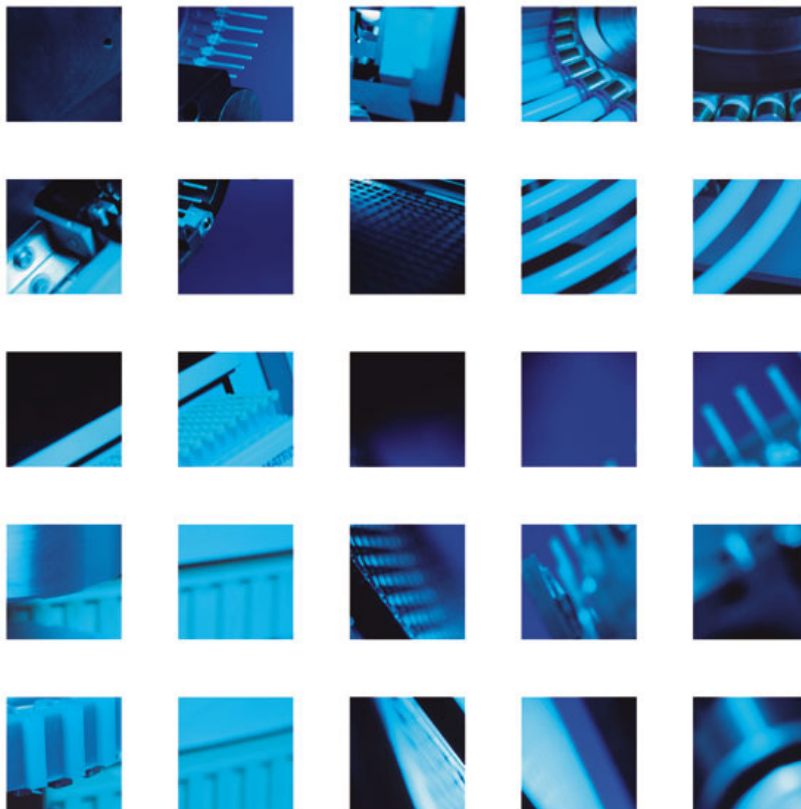


TTP LABTECH

TTP LABTECH LTD

mosquito Standard Operator Manual & Training

Version 3.2.0



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Reference 6773
Author Simon Carter

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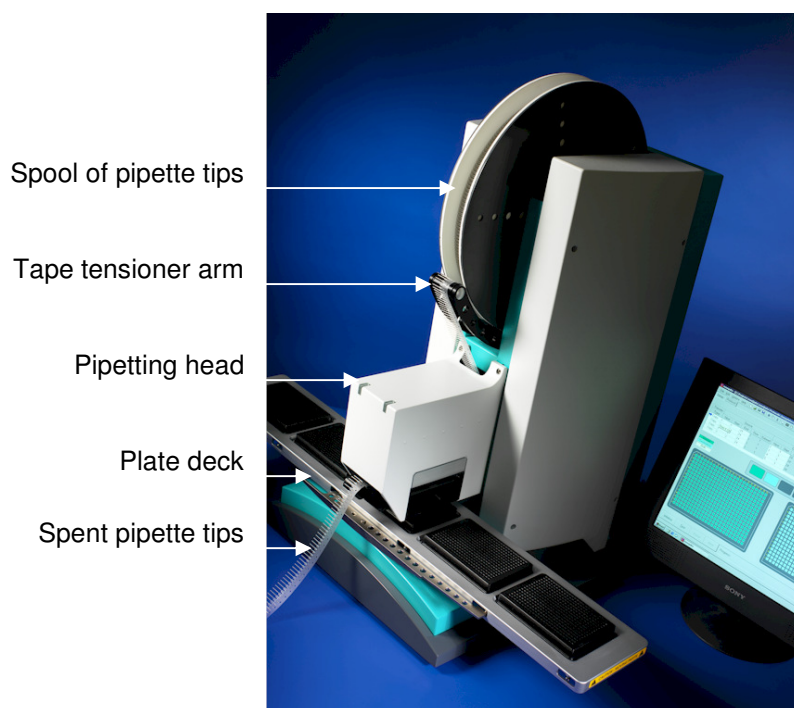
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1 MOSQUITO OVERVIEW

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 Hardware Overview

The mosquito nanolitre pipettor has a volume range of 50nL–1.2 μ L and uses disposable precision pipettes with a pitch of 4.5mm or 9mm (compatible with 384-well and 96-well plates respectively). This allows the instrument to pipette with 16 or 8 tips simultaneously to perform, for example, a 384-well plate-to-plate copy in less than 3 minutes with zero cross-contamination guaranteed.

1.1.1 Deck Types

The mosquito instrument is available with the following deck types:

- 2-position deck
- crystallography deck
- 5-position deck

- custom decks are also available.

1.1.2 Plate Types

The mosquito instrument can perform transfers in or between most plates conforming to standard SBS plate dimensions. Specialist plates may also be accommodated.

TTP LabTech offers proprietary ultra-low-volume plastic ware for distribution of materials in less than 5µl volume; details are available on request.

1.2 Software Overview

This manual describes software release version 3.2.0.

