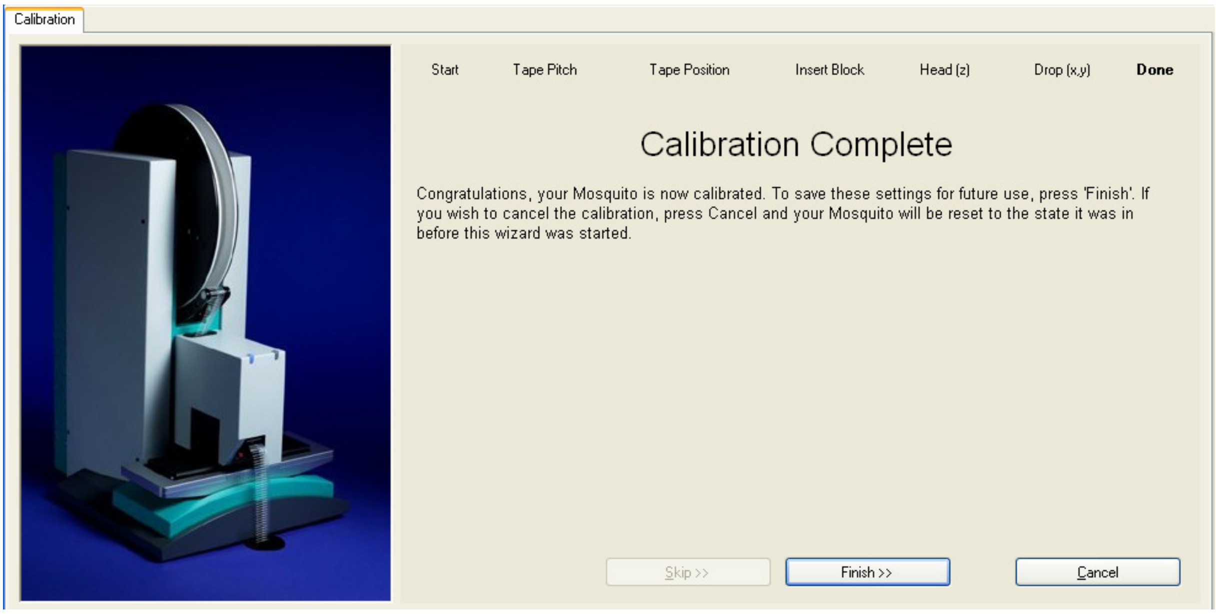
If Mosquito has become so far out of calibration that you cannot reach the marks on the calibration block using the buttons (this should not normally happen) then take the following steps:

- Use the buttons to move as close as possible to the desired position
- Press Next >> and continue to the end of the calibration and save your settings
- Restart the calibration wizard and skip to the drop position screen
- The cross-hair will have re-centered and you can now move the deck further in the required direction
- Repeat this process as often as required

6.6 Finishing the Calibration

You now have only two options, either:

- Press **Finish >>** to complete the calibration and save your settings.
- If you are unhappy with any aspect of the calibration, press **Cancel** and the calibration will be discarded.



Calibration Complete

Congratulations, your Mosquito is now calibrated. To save these settings for future use, press 'Finish'. If you wish to cancel the calibration, press Cancel and your Mosquito will be reset to the state it was in before this wizard was started.

7 PLATE SETTINGS

The **Options|Plates** menu displays a window which allows the operator to set up new SBS plate types or to adjust settings to optimise the mosquito system for different plate types.

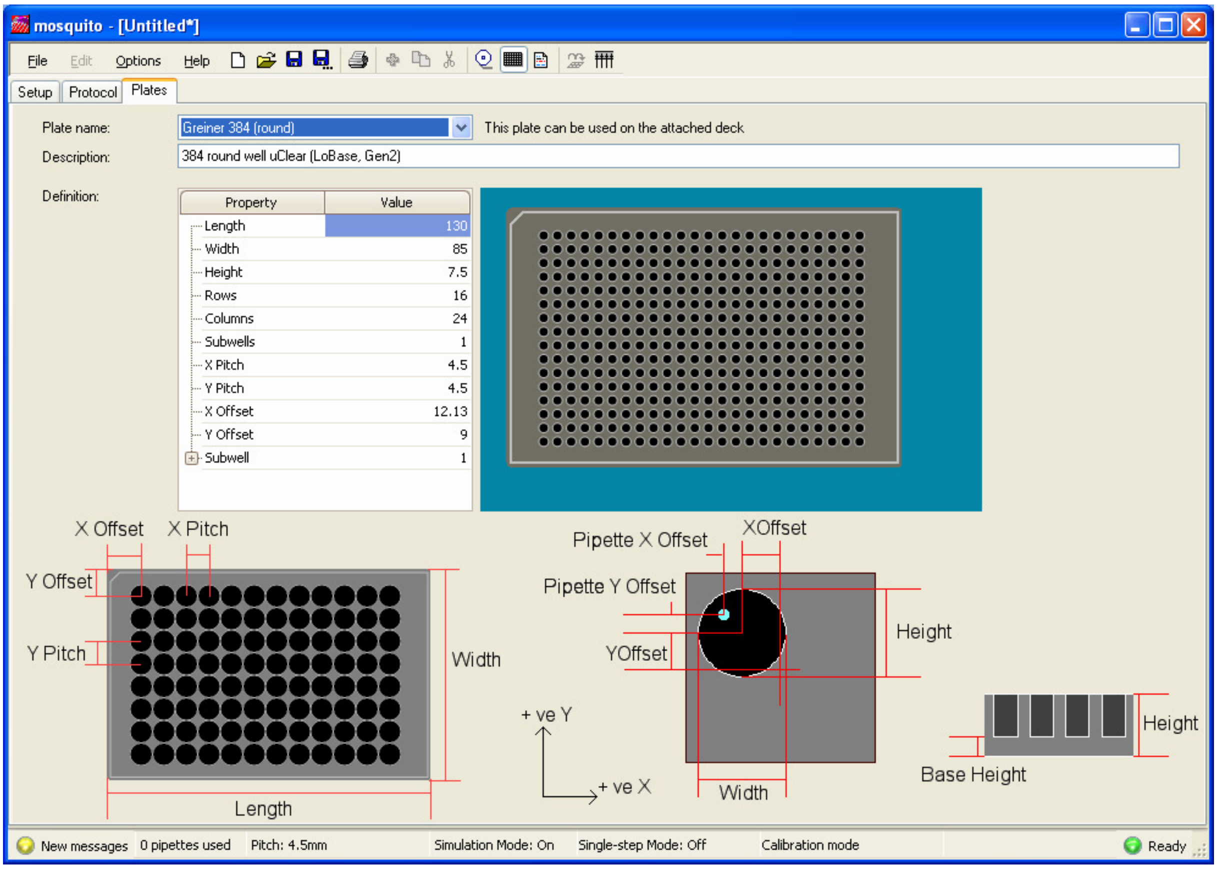


Plate name: Greiner 384 (round) This plate can be used on the attached deck

Description: 384 round well uClear (LoBase, Gen2)

Definition:

Property	Value
Length	130
Width	85
Height	7.5
Rows	16
Columns	24
Subwells	1
X Pitch	4.5
Y Pitch	4.5
X Offset	12.13
Y Offset	9
Subwell	1

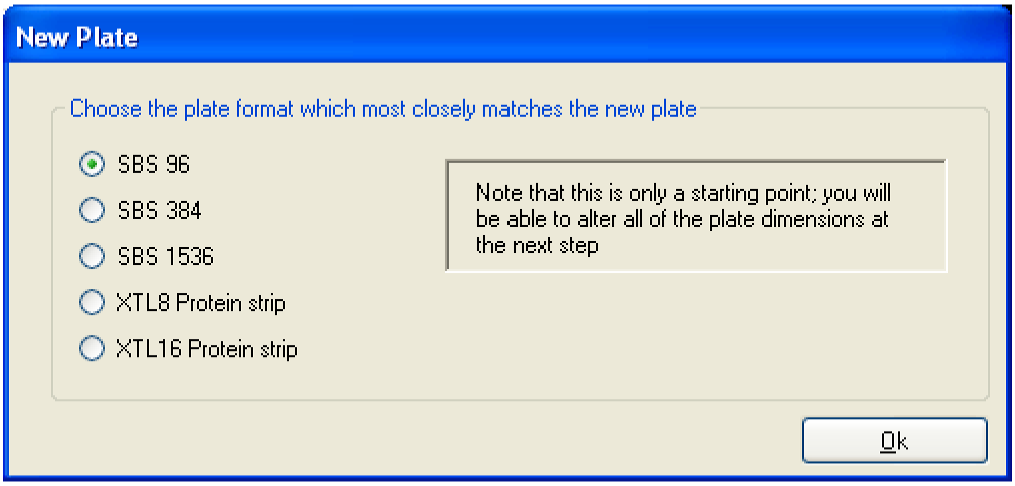
New messages 0 pipettes used Pitch: 4.5mm Simulation Mode: On Single-step Mode: Off Calibration mode Ready

7.1 Adding New Plate Types

A new plate may be added to the plate database using either the **New** option, or the **Save As** option.

Both options request a name for the new plate type. Plate types should be given a descriptive file name.

Selecting **New** will prompt for the plate type which the new plate most closely matches:



New Plate

Choose the plate format which most closely matches the new plate

- ● SBS 96
- ○ SBS 384
- ○ SBS 1536
- ○ XTL8 Protein strip
- ○ XTL16 Protein strip

Note that this is only a starting point; you will be able to alter all of the plate dimensions at the next step

mosquito will automatically generate some approximate measurements.

Once the new plate file is created, the plate details can be updated by typing in the changed values on the **Plates** tab.

After editing, the new plate type should be saved before quitting the **Plates** tab. To save the file use the **Save** icon (💾) or **File|Save** from the main menu.

7.2 Deleting Plate Types

Plate types may not be deleted from within the mosquito software. However a plate file may be deleted using Windows Explorer.

Plate files are saved to the **\mosquito\Plates** directory. Use Windows Explorer to navigate to the **\mosquito\Plates** directory. Highlight the file to be deleted and select **File|Delete**.

7.3 Editing Plate Details

Plate details may be edited directly from the **Plate** tab by typing in updated values.