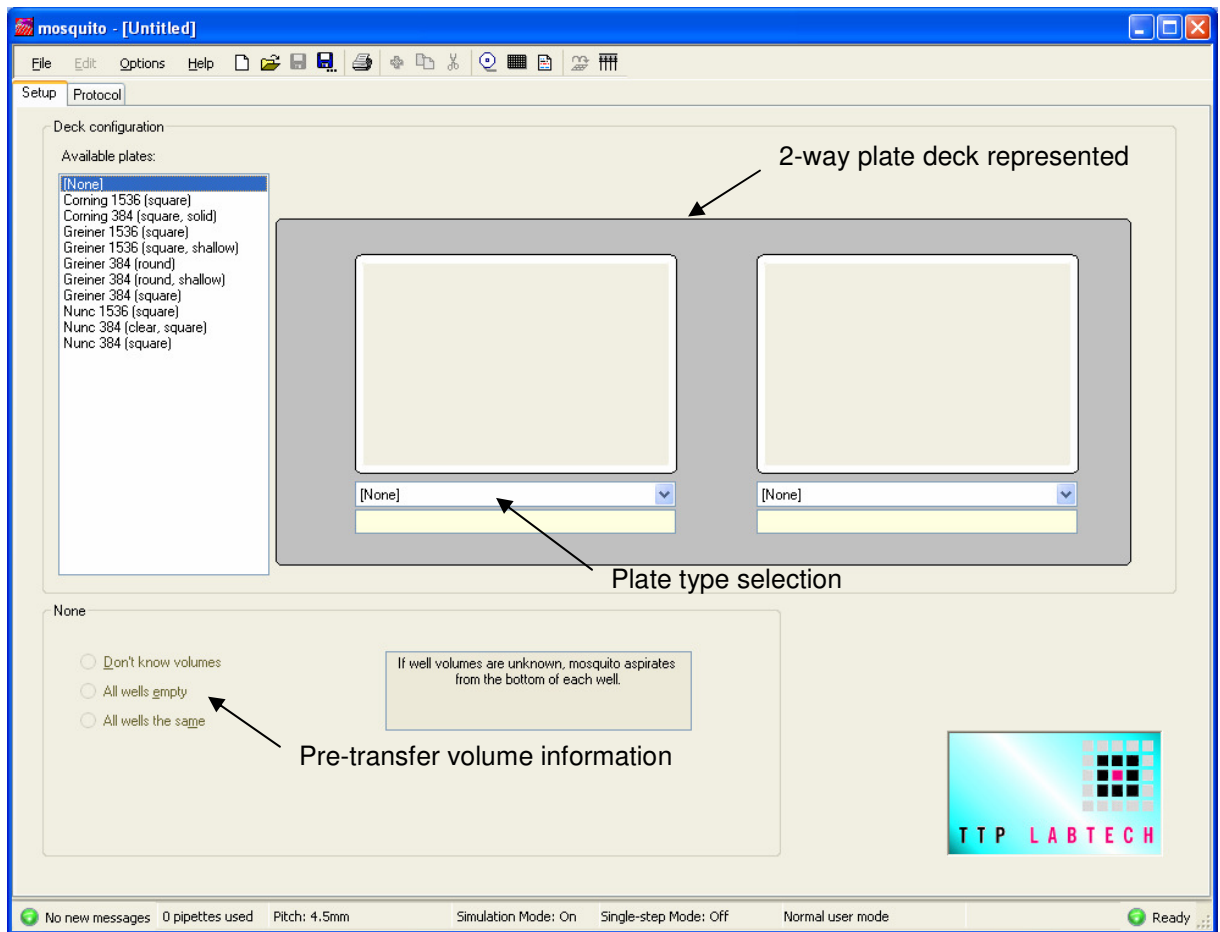
The mosquito software is simple and easy to use, relying on two tabs for setting up, editing and running transfers and protocols. These are the **Setup** tab and the **Protocol** tab.

Settings to allow mosquito to address new plate types can be added using the **Options | Plates** menu.

Access to troubleshooting functions and tools to calibrate the instrument are provided via the **Options | Calibrate** menu. These are not required for general use.

1.2.1 Setup Tab

The **Setup** tab allows the user to configure the plate types to be used in a protocol and the starting conditions from a list of available plates.



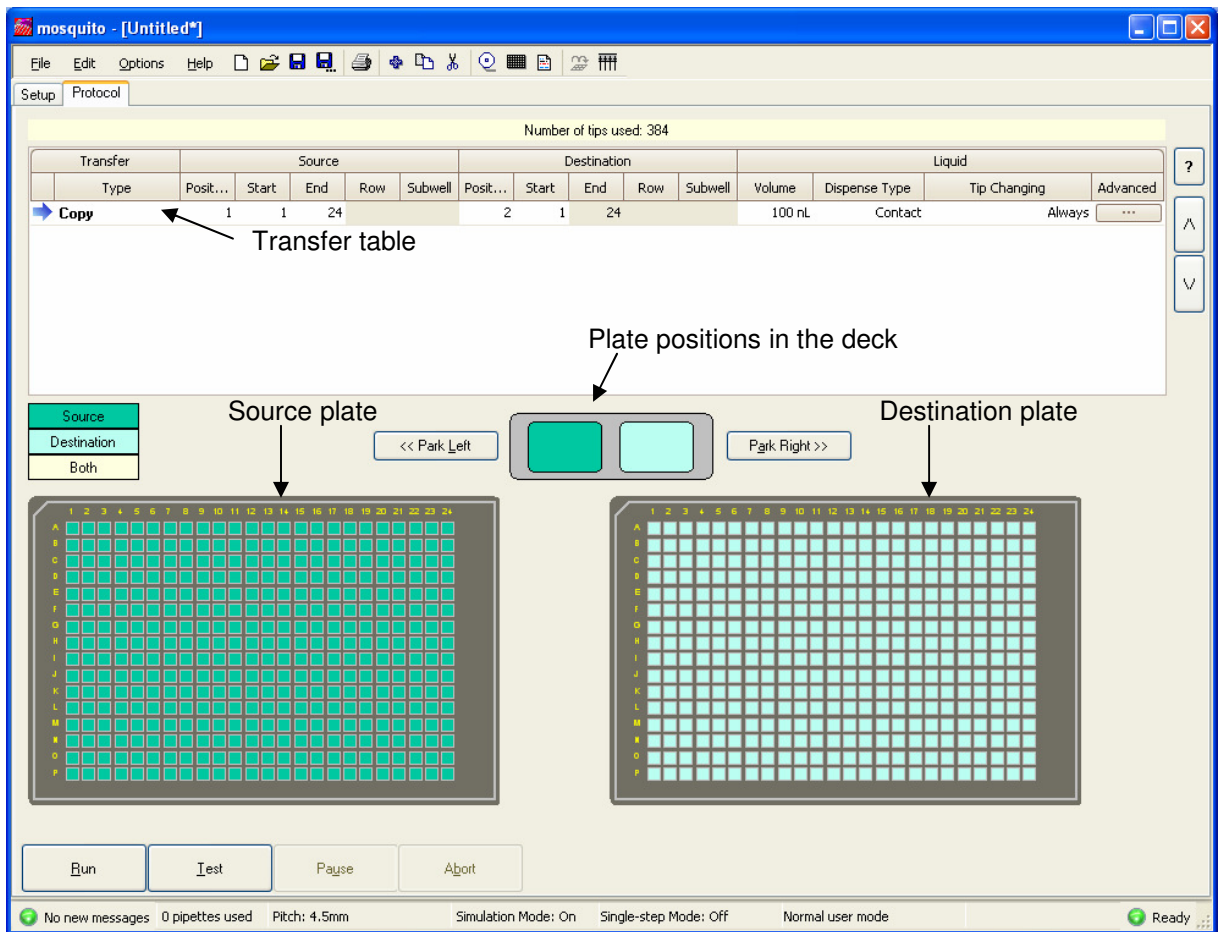
The Deck Configuration section allows the user to specify the type and position of plates on the moveable tray, or “deck”. The number of plate positions on the deck varies with the deck type.

Setting initial well volumes for each plate is optional but, if done, allows for accurate sample tracking and optimised dispensing.

1.2.2 Protocols Tab

The **Protocols** tab allows the user to specify the number of transfers to be performed in the protocol, the volumes to be transferred, and the way samples are transferred.