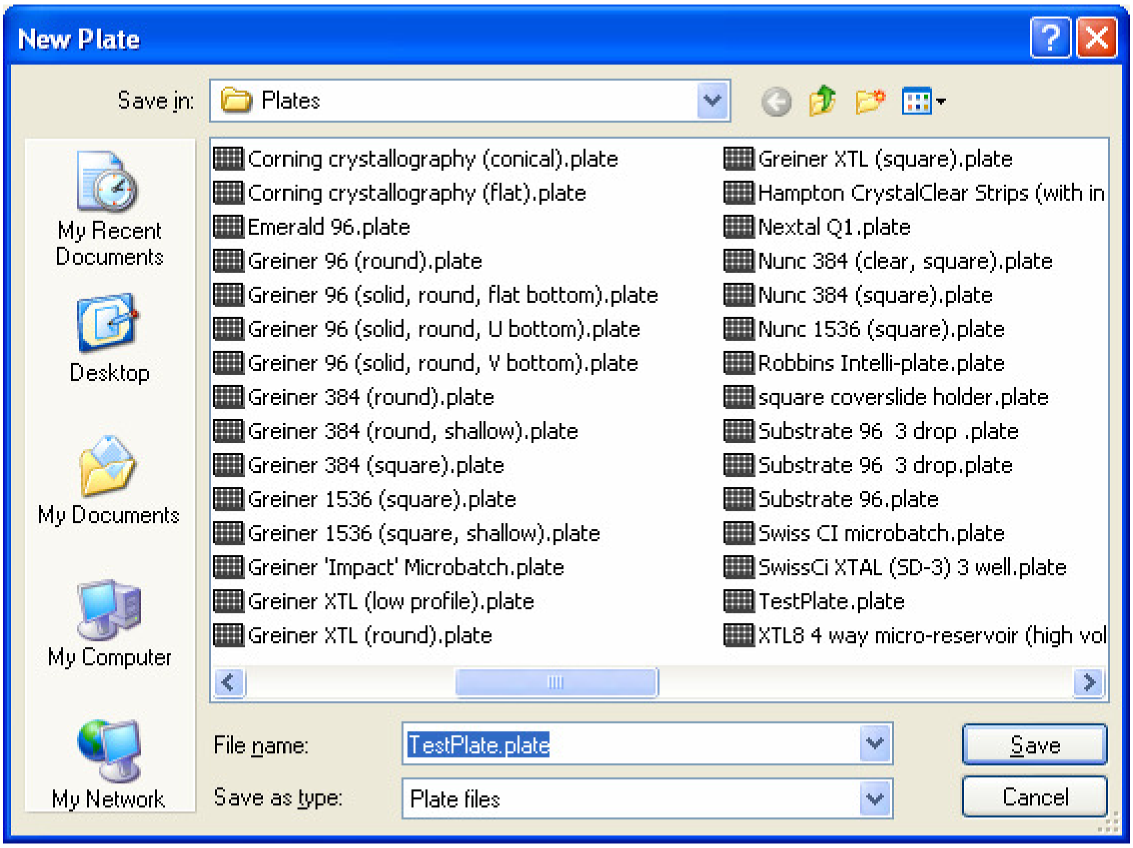
The well X and Y pitch should not be edited for the default plate types as these are set by the SBS standard.

7.4 Saving Changes to Plate Details

7.4.1 Normal Saving

To save the changes over the top of the previous values, click on the **Save** icon (💾), or select **File|Save** from the main menu.

To keep the original plate settings and create a new plate with the current settings, click on the **Save As** icon (💾), or select **File|Save As**. A pop-up dialog box requests a name for the new plate type:



Save in: Plates

Corning crystallography (conical).plate
Corning crystallography (flat).plate
Emerald 96.plate
Greiner 96 (round).plate
Greiner 96 (solid, round, flat bottom).plate
Greiner 96 (solid, round, U bottom).plate
Greiner 96 (solid, round, V bottom).plate
Greiner 384 (round).plate
Greiner 384 (round, shallow).plate
Greiner 384 (square).plate
Greiner 1536 (square).plate
Greiner 1536 (square, shallow).plate
Greiner 'Impact' Microbatch.plate
Greiner XTL (low profile).plate
Greiner XTL (round).plate
Greiner XTL (square).plate
Hampton CrystalClear Strips (with in
Nextal Q1.plate
Nunc 384 (clear, square).plate
Nunc 384 (square).plate
Nunc 1536 (square).plate
Robbins Intelli-plate.plate
square coverslide holder.plate
Substrate 96 3 drop .plate
Substrate 96 3 drop.plate
Substrate 96.plate
Swiss CI microbatch.plate
SwissCi XTAL (SD-3) 3 well.plate
TestPlate.plate
XTL8 4 way micro-reservoir (high vol

File name: TestPlate.plate

Save as type: Plate files

Plate types are saved in the **Plates** directory as separate files identified by the .plate file extension.

7.4.2 Saving as a text file

It is possible to save plate files as text files for easy readability in a text editor such as Notepad or Microsoft Word(tm).

To do this select **File | Export to text file** *while the Plates tab is open*. You will be prompted for a filename and a location to create the file; the current plate will then be saved as a text file.

Note: at the present time it is not possible for mosquito to read in the text version of the plate file. mosquito works only with the original *.plate file.

7.5 Reversing Changes to Plate Files

Backups of all original plate files are installed in the Backup directory, which has the default location C:\Program Files\TTPLabtech\Mosquito\Plates\Backups.

If a plate file is accidentally modified, then the original settings can be restored as follows:

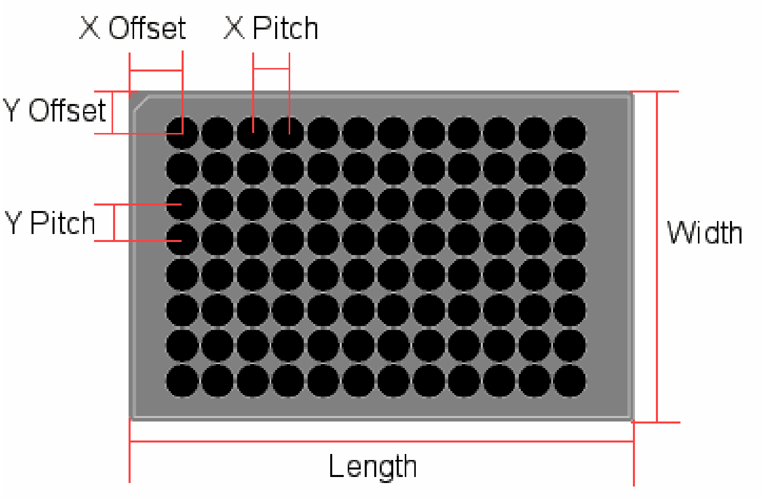
- Exit the mosquito application
- Use Windows Explorer to navigate to the C:\Program Files\TTPLabtech\Mosquito\Plates\Backups directory

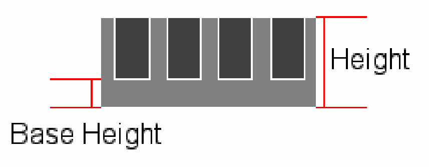
- Copy the relevant plate file to the:
  C:\Program Files\TTPLabtech\Mosquito\Plates directory, overwriting the existing file.
- Restart the mosquito application.

8 CREATING A NEW PLATE TYPE AND TAKING PLATE MEASUREMENTS

Configuring a new plate type requires very precise measurements, such as those obtained with a shadowgraph or Vernier calipers, and may require considerable fine tuning.

The various plate measurements are illustrated below:





All measurements should be given in millimetres.

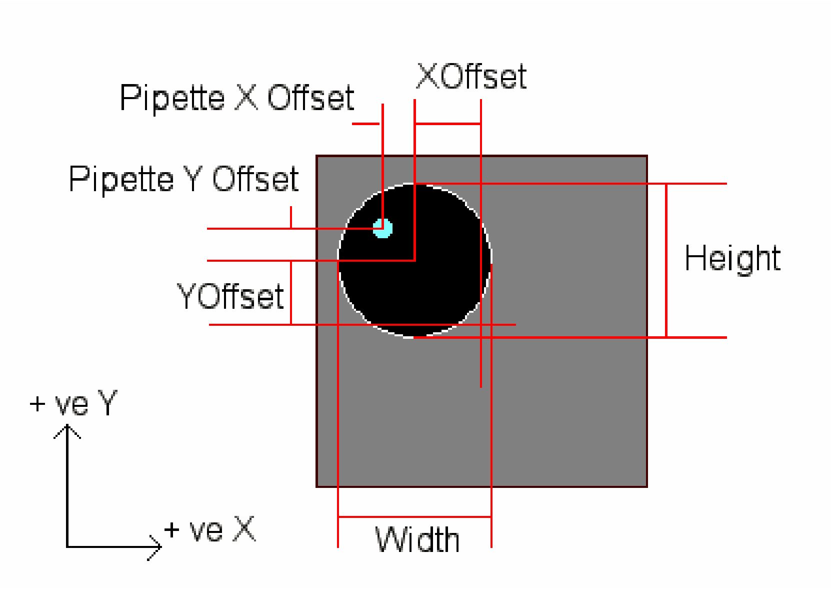
In addition to the measurements illustrated, there are some additional parameters required:

Rows	Number of rows of wells on a plate e.g. 8 for a 96 well plate
Columns	Number of columns of wells on a plate e.g. 12 for a 96 well plate
Subwells	Every well has at least one subwell; and crystallography plates may have more than one. The Subwell parameter is only visible to users of mosquito crystallography instruments.

8.1 Subwell Parameters for Complex Plates

A well is a region on a plate which contains one or more subwells. For simple plates the single subwell and the well coincide exactly, but this will not be the case for plates which have more than one subwell per well, such as the Greiner Crystal Ledge plates.

The meanings of the subwell parameters are summarised in the diagram and explained in the table below:



Width	Horizontal size in mm of the subwell
Height	Vertical size in mm of the subwell
X Offset	Horizontal distance of the centre of the subwell from the centre of the well region
Y Offset	Vertical distance of the centre of the subwell from the centre of the well region
Pipette X Offset	It is sometimes desirable to aspirate and dispense at a point which is not at the centre of the subwell. The Pipette X Offset gives the left-right distance from the centre of the subwell at which to pipette.
Pipette Y Offset	As the pipette X offset, but in the Y direction

There are some additional subwell parameters which are not illustrated on the diagram. These are explained in the following table:

Volume	The maximum volume in microlitres of the subwell. This is used to guard against overfilling a well and to determine how to withdraw pipettes from a well when the volumes are not known.
Area (mm^2)	The cross sectional area of the subwell. This parameter is used to determine the height at which to position pipettes when aspirating and dispensing and well volumes are known.

9 ERROR HANDLING & RECOVERY

9.1 Contacting Customer Support

TTP LabTech are available to answer customer support queries during the company's UK working hours. Medical Equipment Repair Association (MERA) provide a customer support service in the US covering the majority of US working hours.

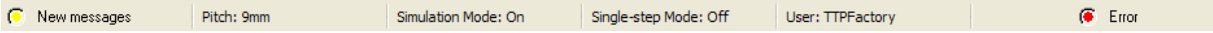
Please email or telephone us with as much information as possible about the problem.

Contact details are:

Catherine Boddington TTP LabTech Ltd Melbourn Science Park Melbourn Royston Herts SG8 6EE United Kingdom	MERA P.O. Box 167 759 191/2 Street Chetek, WI 54728 USA
Telephone: +44 (0) 1763 266708	Telephone: 800 776 7158
Fax: +44 (0) 1763 266755	Email: meradispatch@meraserv.net
Email: mosquitosupport@ttplabtech.com	
Website: http://www.ttplabtech.com	
Availability:	Availability:
8:30am-5:30pm UK Time Monday-Friday.	7am-midnight US Central Time Monday-Friday. There is also a 24-hour fault reporting service but expert advice is not available outside normal hours.

9.2 Error Status

If the software shows a red error light on the status bar, the instrument has detected a problem.



New messages | Pitch: 9mm | Simulation Mode: On | Single-step Mode: Off | User: TTPFactory | Error

When the system is in the error state:

- Check the pipettes and plate deck for obstructions.
- Check the cables and connections to the instrument.
- Click on the Error status light. This displays the Error log window. The most recent message may provide a clue to the part of the instrument that is malfunctioning.
- Reset the instrument using the **Reset/Initialise** button on the **Protocol** tab of the software.
- View the log tab (see Section 9.2.2) to see if there is any useful information.

If resetting the instrument does not cure the error, contact your service representative. **Do not continue pressing Reset.**

A full reset is recommended whenever the instrument has:

- Been interrupted by external interference, for example the plate deck has been nudged.
- Been unattended for more than a few hours or to ensure that the system is operating correctly.
- Stalled.

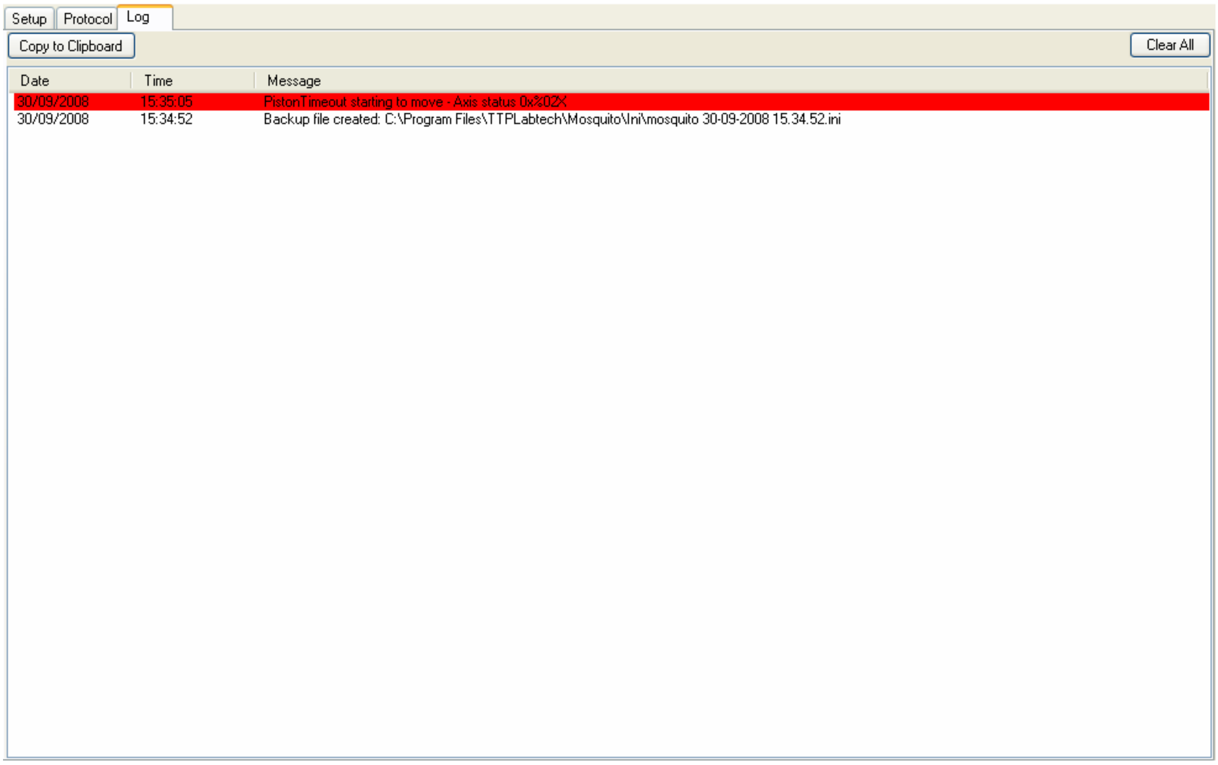
The instrument may stall and display the Busy status light. The problem may be identified using the **Calibration** window (see Section 5) to check the status indicators displayed on the Head, Tape, Spool and Plate panes.

9.2.1 Acknowledging Errors

The message status LED at the left hand end of the status bar shows when any new errors or warnings have been reported to the log. Click this LED to open the log tab to review any errors or warnings. To acknowledge them and clear the message status LED right-click on the error and select **Acknowledge Item.**

9.2.2 Log Screen

Mosquito reports errors and warnings to a log file, the most important of which can be viewed on the log tab by selecting **Options | Log** or clicking the 📄 button.



Date	Time	Message
30/09/2008	15:35:05	PistonTimeout starting to move - Axis status 0x%02X
30/09/2008	15:34:52	Backup file created: C:\Program Files\TTPLabtech\Mosquito\Ini\mosquito 30-09-2008 15.34.52.ini

9.3 Troubleshooting

9.3.1 Persistent Error on Initialise

If there is a persistent error on initialising the instrument:

- If the log tab reports that the output interlock is disengaged then the front sprocket knob is not fully engaged. Manually check that it is in position and pushed fully home.
- If the toggles are engaged but the problem persists, uncheck the interlock boxes on the **Options | Application Preferences** dialog tab. Do not run the instrument like this for long.
- Check that the dancer arm is not clicked fully home.

9.3.2 No Connection

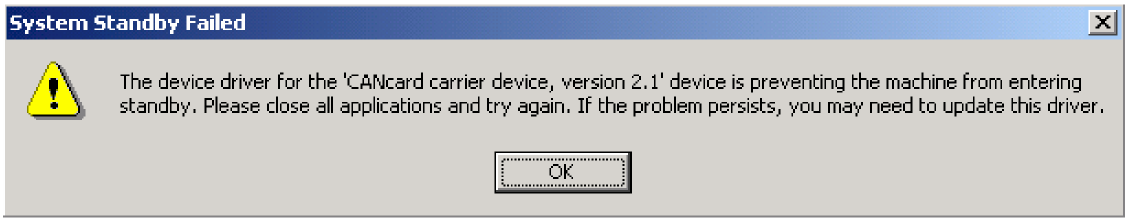
No connection between the PC and the laptop is indicated by no action from the instrument and the status indicator showing 'Busy' or 'Error'. On starting the mosquito application it will report that no instrument is connected.

Check that power is on to the instrument by checking that the fan at the back of the instrument is running and that the cables are all connected.

Power the instrument off and on and restart the mosquito software. If there is still no connection contact a service representative (see Section 9.1)

9.3.3 Error Appears on PC Screen

If the following error appears on the PC screen after a while:



System Standby Failed

The device driver for the 'CANcard carrier device, version 2.1' device is preventing the machine from entering standby. Please close all applications and try again. If the problem persists, you may need to update this driver.

Check that the laptop is not operating on batteries. If it tries to hibernate or standby it is prevented from doing so by the CAN interface.

To rectify the error, click OK, and, if necessary, power off the instrument and reboot the laptop before reconnecting.

9.3.4 Bent Tips or the Piston Breaks Through Sheath

This problem occurs when the pipette tips are hitting a surface.

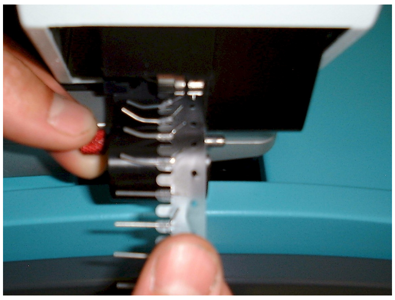
- ❑ Check the tips go into wells and are not trying to go too deep.
- ❑ If they are, recalibrate the instrument head (see Section 5). To remove bent tips from the head, see Section 9.3.6.

9.3.5 Tape Fails to Load

If the tape doesn't wind on at all:

- ❑ Check whether there is any noise:
  - o If yes, wait and start the tape feed again as the tape leader has missed the sprocket.
  - o If not, try re-feeding the leader tape, as it has not triggered the sensor.

If the index winds in but the leader tape doesn't emerge and a juddering noise ensues, the leader tape has jammed in the head.



- Go to the **Interlocks** tab of the preferences dialog and override (check) the **Output Interlock (**see section 4.2.2).
- Unclip the front tape guide by pulling out the red pin (see picture). This may release the leader tape.
- If this fails, power the instrument down, pull the tape out and restart the instrument.

If removing the pipette tape proves difficult, the head cover can be removed to give better access. Contact TTP LabTech for assistance.

To reload pipette tape, a new leader tape is required.

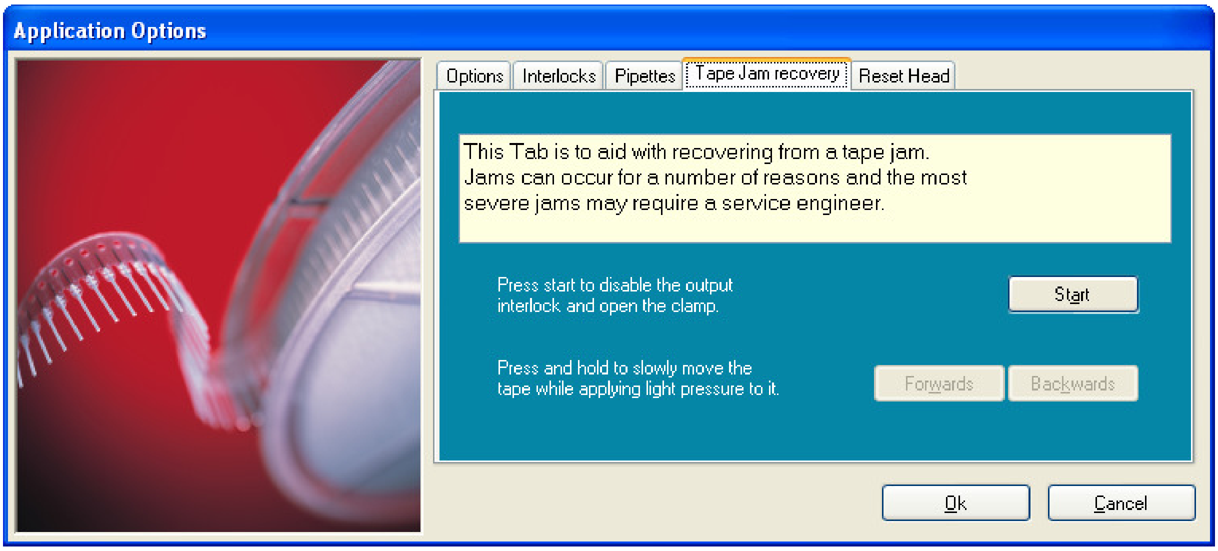
Occasionally when troubleshooting or developing a protocol the tips bend and jam for indexing:

❑ Check that the head cover splash guard is correctly aligned under the tips.