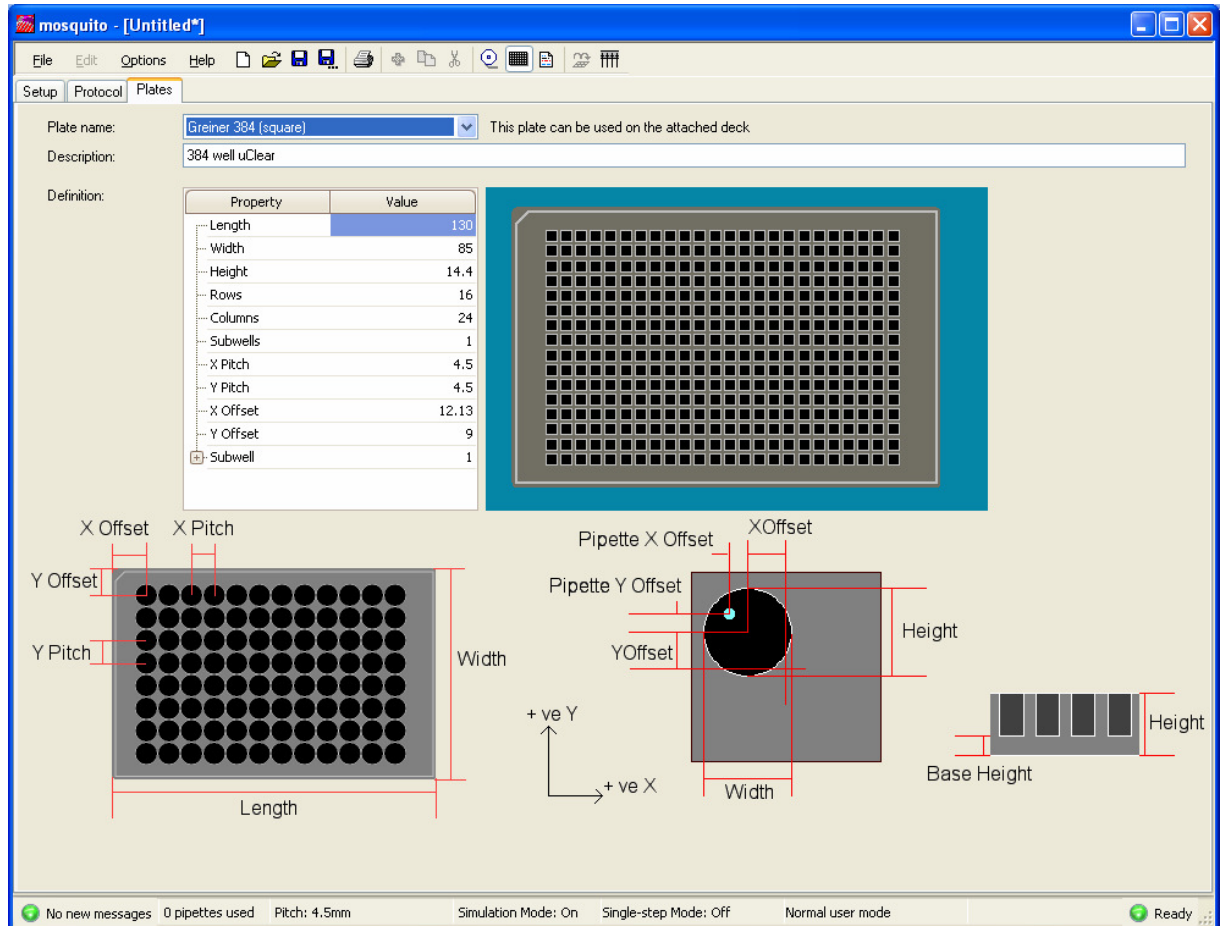
New transfers can be added to a protocol, copied or deleted, to suit the needs of the user. The details of each transfer operation are set up in the transfer table at the top of the screen.



The protocol illustrated above has one transfer – a simple 384-well plate copy.

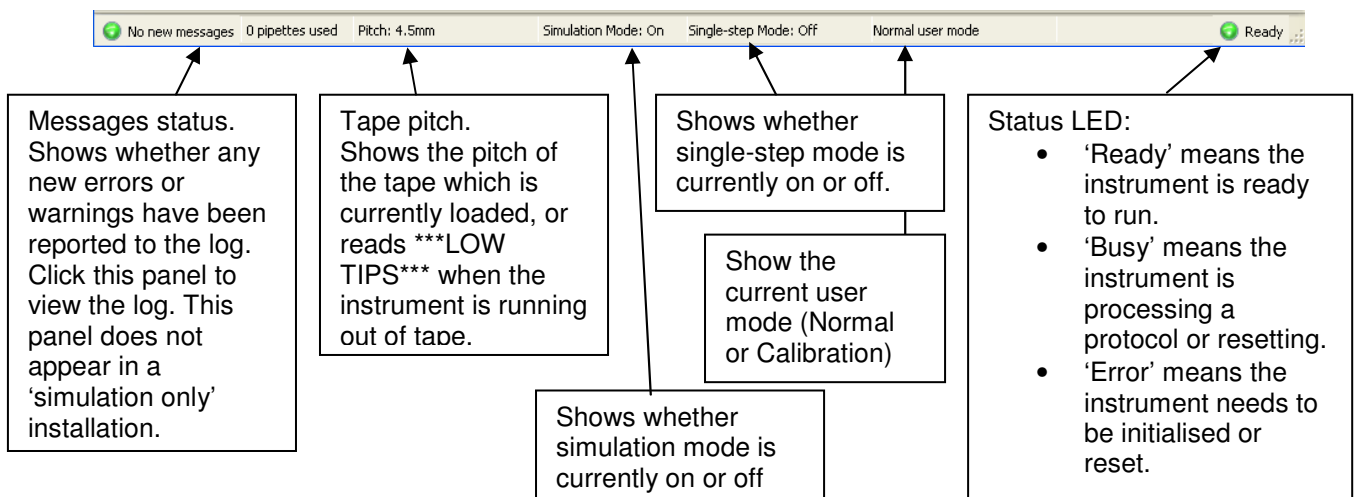
1.2.3 Plate Settings

The **Options|Plates** menu displays a window which allows the operator to view SBS plate types specified for use with the system.



1.2.4 The Status Bar

The bottom portion of the screen is the status bar. The meanings of the various panels are as follows:



The connection between the PC and the instrument can be broken by disconnection or power down of one of the items. If this occurs, the mosquito software must be restarted to re-establish the connection before processing can continue.