9.3.6 Clearing a Tape Jam

To clear a severe tape jam or remove bent tips from the instrument head:

❑ Select **Options | Preferences** and the **Tape Jam Recovery** tab. (You must be in Calibration Mode to see this tab). The following screen is displayed:



Application Options

This Tab is to aid with recovering from a tape jam.
Jams can occur for a number of reasons and the most severe jams may require a service engineer.

Press start to disable the output interlock and open the clamp.

Press and hold to slowly move the tape while applying light pressure to it.

❑ Press **Start** to open the clamp and override the tape interlocks.

- ❑ If the jam is at the rear of the head then apply gentle pressure to the output stream of pipette tips by pulling on it and press the **Forwards** button to advance the tape. *Take care to avoid the sharp tips*.
- ❑ If the jam is at the front of the head then you may need to pull the tape backwards and hold the **Backwards** button.

Warning: If the **output** sprocket is disengaged then the pipette tape must be pulled through manually as the jog Forwards button is pressed. When the **input** sprocket is disengaged, pipette tape should NEVER be driven forward or back.

If this fails to clear the jam, it may be necessary to remove the head cover and clear the head. Please contact TTP LabTech for advice (see Section 9.1).

9.3.7 Pipettes Fails to Reach Deep Enough Into Wells

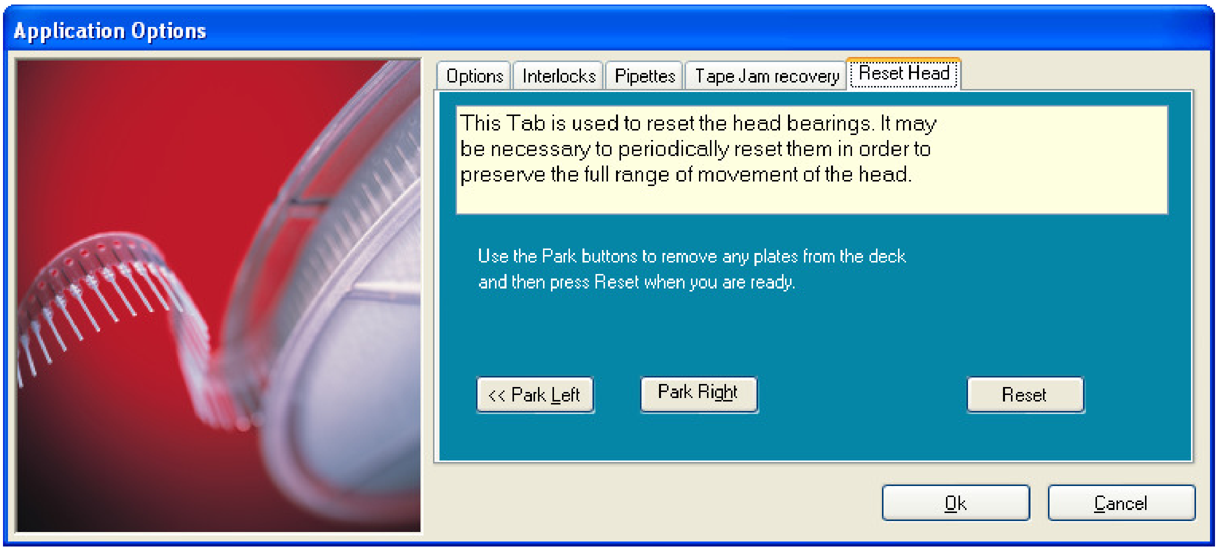
Under some circumstances the head may not go low enough during a run to aspirate correctly or dispense accurately, especially when running protocols which previously ran correctly.

Another symptom of this problem is the error 'Head Axis hit limit' appearing in the log screen (section 9.2.2), especially at the end of a run.

A possible cause of this is the behaviour of the head drive bearings which occasionally need resetting by driving them to their lowest limit. A tool has been provided for this.

It is recommended that you contact TTP LabTech before following this procedure.

Select **Options | Preferences** and then the **Reset Head** tab:



This Tab is used to reset the head bearings. It may be necessary to periodically reset them in order to preserve the full range of movement of the head.

Use the Park buttons to remove any plates from the deck and then press Reset when you are ready.

NOTE: Use the park buttons to move the deck and remove any plates before proceeding.

When you have removed all plates from the instrument press the **Reset** button. The mosquito head will repeatedly drive quickly down and slowly up again.

It is not normally necessary to recalibrate the instrument after following this procedure.

9.3.8 Optimisation Faults

9.3.8.i Tips Produce Erratic Drops on Dry Surfaces.

The problem may have a number of causes:

- ❑ There may be inadequate liquid in the source wells.
- ❑ The aspirate height may be set too high.

First, check the volume in the source wells using the **Setup** tab.

Alternatively, the dispense height may be set incorrectly. Use the **Plates** tab to adjust the well depth setting for the type of plate in use. A range of about 150µm is usually adequate. **Note**: the new setting should be saved as a new plate type.

A continuous row where drops have not dispensed indicates that one of the pipette jaws is not properly functioning. If the problem persists, please contact a service representative (see Section 9.1).

9.3.8.ii Volumetrics are Unsatisfactory

- ❑ Check that the pipette tips are aspirating centrally from the well, as aspirating from the sides in extremely curved menisci can be ineffective.
- ❑ Check that the tips are dispensing centrally into the well, as aspirating into edges or corners compromises repeatability.

9.3.9 Tape Indexes Very Slowly

When the software is first run or whenever the instrument has been left idle for 10 minutes or more, the first movement of the tape through the head will be quite slow. This is by design; if the pipettes are held in the clamped position for any length of time they lose some of their natural elasticity and need to be moved slowly to prevent jamming.

Whenever this occurs a warning message is reported to the log.

9.4 Diagnostics Tools

The software gives access to a number of diagnostics tools, via the **Options | Diagnostics** menu item. (**Note:** the software must be in Calibration Mode to see and access this menu item.)

Note: Incorrect use of these tools may result in damage to and miscalibration of the instrument; they should only be used under guidance from TTP LabTech.

10 APPENDIX A: AUTOMATION INTERFACE

10.1 Introduction

The mosquito software provides an automation interface for integration with applications written in C#, Visual Basic.NET™ and other .NET programming languages. This interface is accessible by referencing a special .NET assembly, called Mosquito.dll, in the client project.

Visual C# Express and Visual Basic.NET Express can both be used to create client programs which can automate Mosquito.

Note: The Mosquito.dll assembly actually references Mosquito via .NET Remoting. It is also possible to reference Mosquito directly via .NET Remoting in the same way as this dll.

[Note that the legacy COM interface is still provided for existing automation clients – however, this is not recommended for future development. Also, the existing automation client source code:

1) may need to be amended because optional parameters are no longer supported, and
2) will probably need to be recompiled after updating the project reference (if in VB6) or equivalent, due to slight differences at the binary level to the COM interface in previous versions.]

10.2 Mosquito Integration DLL

The mosquito installation installs the Mosquito.dll into the .NET Global Assembly Cache (GAC). This means that any client program that references Mosquito.dll does not need to copy it to its own directory, as it will be loaded from the GAC automatically.

To use the mosquito automation interface from Visual C# add a reference to the Mosquito.dll and MosquitoSharedTypes.dll to your C# project (by selecting **Project|Add Reference,** browsing to C:\Program Files\TTPLabtech\Mosquito and selecting Mosquito.dll and MosquitoSharedTypes.dll). The 'Copy Local' setting on the referenced assemblies should be set to false.

10.3 Automation Hierarchy

Four core objects are exposed by mosquito: `CoMosquito, CoPipettor, CoDeck and CoProtocol`. In addition, CoProtocol allows the creation or referencing of Transfer objects, which have the `ITransfer` interface.

The CoMosquito object represents the high-level control of the instrument and provides methods to load protocol files, run the protocol and park the deck.

The CoPipettor object represents a lower level of control of the instrument and provides methods to move the deck to a particular position, aspirate, dispense and so on.

The CoDeck object represents the mosquito deck which contains the plates, and allows the plate type and name to be set for a particular nest, and the setting of the various volume options and parameters for each next position.

The CoProtocol object represents the current protocol. It provides method to access, create or remove Transfers within the protocol.

The ITransfer interface provides access to the individual transfers within a protocol, and allows the various properties for a transfer to be read or set.

10.4 CoMosquito

The CoMosquito object implements the IMosquito interface.

The `CoMosquito` object has a number of methods and fires one outgoing event.

10.4.1 IMosquito Methods

Note Methods marked * are asynchronous. For example, Run will return immediately without waiting for the operation to complete. You must process OnUpdate events and check the IsReady flag to determine when an operation has completed.

```
bool Load(string FileName)
```

Loads the protocol file Filename; returns `true` if successful, `false` otherwise.

```
*string Run()
```

Runs the currently loaded protocol; returns any error message immediately if the run failed or an empty string if the run starts correctly.

```
*void ParkLeft()
```

Parks the deck one position to the left of the current one.

```
*void ParkRight()
```

Parks the deck one position to the left of the current one.

```
*void ParkAt(int Position)
```

Parks the deck at the given position number, starting from zero.

```
*void Pause()
```

Pauses the current run.

```
*void Resume()
```

Resumes the current run.

`*void Reset()`

Resets the instrument and re-homes all of the drives. Use this method to attempt to recover from an error condition.

Do not call Reset repeatedly. If Reset fails then there is a physical reason for the failure which must be attended to manually.

`*void Shutdown()`

Place the instrument into a quiescent state prior to being turned off.

You should never need to call this method. Exiting the mosquito application will do this automatically.

`bool IsRunning()`

Returns `true` if the instrument is running a protocol.

`bool IsOk()`

Returns `false` if the instrument is in an error state, `true` otherwise.

`bool IsReady()`

Returns `true` if the instrument is not in an error state and is not moving, i.e. is ready to run a protocol (whether that protocol is valid or not).

`int GetTotal()`

Returns the total number of steps to be performed in the currently running protocol.

`int GetElapsed()`

Returns the number of steps which have been executed in the currently running protocol. This is useful for giving progress indication in conjunction with `GetTotal().`

`bool IsPaused()`

Returns `true` if the currently running protocol is paused, `false` otherwise.

`bool HasLowTips()`

Returns `true` if the instrument is running out of pipettes, `false` otherwise.

`CoPipettor GetPipettor()`

Returns a reference to the low-level automation object CoPipettor; see section 10.5.

10.4.2 IMosquito Events

```
public delegate void NotifyEventHandler(bool IsReady, bool IsOk);

public event NotifyEventHandler OnUpdate;
```

`OnUpdate` is fired whenever the state of the instrument changes. For example:

- when a protocol starts running
- when the instrument enters or leaves an error state
- whenever a step or phase of a step in the running of a protocol is completed.

The `IsReady` and `IsOk` parameters give the same result as calling the `IsReady()` and `IsOk()` methods; thus there is no need to call `IsReady()` or `IsOk()` while processing an `OnUpdate` event.

10.4.3 Mosquito Visual C#™ Example

A small Microsoft Visual C# application has been provided to demonstrate the use of the IMosquito interface; The default installation location is C:\Program Files\TTPLabtech\Mosquito\CSharp Example. The code is well commented, and should be self explanatory.

10.5 CoPipettor

The CoPipettor class implements one interface, IPipettor.

CoPipettor is non-creatable. Code like the following will fail whether the mosquito application is already running or not:

```
CoPipettor Pipettor_m = new CoPipettor();
```

Instead, to get a reference to the pipettor object call the GetPipettor method of the CoMosquito object:

```
CoMosquito Mosquito_m;
IPipettor Pipettor_m;
Mosquito_m = new CoMosquito();
Pipettor_m = Mosquito_m.GetPipettor();
```

10.5.1 IPipettor Methods

Note Methods marked * are asynchronous. For example, Aspirate will return immediately without waiting for the aspirate operation to complete. You must process OnUpdate events and check the IsReady flag to determine when an operation has completed.

```
void SetPlate(int Position, string Plate)
```

Specifies the plate in the given position, starting from zero. The plate name should be the fully-qualified path.

Raises exceptions if

- the tape used by mosquito has too small a pitch to enter the wells of the plate specified
- if the plate file cannot be found or loaded.
- If the selected nest position is invalid

```
*void Begin()
```

Start running a job via the IPipettor interface; re-homes the instrument as necessary. Raises an exception if the instrument is already running.

```
*void End()
```

End a job running via the IPipettor interface; parks the instrument as necessary and returns control to the user interface. Returns an exception if the instrument is not already running.

```
*void Index()
```

Causes the instrument to change the pipette tips

```
bool IsRunning()
```

Determines whether the instrument is running, either via the IPipettor interface, the IMosquito interface or the user interface.

```
bool IsOk()
```

Returns true if the instrument is ok, false if it is in an error state.

```
bool IsReady()
```

Returns true if the instrument is ready, i.e. is not currently moving or expecting a command. The instrument will not be ready after Begin() has been called until End() is called

```
*void MoveTo(int Position,
       int Column,
       int Row,
       int Subwell)
```

Moves the deck so that the specified position is under the head. Note that all parameters are 0-based, unlike the mosquito user interface which is 1-based.

Raises exceptions if

- the move position is invalid
- there is no plate at the specified position

```
 *void Aspirate(
         double Volume,
         double WellVolume, // default 0
         bool OverAspirate, // default true
         bool UseManualHeight, // default false
         double ManualHeight, // default 0,
         bool TipTouch, // default false
         double ClearSpeed, // default 5
         double ClearDist, // default 2
         int MixCycles, // default
         int MixVolume, // default
         double PistonSpeed, // default 25
         bool IsMultiAspirate,
         bool HasBlowback)
```

Aspirates the given volume in nanolitres from the current position. The WellVolume is used to specify the volume of liquid in the well should it be known; the remaining parameters correspond directly to those parameters in the advanced transfer options (see Section 2). Suggested defaults are shown.

Raises an exception if no plate has been set at the current position.

```
*Dispense(double Volume,
        double WellVolume, // default 0
        bool ContactFirst, // default False
        bool UseManualHeight, // default False
        double ManualHeight, // default 0
        bool TipTouch,  // must be False
        double ClearSpeed, // default 5
        double ClearDist, // default 1
        int MixCycles // default 0
        int MixVolume // default 100
       double PistonSpeed) // default 25
```

Dispenses the given volume in nanolitres from the current position. The WellVolume is used to specify the volume of liquid in the well should it be known; the remaining parameters correspond directly to those parameters in the advanced transfer options (see Section 2). Suggested defaults are shown.

Raises exception if no plate has been set at the current position.

10.5.2 IPipettor Events

```
public delegate void NotifyEventHandler(bool IsReady, bool IsOk);

public event NotifyEventHandler OnUpdate;
```

`OnUpdate` is fired whenever the state of the instrument or the low level controller changes. For example:

- when a protocol starts running
- when the instrument enters or leaves an error state
- whenever a step or phase of a step in the running of a protocol is completed.

The `IsReady` and `IsOk` parameters give the same result as calling the `IsReady()` and `IsOk()` methods; thus there is no need to call `IsReady()` or `IsOk()` while processing an `OnUpdate` event.

It differs from IMosquito.OnUpdate in that the low-level controller will generate rather more updates – for example, after every aspirate, dispense or move operation.

10.5.3 Using IPipettor

The IPipettor interface offers great flexibility and control over the mosquito instrument and a full example is outside the scope of this manual. The outline usage of this interface should be as follows:

```
//
// Set which plates are in which positions to allow mosquito to
// perform height and position calculations automatically
//

      Pipettor.SetPlate (0, "Corning 96 Round.plate");
      Pipettor.SetPlate (1, "Corning 96 Round.plate");

//
// Home the instrument and start running
//
      Pipettor.Begin();

//
// Wait until OnUpdate has been called with IsReady = True and IsOk
= True
// Numerous implementations possible including one like the
following
//
//    while ((!MosquitoReady || !PipettorReady)
//           && MosquitoOk && PipettorOk)
//     {
//            Application.DoEvents();
//     }
//
```

```
//
// where MosquitoReady and the other parameters are class member
// Booleans which are set during calls to Pipettor_OnUpdate.
//

//
// Move the instrument
//
     Pipettor.MoveTo(0, Column, 0, 0)

//
// Wait until ready again, then
//
     //
     // Aspirate
     //
          Pipettor.Aspirate(100, ....other params not shown...)

     //
     // Wait until ready again
     //
     // Then move, wait until ready, dispense, etc.
     //

     //
     // Finish
     //
          Pipettor.End();
```

10.6 CoDeck

CoDeck implements the IDeck interface. It implements a number of methods, but has no events. CoDeck can be created directly, and has a default constructor.

10.6.1 IDeck related Enums

```
public enum EStartingPlateVolumeOption
{
    Empty = 0,
    Unknown = 1,
    Known = 2
}
```

This corresponds to the ‘Initial well volumes’ choice for a particular plate on the Setup tab. ‘Empty’ = ‘All Wells Empty’, ‘Unknown’ = ‘Don’t know volumes’, and ‘Known’ = ‘All wells the same’.

10.6.2 IDeck Methods

```
void SetPlateType(int Position, String PlateFile)
```

Sets the plate type at the nest position specified to the plate specified. The Position is 0-based (so 0 means the first nest). The PlateFile parameter is the name of the plate file to be used, without any extension (but with spaces and brackets exactly as in the file) – e.g. "Greiner 384 (square)".

```
string GetPlateType(int Position)
```

Gets the name of the plate-file at the 0-based nest position specified.

```
void SetPlateName(int Position, String PlateFile)
```

Sets a name for the plate at the 0-based nest position specified.

```
string GetPlateName(int Position)
```

Gets the name for the plate at the 0-based nest position specified.

```
void SetPlateStartingVolumeOption
     (int Position,
      EStartingPlateVolumeOption VolumeOption)
```

Sets the 'Initial Well Volume option' for the plate at the 0-based nest position specified. See 10.6.1 above.

```
EStartingPlateVolumeOption GetPlateStartingVolumeOption
     (int Position)
```

Gets the 'Initial Well Volume option' for the plate at the 0-based nest position specified. See 10.6.1 above.

```
void SetPlateStartingVolume(int Position, int Volume)
```

Sets the Initial Well Volume for the plate at the 0-based nest position specified. This is only relevant if the StartingPlateVolumeOption is set to 'Known'. See 10.6.1 above.

```
int GetPlateStartingVolume(int Position)
```

Gets the Initial Well Volume for the plate at the 0-based nest position specified. This is only relevant if the StartingPlateVolumeOption is set to 'Known'. See 10.6.1 above.

10.7 CoProtocol

CoProtocol implements the IProtocol interface. It implements a number of methods, but has no events. CoProtocol can be created directly, and has a default constructor.

10.7.1 IProtocol Methods

```
ITransfer GetTransfer(int Index)
```

Returns an ITransfer reference to the transfer at the protocol index position specified. This Index position is 0 based.

```
int GetTransferCount()
```

Returns the number of transfers in the current protocol.

```
ITransfer AddTransfer()
```

Creates a new Transfer object, which can have its properties set. It will only be added to the current protocol when the Commit function on the Transfer is called.

```
void RemoveTransfer(int Index)
```

Removes the transfer at the 0-based protocol index given.

```
void BeginUpdate()
void EndUpdate()
```

These two functions should be used when adding many transfers at once, and/or if doing lots of manipulation of transfers in the protocol. If the calls to add or modify the transfers are surrounded by BeginUpdate before the adding starts, and EndUpdate once it's finished, the UI will not continually get updated, and the performance is vastly improved. If these calls are omitted (which is not recommended) performance is extremely sluggish, and the UI will take a long time to update with all of the added protocols.