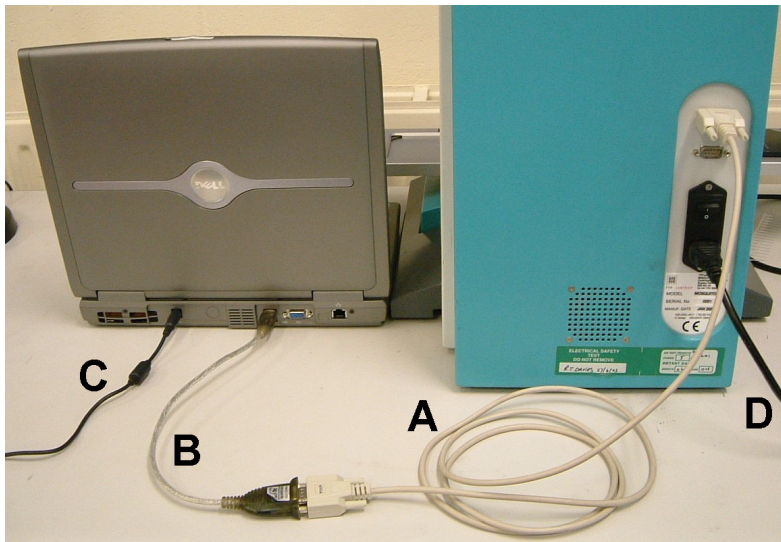
1.3 Setting Up Mosquito

1.3.1 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

1.3.2 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 open the mosquito application software by double-clicking on the application icon: 
- Note:** the instrument must be turned on approximately 10 seconds before the software is opened.
- When the software is open, a prompt asks if you want to initialise the instrument. Click Yes.

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

1.3.3 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 PROTOCOLS

The mosquito software is capable of naming and storing a selection of user-definable settings for a transfer Protocol. These named Protocols contain all the data required to perform pipetting operations, including volume settings, plate types, plate positions and so on.

A protocol is a series of user-specified transfers between wells. A transfer is a set of similar pipetting operations transferring sample liquid from source to destination. The exact sequence of operations depends on the parameters specified for the transfer. The settings for a protocol are stored in a file with the extension “.protocol”.

2.1 Standard Operation

In normal operation, the user can simply:

1. Select a pre-defined protocol from the **File|Open** menu.
2. Load the plates to be used on the instrument plate deck.
3. Adjust the plate types and positions saved with the protocol, if required, using the **Setup** tab.
4. Adjust volume settings and other transfer details, if required, using the **Protocol** tab.
5. Run and oversee the progress of the operation using the **Protocol** tab.

Protocols may be shared with other users, although they will not necessarily run on mosquito instruments with different decks or with a different type of pipette tape loaded.

2.2 Opening a Protocol

A new protocol is created by selecting the **New** icon: , or **File|New** from the main menu.

A saved protocol is opened by selecting the **Open** icon: , or **File|Open** from the main menu.

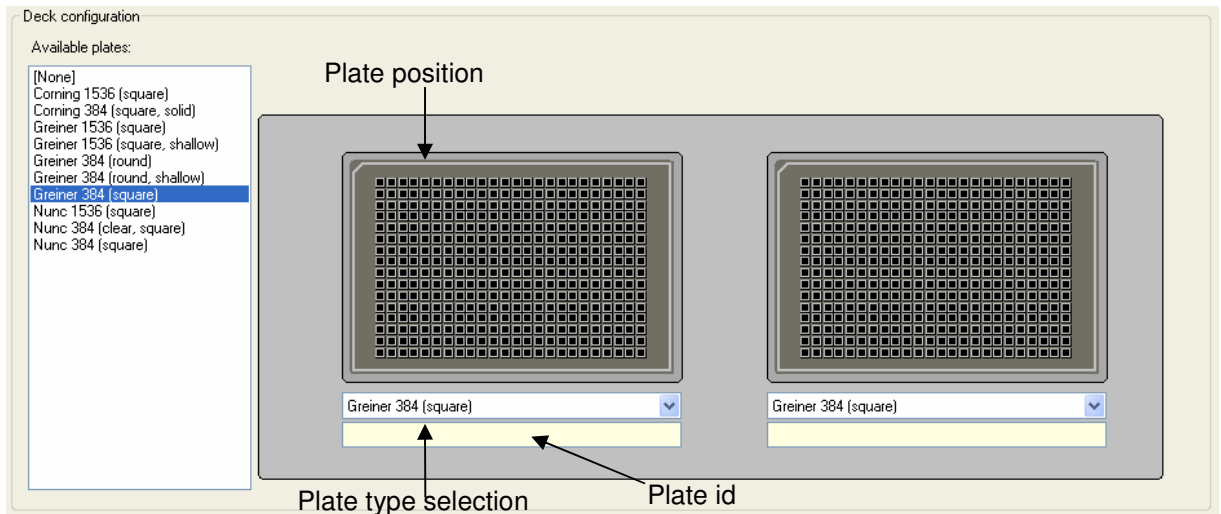
The protocol can be edited and saved by the user.

2.2.1 Setting the Starting Conditions

The types of plates used, and their layout on the instrument deck, are set up using the Deck Configuration settings on the **Setup** tab.

To add a plate type to a plate position in the deck, select the plate type required from the drop-down list. The software provides a ready defined selection of useful plate types.

Note: Plate types are available for selection only if the instrument pipette tape and deck configuration is compatible with the type of plate.



Hints:

- You can drag and drop from the plate list to a position, or from one position to another. If you hold down CTRL while dragging from one position to another then the plate will be copied, together with its volume information.
- If you drag a plate from a position and drop anywhere else then the plate will be removed from that position.
- You can double-click a plate in the list or right click a position to open the **Plates** window at that plate to view its measurements.
- You can enter an id for the plate in the yellow box. This is just for your information; it might be a barcode or can be any text string you like.

It is not necessary to specify initial well volumes but, if done, it allows accurate sample tracking and optimised dispensing. The optimum transfer is performed when the pipette tip is inserted just below the surface of the liquid, and this can be calculated if good starting condition information is input by the user.

Initial well volumes are set by selecting the 'All wells the same' option and entering the well volume in microlitres.

Position 1: Initial well volumes

Don't know volumes
 All wells empty
 All wells the same




All well volumes are the same.

microlitres


If the volume in the wells is unknown, or the plate is empty, then one of the other options should be selected.

2.3 Specifying Transfers


The protocol transfer details are specified from the **Protocol** tab.

- To add a new transfer to the table of transfers, select **Edit | Add Transfer** or click the **Add Transfer** button  on the toolbar. The type of transfer added to the table is set to 'Copy' by default.
- Transfers are copied by highlighting the transfer in the table and using the **Copy Transfer**  button.
- Transfers are removed by highlighting the transfer and using the **Remove Transfer**  button.

Number of tips used: 384

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

The transfer types are:

Transfer	Description
Copy	Transfers sample from one or several source columns on one plate to replica destination columns.
Mirror	This transfer is also a copy, but it reverses the order of the columns for the destination to form a mirror image of the source.
Pool	Transfers sample from many source columns on one plate to one destination column.
Aliquot	Transfers sample from one source column on one plate to many destination columns.
Pause	Pauses the protocol either for a specified number of seconds or until the Resume button is pressed.
Comment	Has no effect; it is just used to enter comments (by pressing the  button) to explain the workings of a protocol.
Change Pipettes	Forces the instrument to change the pipettes at this point in a protocol. This option is not normally required; the default behaviour set via the Tip Changing option is normally adequate
Multi-Aspirate	Perform an additional aspirate step. This allows the instrument to aspirate material from two or more source wells prior to dispensing; see the advanced manual for details
Reverse Aliquot	Transfers sample from one source column on one plate to many destination columns, but it reverses the order of the columns for the destination, dispensing from right to left. This may be combined with Multi-Aspirate operations.
Worklist	Specifies a worklist file which defines the sequence of operations mosquito should perform; see the advanced manual for details .