10.7.2 Using CoProtocol

The following code snippet indicates typical usage of CoProtocol, and ITransfer (see next section).

```
CoProtocol MyProtocol = new CoProtocol();

// start making changes to the protocol
MyProtocol.BeginUpdate();

// Add a transfer
```

```
ITransfer transfer = MyProtocol.AddTransfer();
transfer.Type = 1; // Copy
transfer.SrcRow = 0;
transfer.SrcStartCol = 3;
transfer.SrcEndCol = 5;
transfer.DstNest = 1;

// etc.

transfer.Dispose(); // this commits the transfer and releases
its resources

// Add another transfer
transfer = MyProtocol.AddTransfer();

transfer.Type = 1; // Copy
transfer.SrcRow = 0;
transfer.SrcStartCol = 6;
transfer.SrcEndCol = 8;
transfer.DstNest = 2;

// etc.

transfer.Dispose();

// more adding of transfers (not shown)……

// Finish adding/modifying the protocol – this tells the UI we
// are finished, and allows it to update itself and validate
// the protocol.
MyProtocol.EndUpdate()
```

10.8 CoTransfer

CoTransfer implements the ITransfer interface. It implements one method and several properties, but has no events. Properties are shown in the interface listing as:

```
Type PropertyName {get; set;}
```

which means that it is possible to get the value, and set a value. They would be used as follows (using the property 'SrcNest' as an example):

```
CoProtocol MyProtocol = new CoProtocol();
ITransfer MyTransfer = MyProtocol.GetTransfer(1);
int PreviousSrcNest = MyTransfer.SrcNest;
MyTransfer.SrcNest = 1;
```

CoTransfer cannot be created directly – references to an ITransfer are obtained via CoProtocol's GetTransfer and AddTransfer methods.

10.8.1 ITransfer related Enums

```
public enum ETransferType
{
    Buffer = 0,
    Copy = 1,
    Mirror = 2,
    Assemble = 3,
    Split = 4,
    Pause = 5,
    Comment = 6,
    ChangePipettes = 7,
    MultiAspirate = 8,
    ReverseSplit = 9,
    Worklist = 10,
    BulkDispense = 11
}
```

This enum is used for ITransfer's Type property. It corresponds to the options in the Type column in the Protocol grid.

Note that some of the options are named differently: 'Assemble' = 'Pool', 'Split' = 'Aliquot', 'ReverseSplit' = 'Reverse Aliquot'.

```
public enum ETipChangeStrategy
{
    ePostDispense = 0,
    ePreAspirate = 1,
    ePostTransfer = 2,
    eNever = 3
}
```

This enum is used for ITransfer's TipChangeStrategy property. TipChangeStrategy corresponds to the 'Tip Changing' column in the Protocol grid.

The following shows how the above enum values map to the options in the grid:

Enum value	**Tip Changing option**
ePostDispense	Always
ePreAspirate	Multi-Dispense
ePostTransfer	Between transfers
eNever	Never

10.8.2 ITransfer Methods and Properties

10.8.2.i Methods

```
void Dispose()
```

This closes and releases the reference to the Transfer in the protocol. This **must** be called to release memory and resources. If the transfer had not been committed, then Dispose will automatically perform the commit.

Once an ITransfer reference has been disposed, it should not be used any further. (Of course, the variable used to store the reference can be set to another ITransfer.)

```
void Commit();
```

This commits any changes which have been made to the transfer. Also, in the case of an ITransfer obtained via IProtocol.AddTransfer, the Transfer is added to the end of the protocol.

All validation is also performed at the time of the commit, and not on each individual set.

If validation fails an exceptions is thrown with details, and the transfer is not added to the protocol.

Note: Generally it would be more usual to call Dispose, unless for some reason you need to keep the reference to make further modifications after committing the existing ones.

10.8.2.ii Properties

The following Properties correspond to each of the properties which can be set on a transfer in the UI. They are generally self-explanatory, but anything unusual or noteworthy is indicated, such as valid ranges.

Note that all values are '0-based' meaning they start counting from 0 (even though they are generally not in the UI) unless otherwise indicated. E.g. setting SrcStartCol to 1 would show '2' when shown in the UI, and would indicate the 2nd column.

```
int Volume { get; set; }

ETransferType Type { get; set; }

int SrcNest{ get; set; }
```

Nest equates to 'Position' in the Protocol grid, and is 0-based. Range is dependent upon puck-type.

```
int SrcRow{ get; set; }
int SrcStartCol { get; set; }
int SrcEndCol { get; set; }
int SrcSubWell { get; set; }
```

Range of the above depends on plate.

```
int DstNest { get; set; }
```

```
int DstRow { get; set; }
```

```
int DstStartCol { get; set; }
```

```
int DstEndCol { get; set; }
```

```
int DstSubWell { get; set; }
```

```
int MixCycles { get; set; }
```

Range is 0 - 10

```
int MixVolume { get; set; }
```

Range is 50 - 1000

```
double MixMove { get; set; }
```

Range is 0 - 5

```
int    AspMixCycles { get; set; }
```

Range is 0 - 10

```
Int    AspMixVolume { get; set; }
```

Range is 50 - 1000

```
bool   UseAspManualHeight { get; set; }
```

```
double AspManualHeight { get; set; }
```

UseAspManualHeight must be true for AspManualHeight to take effect. Range is -20 to 20.

```
bool   UseDispManualHeight { get; set; }
```

```
double DispManualHeight { get; set; }
```

UseDispManualHeight must be true for DispManualHeight to take effect.

```
int    PauseDuration { get; set; }
```

This is in seconds. Only for use with the Pause transfer type.

```
bool   PauseNeedsHome { get; set; }
```

This is equivalent to 'Home deck after pausing' in the Pause transfer options.

```
double AspClearSpeed { get; set; }
```

Range is 2 - 10

```
double DispClearSpeed { get; set; }
```

Range is 2 - 10

```
double AspClearSurfDist { get; set; }
```

Range is 0 - 20

```
double DispClearSurfDist { get; set; }
```

Range is 0 - 20

```
double PistonAspirateSpeed { get; set; }
```

Range is 1 - 25

```
double PistonDispenseSpeed { get; set; }
```

Range is 0 - 20

```
string Comment { get; set; }
```

Only for use with the Comment transfer type.

```
string Worklist { get; set; }
```

The full path to a worklist file – only for use with the Worklist transfer type.

```
ETipChangeStrategy TipChangeStrategy { get; set; }
```

See 10.8.1 above

```
bool   IsDryDispense { get; set; }
```

This relates to the ‘Dispense Type’ column in the protocol grid. If IsDryDispense is true, the Dispense Type is set to ‘Droplet’, otherwise it is set to ‘Contact’.

```
bool WaitInWellAfterDispense { get; set; }

bool SpecifyPauseParkPosition { get; set; }

int PauseParkPosition { get; set; }
```

SpecifyPauseParkPosition must be true for PauseParkPosition to take effect. Only for use with the Pause transfer type.

```
double BulkDispenseVolume { get; set; }
```

The volume to bulk dispense in microlitres. This is only for use with the Bulk Dispense transfer type, which in turn is only available when the optional Bulk Dispenser unit is fitted. Range is 1 - 400.

11 APPENDIX B: COMMAND LINE INTERFACE

Mosquito 2.5 supports a limited command line interface. In general, the IMosquito automation interface (see Appendix A: Automation Interface) is preferred but the command line is available when it is not possible to use the automation interface.

The format of commands is as follows:

mosquito.exe [/h] [/i] [/r] [/x] [filename] [Source=n] [DeleteRows=n1,,n2,n3]

The plate or protocol file *filename* will be loaded if it can be found.

/? /h /H show a help dialog explaining these parameters

/i /I initialise the instrument

/r /R run the current protocol (if filename is specified, load that first)

/x /X with /r, run the current protocol and exit the application when the run has finished. Note: if the run does not finish ok then the application will not exit.

This interface will work whether the mosquito is already running or not.

Options used to modify a currently loaded protocol:

Source=n For each row in the transfer with only a single source column will set the source column to the value n (starting from 0)

DeleteRows=n1,n2,n3,... Delete the specified rows from the currently loaded protocol, starting from 0

11.1 Command Line Example

From a command prompt at the \program files\mosquito directory the command

```
mosquito /i /r /x “protocols\my protocol.protocol”
```

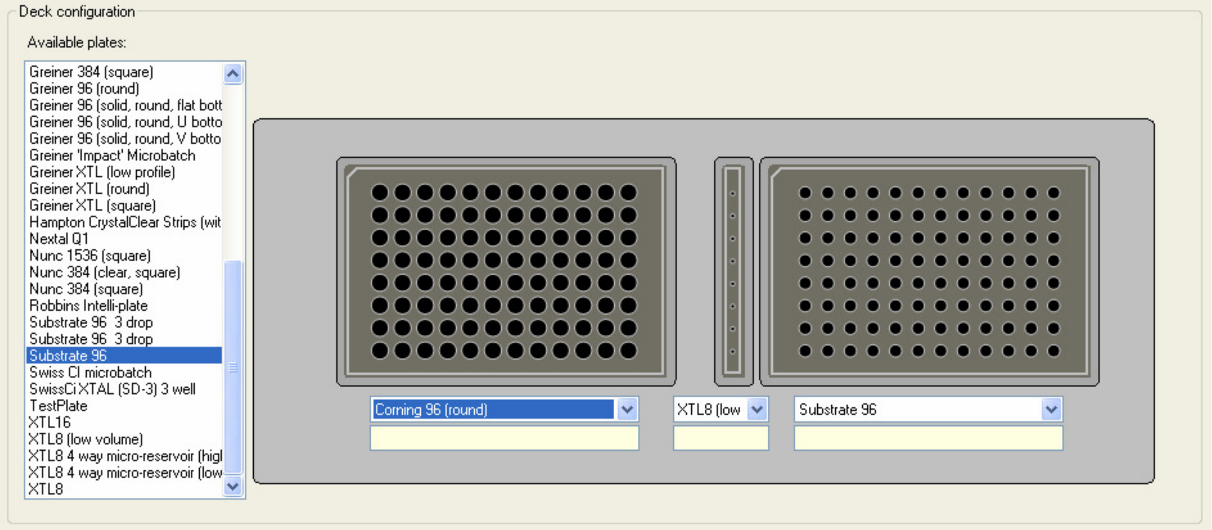
will start mosquito, initialise the instrument, load “my protocol”, run it and then exit.

12 APPENDIX C: EXAMPLE PROTOCOLS

12.1 Hanging Drop Crystallography

The example described here uses the crystallography configuration of mosquito, including a customised deck.

mosquito is capable of addressing hanging drop crystallography in an unique way. The instrument can accurately pipette droplets of protein solution and reagent to the same locations on a plate seal. These locations are arranged in a mirror image of the plate wells, so that, when the seal is turned over to attach to the screen plate, each droplet has the same reagent in it as the reservoir underneath. This translation is impossible with 96-array pipettors.