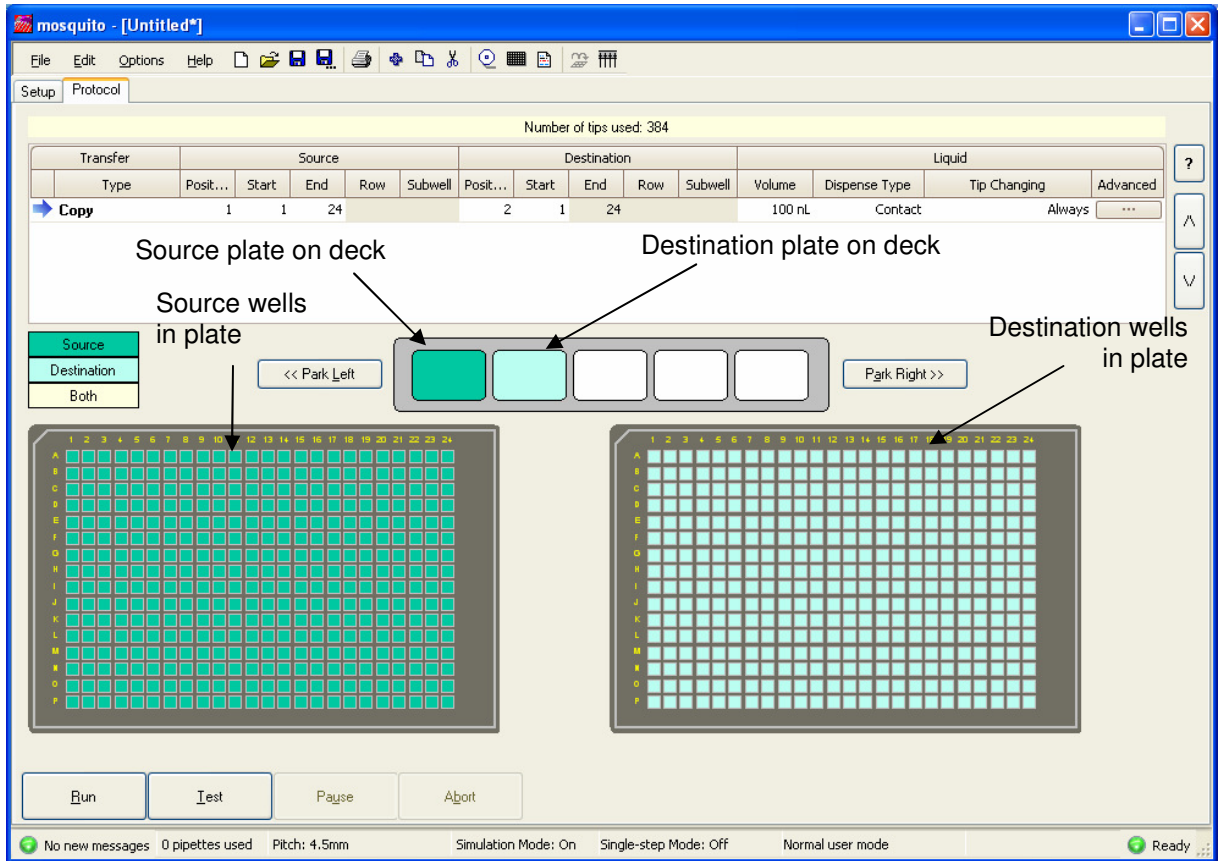
2.3.1 Source Wells

The convention is to use the left-hand plate as the source, and transfer samples to the right-hand side of the plate, or to the right-hand plate. However, mosquito can transfer from right to left, if required.

To specify the source wells for a transfer, enter values in the Source columns of the table:

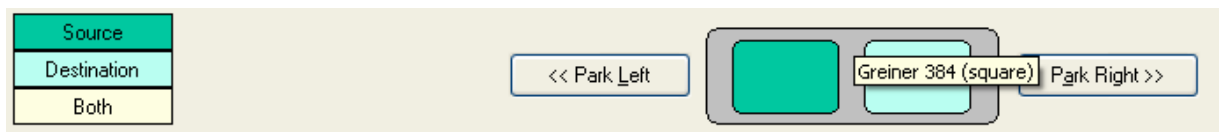
- Enter the plate deck position number of the plate which is the source for the transfer in the Position column. The leftmost position is position 1.
- Specify the start column number for the source wells in the Start column.
- Specify the source wells end column number (if appropriate) in the End column.

The source wells specified are displayed on the large plate representation on the left (labelled “source wells in plate”).



Note that the detailed plate representation always displays the source wells on the left and the destination wells on the right. Where source and destination are in the same plate, the plate is represented twice.

The deck layout graphic shows the actual position of the source/destination plates on the deck.



2.3.2 Destination Wells

To specify the destination wells for a transfer, enter values in the Destination columns of the table.

- Enter the deck position number of the plate which is the destination for the transfer in the Position column.
- Specify the start column number for the destination wells in the Start column.
- Specify the destination wells end column number (if appropriate) in the End column.

The specified destination wells are displayed on the large plate representation on the right.

If the source plate is the same as the destination plate, the large plate is represented twice with the source and destination wells separately displayed.

2.3.3 Drag and Drop Selection

It is possible to select the source and destination positions by drag and drop. For example, simply drag the position from the deck layout graphic to the left plate image to select that position as the source, or drag it to the 'source' section of the transfer in the list at the top of the screen.

It is also possible to select the source or destination wells by drag and drop. Hold the left mouse button down and drag the mouse to select a range of wells, or click once, move the mouse and click again while holding **Shift**.

It is possible to select a particular row and/or subwell by this method.

If you hold **Ctrl** down while clicking on a well a copy of the current transfer will be created but with the clicked column selected instead of the previous one.

2.3.4 Transfer Volumes

For Copy, Mirror and Aliquot transfers, the Volume column specifies the amount to be dispensed in each individual well, and has a range 0-1200nL. The volume for a transfer is adjusted by typing the required value into the Volume column of the table.

For Pool transfers, the Volume column specifies the amount to be aspirated from each individual well, NOT the total volume dispensed into a well for mixing. It has a range 0-1200nL.