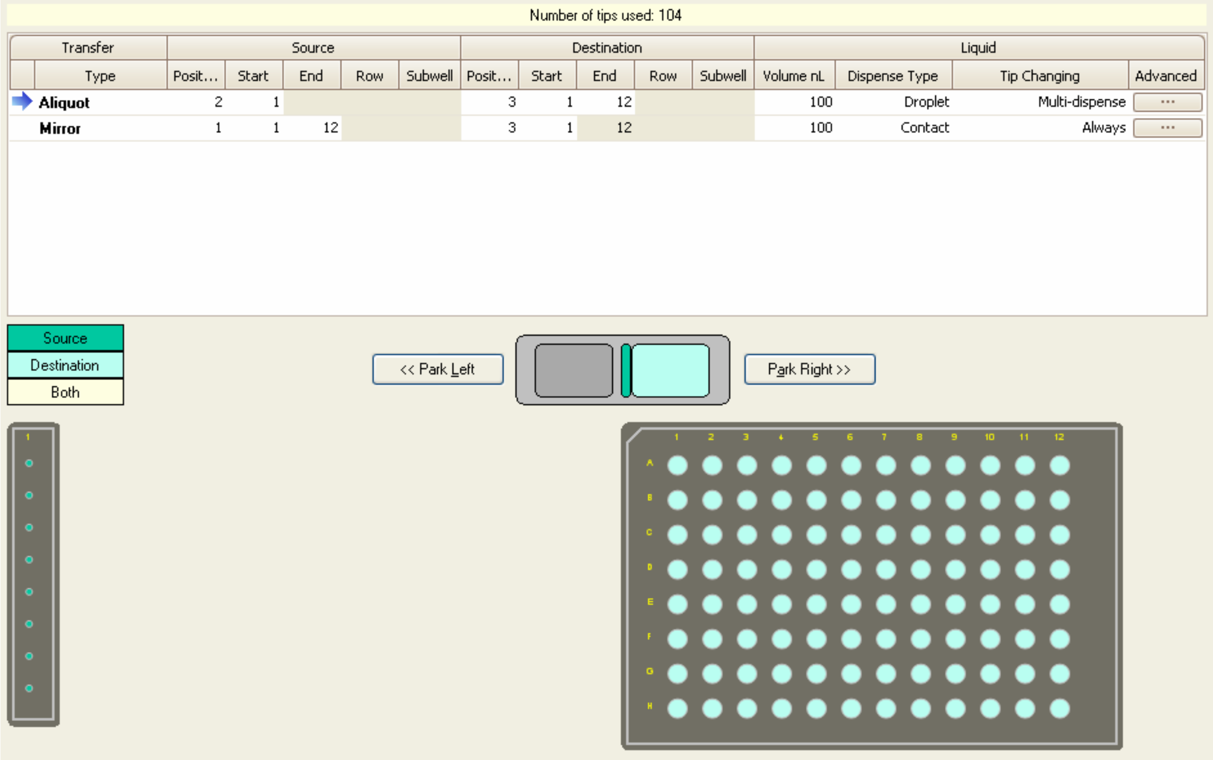


This crystallography mosquito has a special crystallography deck which holds two SBS format microtitre plates and an additional single-column position with options for holding either 200 µl Eppendorf strips, or a custom 4µl/well consumable.

The protocol is set up as follows:

The plate deck holds a 96-well plate of crystallising agents in position 1, protein solution in the single column position 2, and a plate seal held in place on a blank, flat SBS-sized block in position 3.

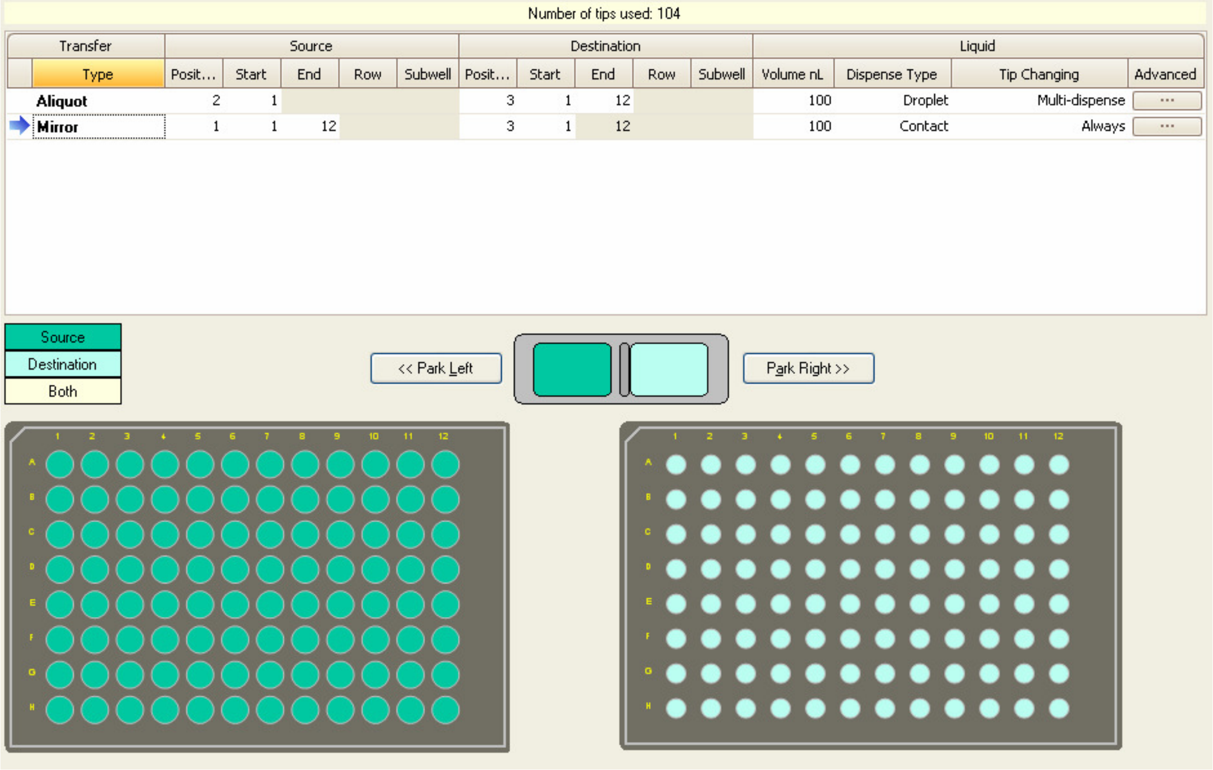
The first transfer step transfers aliquots of protein solution from the single column position to all the positions on the plate seal, and is shown below:



Number of tips used: 104

Transfer		Source					Destination					Liquid			
	Type	Posit…	Start	End	Row	Subwell	Posit…	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
➡	**Aliquot**	2	1				3	1	12			100	Droplet	Multi-dispense	…
	Mirror	1	1	12			3	1	12			100	Contact	Always	…

Using fresh tips, the second transfer step mirrors aliquots of the crystallising agents from a 96-well plate to all the positions on the plate seal, and is shown below:



Number of tips used: 104

Transfer		Source					Destination					Liquid			
	Type	Posit…	Start	End	Row	Subwell	Posit…	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
	Aliquot	2	1				3	1	12			100	Droplet	Multi-dispense	…
➡	**Mirror**	1	1	12			3	1	12			100	Contact	Always	…

The protocol may be saved and run.

The droplets on the plate seal coincide to provide the starting conditions for the preparation. The seal can then be placed over the plate of crystallising agent and incubated.

12.2 Sitting Drop Crystallography

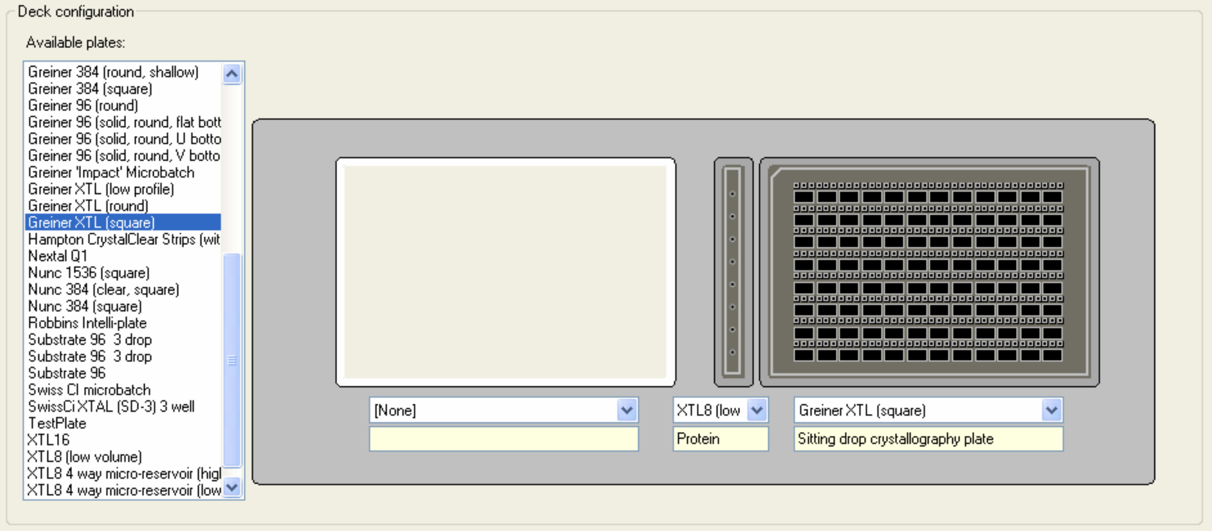
The example described here uses the crystallography configuration of mosquito, which has a moveable y-axis drive and, when fitted with 96 pitch (9 mm) pipette tape, can address Greiner crystallography plates. These plates are on a standard 96-well format but each principal well contains three smaller droplet wells for sitting drop crystallography applications.