2.3.5 Dispense Type

The dispense optimisations are:

- "Contact first" inserts the pipette tip into the liquid, or touches the bottom of a well, then dispenses, before slowly withdrawing the pipette.
- "Droplet first" dispenses the sample just above the well bottom or liquid surface, so it forms a droplet on the end of the pipette, then touches the droplet on the well bottom or liquid before withdrawing.

In most cases, the "contact first" dispense method produces the best volumetric results.

To change the dispense type, click on the Dispense Type column to bring up the drop down options, and then select the option required.

2.4 Using a Barcode Scanner

If your Mosquito is fitted with a barcode scanner, it is possible to use it scan each plate on the deck at the beginning of a protocol run. The numeric value of the barcode for each plate will be saved in the report file (Which can be viewed by going to **File | View Report** or looking at the files in the **Reports** directory)

To enable the barcode scanner:

- Go to the “Options” menu
- Select “**Barcode Options**”. If this option is not present, then your mosquito is not configured to use a barcode reader, or you are using out of date software. In either case, please contact our support team who will be happy to help.

You should see a window like this:



To enable barcode scanning, check the button marked “Scan plate barcodes before running protocol”.


If you wish to abort the run if a barcode read is unsuccessful, also check “Abort run if barcode read fails”. If you leave this unchecked, you will get an error message in the report if any of the reads fail, but the run will continue.


Important Note

The barcode options are stored **per protocol, not** for the entire application. If you make a new protocol, you will need to set the options again if you wish to use the scanner. Similarly, if you have the “Scan plate barcodes before running protocol” option unchecked and then load a protocol which has barcode reading turned on, the option will become checked.

2.5 Saving a Protocol

Protocols and their settings can be saved for future use.

To replace a previous protocol with the current changes, click on the **Save** icon () , or select the **Save** option from the **File** menu.

To keep the original protocol and create a new protocol with the current settings, click on the **Save As** icon () , or select the **Save As** option from the **File** menu. You will be prompted for a filename and a location to create the file.


2.5.1 Saving as a Text File

It is possible to save protocols as text files for easy readability in a text editor such as Notepad or Microsoft® Word.

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

Note: at the present time mosquito does not read text versions of the protocol file. mosquito works only with the original *.protocol file.

2.5.2 Printing a Protocol

Select **File | Print** or press the Print  button on the toolbar to print out the current protocol in text form.

2.6 Running a Protocol

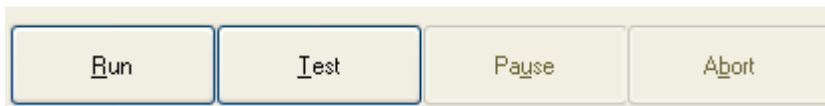
mosquito is ready to run a protocol when the user has checked the plate and volume settings, made any necessary updates and loaded the required plates into the deck positions.

To run the protocol, click the **Run** button on the **Protocol** tab. mosquito performs the transfers in sequence, and the software updates the plate display on the **Protocol** tab to reflect which wells the instrument has already addressed.

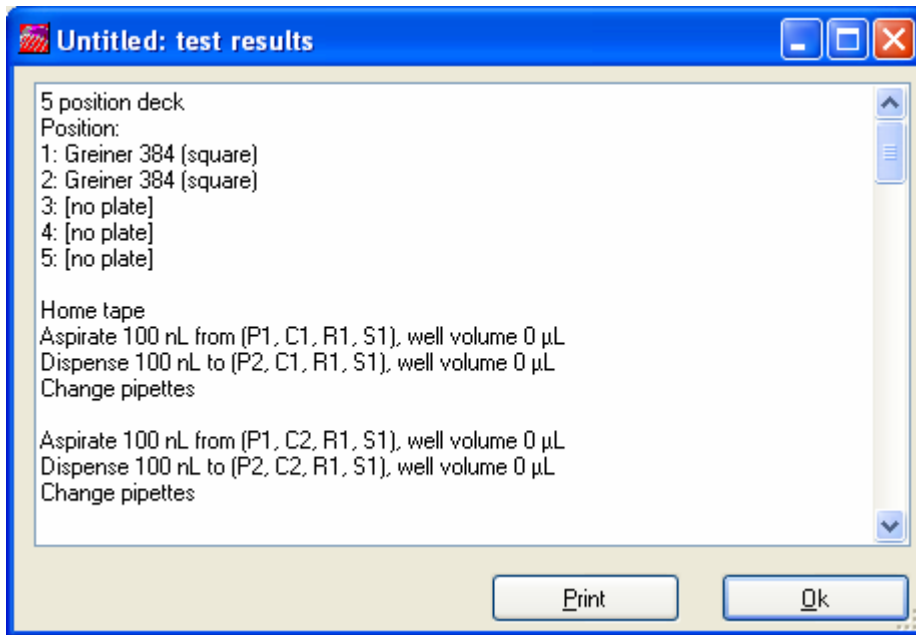
Using the default settings, running a protocol always homes the plate deck drives before processing. Pipettes are **always** changed at the start of a protocol or when a run is aborted, so each protocol starts with clean pipettes.

2.6.1 Testing a Protocol

Once a protocol is configured and before it is run, the user can check the operations that will be performed. This is especially useful for protocols which contain transfers which have been optimised by using the pipette-changing options 'Multi-dispense' or 'Between Transfers'.



Press the **Test** button on the **Protocol** tab to display the sequence of aspirate, dispense and pipette change operations which will be performed.



This test can be printed by pressing the **Print** button.

2.6.2 Pausing a Protocol

A running protocol is paused by clicking the **Pause** button on the **Protocol** tab. The instrument pauses at the next convenient point, and the **Pause** button changes to a **Resume** button.

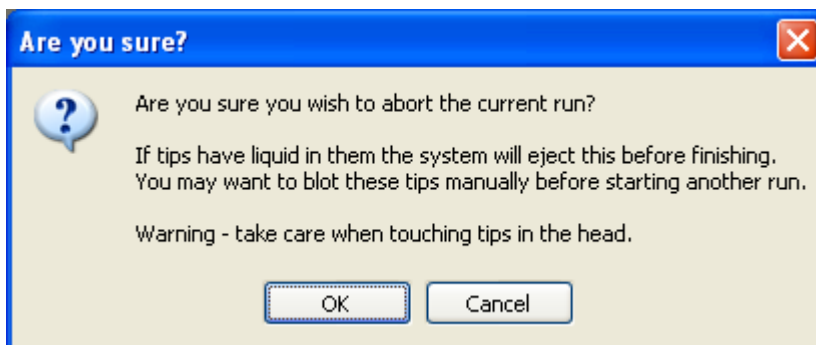
To resume operation, click the **Resume** button.

2.6.3 Stopping a Protocol

A running protocol is stopped by clicking the **Abort** button on the **Protocol** tab. The instrument stops at the next convenient point. **Note:** this is not an immediate action.

The wells on which mosquito was operating when **Abort** was pressed are coloured red.

If the instrument has just aspirated a sample, a dialog box informs the user that the instrument will move to a clean position and then eject the liquid.



Warning: The pipette tips are changed after the liquid is dispensed so keep fingers clear of the instrument head.

3 EXERCISE 1: CREATING A PROTOCOL

3.1 Create a New Protocol

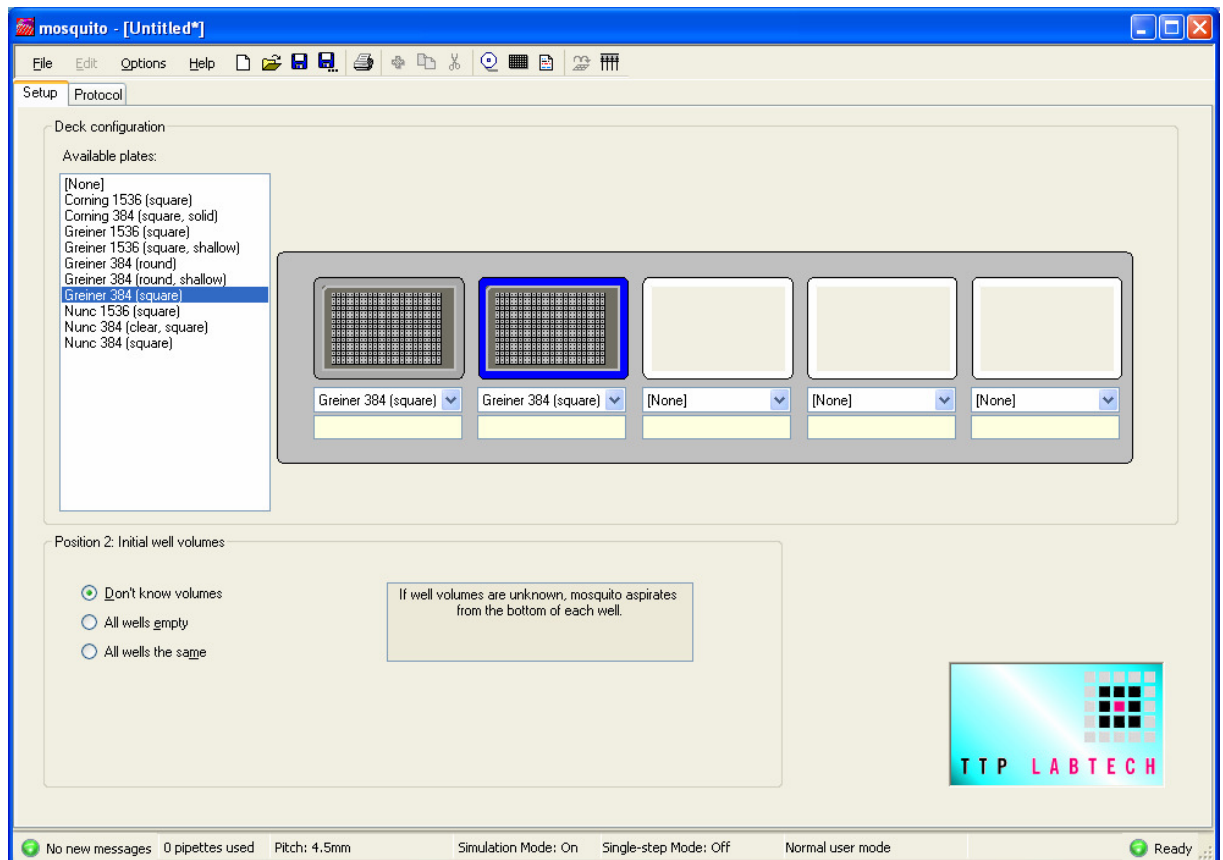
A new protocol is created by selecting the **New** icon: , or **File | New** from the main menu.

A new protocol defaults to an empty deck and an invalid transfer.

3.2 Fill in the Setup Tab

- Click the **Setup** tab to bring it to the front.
- Under Deck Configuration, select Greiner 384 square for both plates.
- Set initial well volumes to 'Don't know volumes'.

The Setup tab should look like (note that Deck Configuration may vary according to the number of plate positions on the instrument):



3.3 Set up the Transfer Table

- Click on the **Protocol** tab to bring it to the front.

- Adjust the source column numbers to cover the whole plate (i.e. start at column 1 and end at column 24). Because the transfer is a Copy, the destination column numbers will automatically adjust to match the source plate.
- The transfer volume is set by typing '500' in to the Volume column.
- The Dispense Type, Tip Changing and Advanced columns may be adjusted if required.

The protocol should be similar to the one displayed below:

Number of tips used: 384

Transfer	Source					Destination					Liquid				
	Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced
→ Copy		1	1	24			2	1	24			500 nL	Contact	Always	...