There is a special format for identifying which of the wells on these crystal ledge plates are to be visited in a transfer. The sub-wells are numbered so:

2	3	4
1		

An example sitting drop crystallography protocol is given below:

The **Setup** tab to is used to select the correct plate type and well volumes:



Deck configuration

Available plates:

Greiner 384 (round, shallow)
Greiner 384 (square)
Greiner 96 (round)
Greiner 96 (solid, round, flat bott
Greiner 96 (solid, round, U botto
Greiner 96 (solid, round, V botto
Greiner 'Impact' Microbatch
Greiner XTL (low profile)
Greiner XTL (round)
Greiner XTL (square)
Hampton CrystalClear Strips (wit
Nextal Q1
Nunc 1536 (square)
Nunc 384 (clear, square)
Nunc 384 (square)
Robbins Intelli-plate
Substrate 96 3 drop
Substrate 96 3 drop
Substrate 96
Swiss CI microbatch
SwissCi XTAL (SD-3) 3 well
TestPlate
XTL16
XTL8 (low volume)
XTL8 4 way micro-reservoir (higl
XTL8 4 way micro-reservoir (low

[None]

XTL8 (low

Protein

Greiner XTL (square)

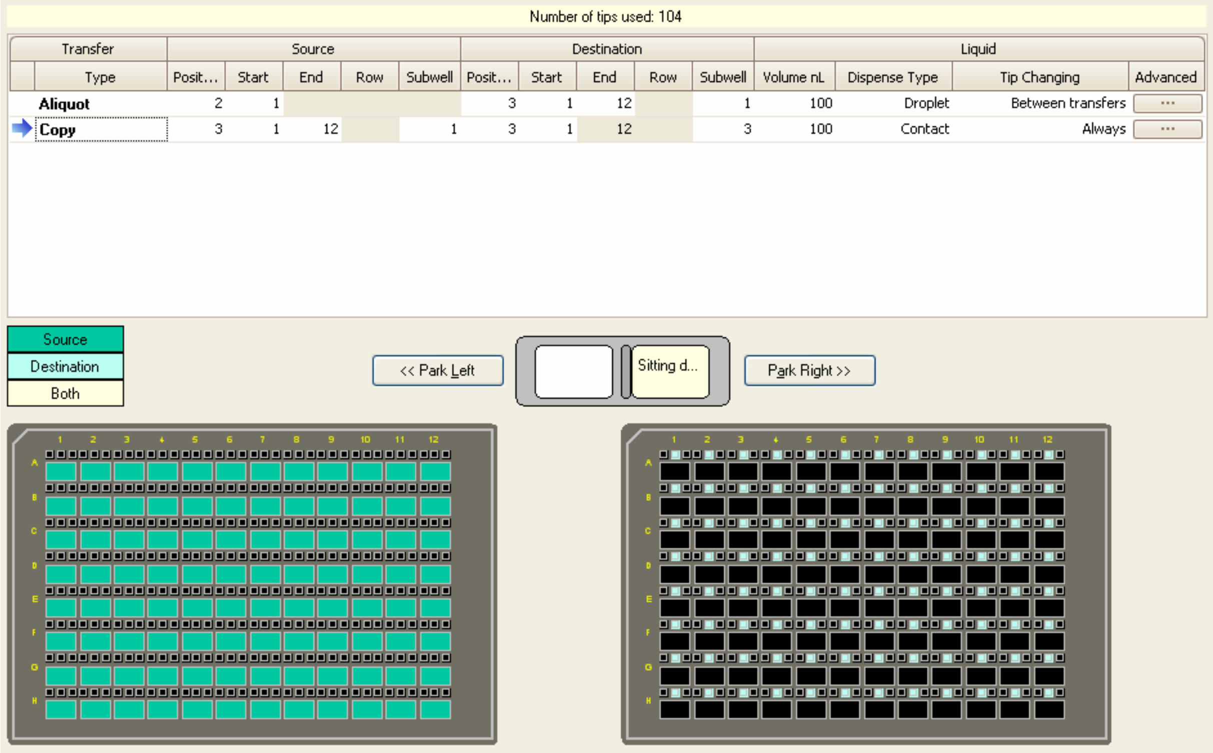
Sitting drop crystallography plate

To pipette material from the liquor well (sub-well 1) to each of the sub-wells numbered 3 across the whole plate, as shown below:

1		

	3	

the **Protocol** tab is used to create a transfer like the following:



Number of tips used: 104

Transfer		Source					Destination					Liquid			
	Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing	Advanced
	Aliquot	2	1				3	1	12		1	100	Droplet	Between transfers	...
➡	Copy	3	1	12		1	3	1	12		3	100	Contact	Always	...

Note: setting the Change Tips option to “Multi-dispense” or “Between transfers” means that the tips are not changed after every dispense; these three transfers will be optimised to minimise tip usage. This would result in slight cross-contamination between the three crystal ledges if different materials are already there. Setting Change Tips to “Always” would prevent this.

13 APPENDIX D: INTERIOR OF THE HEAD

13.1 Illustrations

Warning – If the head cover is removed the user must take care to keep clear of all moving parts.

The interior of the head is as follows:

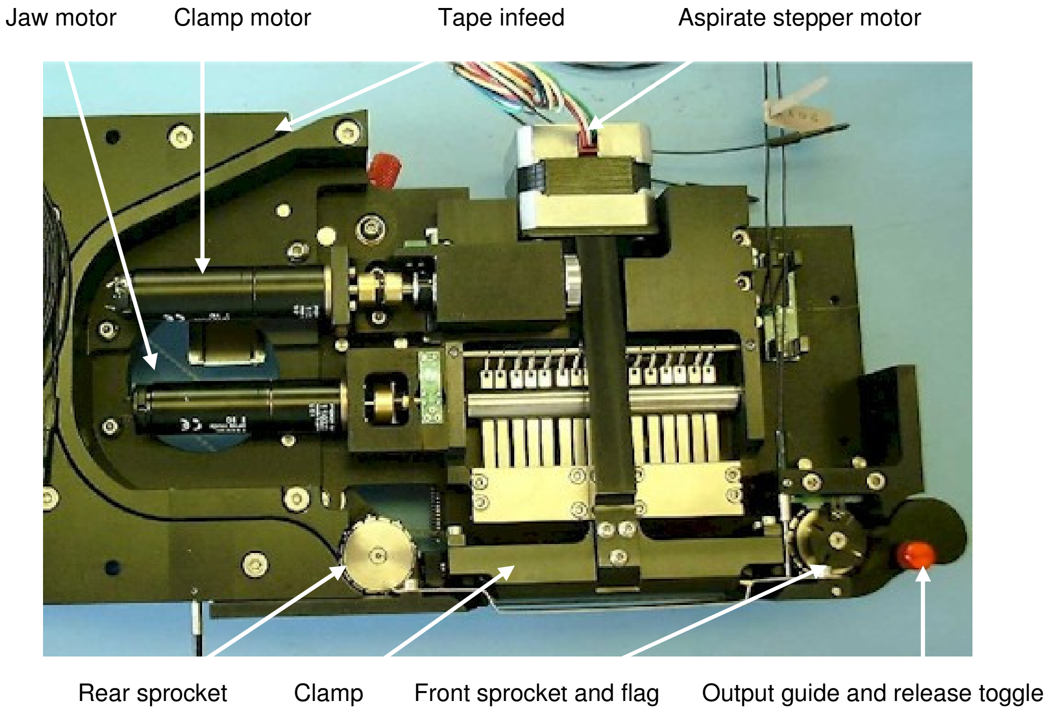


Figure 1: Left-hand side of head

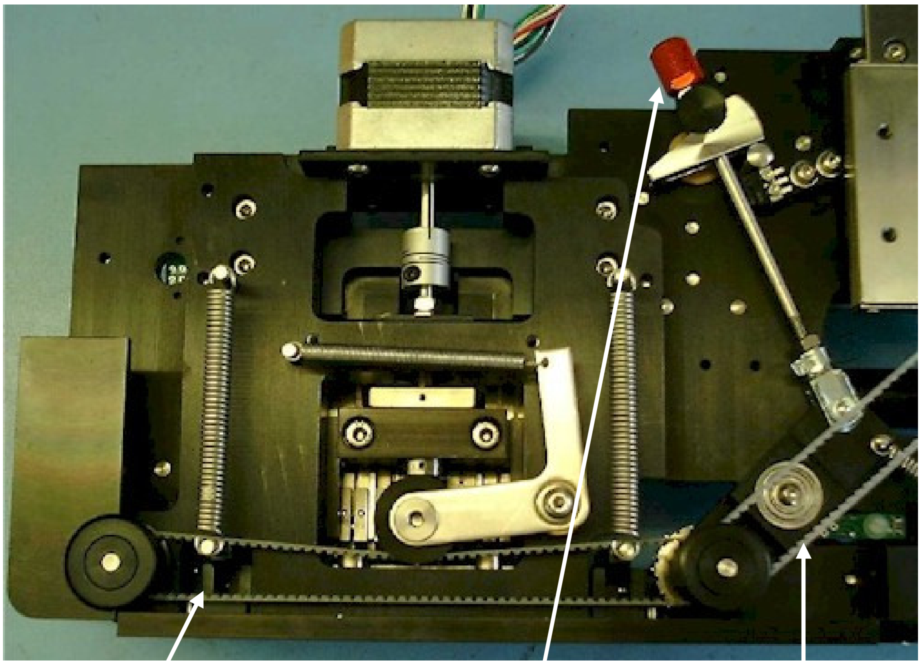


Figure 2: Right-hand side of head

14 APPENDIX E: DEFINITIONS

Aliquot Transfers sample from one source column on one plate to many destination columns.

Copy Transfers sample from one or several source columns on one plate to the specified destination columns.

Dancer arm The tensioner for the pipette tape.

Deck The plate holder tray containing positions for plates.

Home Homing resets the robotic axes as part of initializing the instrument.

Initialising The instrument reset performed at the beginning of each session.

Leader tape A small section of tape used to start a pipette reel.

Mirror This reverses the order of the copy transfer to form a mirror image of the original. It is used for pipetting sample onto a plate lid which is then placed over wells.

Mix A user-defined sequence of aspirates and dispenses within the same well, to mix liquid within the well.

Park The deck has a number of calibrated 'home' positions at which the sprung clips which hold plates in place are released. The deck can be moved between these positions by pressing the Park Left and Park Right buttons on the protocol tab.

Pool Transfers sample from many source columns on one plate to one destination column.

Position An indentation for holding a plate on the plate deck.

Protocol All the data required to perform a single transfer or set of transfers. This may be named and saved by the user for subsequent use.

Spool The reel holding the pipette tape.

Transfer One movement of sample from source to destination. The exact sequence depends on the parameters specified for the transfer.

15 APPENDIX F: HISTORY

Revision	Date	Comments
Draft A	30 January 2004	CEM First issue of document
Draft B	12 February 2004	SRC Minor corrections
Draft C	5 March 2004	SRC Added details of timestamps in report files
Draft D	6 October 2004	SRC Updated for software version 2.5.1.42
Draft E	22 November 2004	SRC Updated for software version 2.5.1.44
Draft F	21 January 2005	SRC Updated for software version 2.5.1.45
Draft G	12 May 2005	SRC Updated for software version 2.6.1.47
Draft H	17 June 2005	ESJT Updated for software version 2.6.1.48
Draft I	8 March 2006	SRC Updates for software version 2.7.51
Draft J	19 October 2006	PGS Updated for software version 2.8.52
Draft K	20 April 2007	PGS Updated for software version 2.8.55
Draft L	1 May 2007	SRC Updated for software version 2.8.56
Draft M	30 September 2008	PJB Updated for software version 3.0.0
Draft N	12 March 2009	PJB Updated for software version 3.0.1
Draft P	21 July 2009	PJB Updated for software version 3.2.0