Source

Destination

Both


<< Park LeftPark Right >>

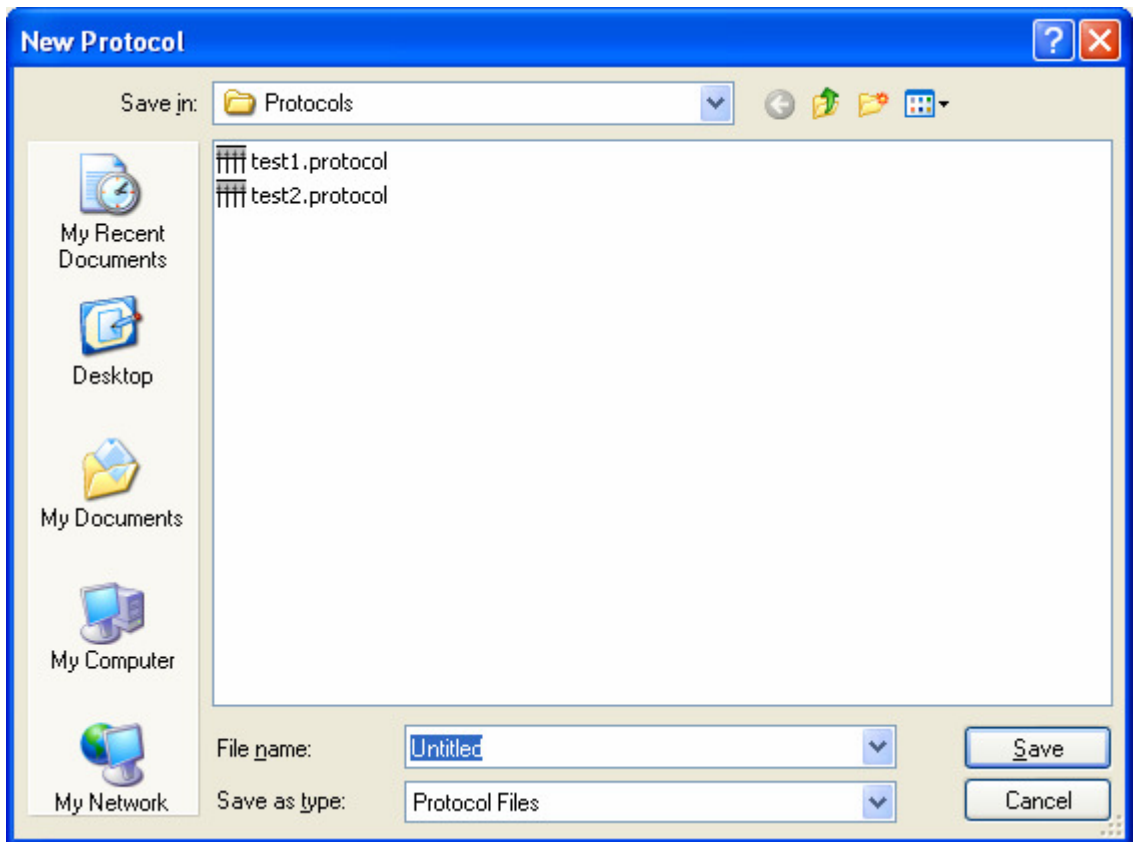
empty

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
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I																								
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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
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3.4 Save the Protocol

- Click on the **Save** icon () , or select the **Save** option from the **File** menu.
- A pop-up dialog box requests a name for the protocol:



- Type in 'Exercise 1' as a file name.
- Click **OK** to save the protocol.

4 PIPETTE USE

There are a number of user-configurable options concerning the pipettes and their replacement for use.

4.1 Pipette Changing During Transfers

The user can specify at what point in the dispense cycle the pipettes are changed using the Tip Changing column. The options are:

- “Always” changes pipette tips after each individual dispense. This is the default setting and ensures there is no cross-contamination between wells.
- “Multi-dispense” changes pipette tips before each aspiration. When aliquotting, this allows several dispenses to be made from one aspiration, which may result in faster processing.
- “Between transfers” changes pipette tips after performing the actions defined in a row of the transfer table.
- “Never” does not change the pipette tips.

To specify when to change tips, click on the column to bring up the drop down options, and then select the option required.

If Tip Changing is set to either ‘Multi-dispense’ or ‘Between Transfers’, mosquito optimises the number of times pipettes are filled and minimises the number of pipettes used, to process the protocol faster and use fewer pipettes. Generally this means that tips will be re-used for transfers using the same source wells. However, the user should check that using these settings will not cause contamination of wells. This is done using the **Test** button (see Section 2.6.1).

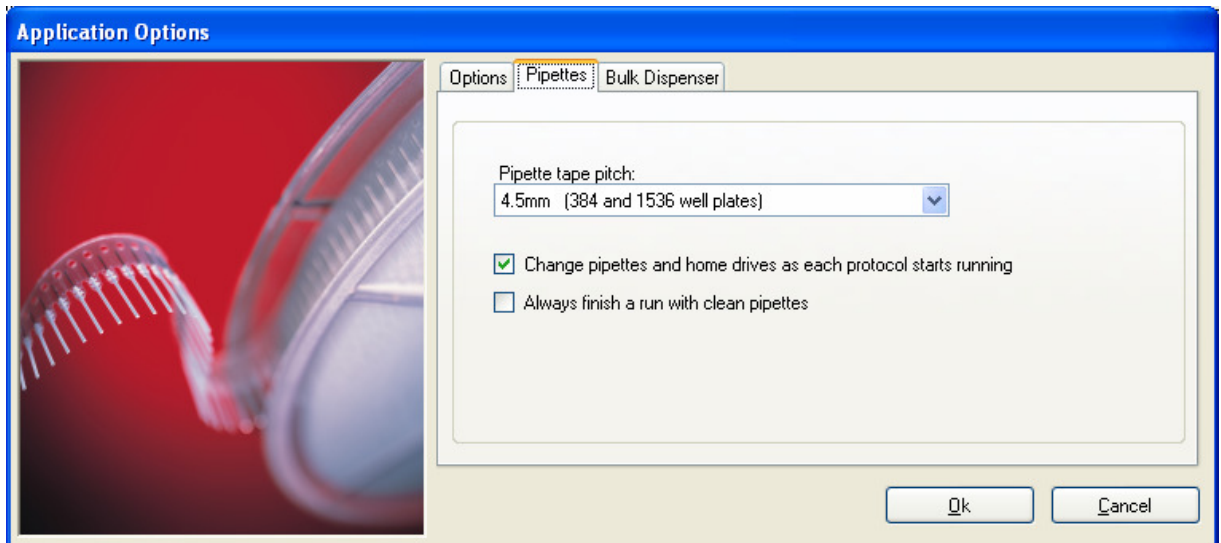
In ‘Between Transfers’ mode, mosquito optimises transfers of the same type that are consecutive in the table AND use the same source wells. Copy and Mirror are considered to be transfers of the same type.

See Section 5.4 for some examples of the effect these options have.

4.2 Changing Pipettes at the Start and End of a Protocol

The mosquito instrument’s drives are homed as each protocol starts running, by default. This ensures that the deck and head are in the correct position and that a run always begins with fresh pipette tips. This behaviour can be disabled if required - for instance, if it is known that the instrument has not been disturbed since a successful run and that there are already clean pipettes in the head.

To disable drive homing and loading clean pipettes at the start of a run select **Options | Application preferences** from the main menu to access the Application Options dialog box, then uncheck the ‘Change pipettes and home drives as each protocol starts running’ check box.



Note: If this setting is disabled, care must be taken not to cause well cross-contamination because dirty pipettes have been reused.

The user can set mosquito to load clean pipettes at the end of every run. To do this select **Options | Application preferences** from the main menu to access the Application Options dialog box, then check the 'Always finish a run with clean pipettes' check box.


It is not normally necessary to check both 'Change pipettes and home drives as each protocol starts running' (see Section 4.1) and 'Always finish a run with clean pipettes'.

Pipette pitch is normally set as part of the tape loading or calibration process, but if an error is made it can be corrected by selecting the correct pitch in the drop-down list.

4.3 Stepping Through a Protocol

It is possible to work through a protocol one step at a time. This is useful when troubleshooting or to check that the plate deck and pipetting head movements are correct.


To turn on single-step mode, select **Options | Enable Single Step Mode** from the main menu. Single-step mode can be turned on (or off) before a run starts or during a run. The status bar will show whether Single-step mode is on or off.

When single-step mode is on, mosquito pauses at the end of each step until the user presses the step button  on the toolbar, F8, or **Options | Single step**.

When you turn single-step mode off during a protocol run, the protocol will immediately begin running as normal.

Selecting **Abort** while single-stepping will turn off single-step mode while the abort operation is performed and turn it back on after the abort is complete.

4.4 Manually Winding on Pipette Tape

When the mosquito instrument has been unused for some time it may be desirable to wind on tape manually. To do this select **Options|Feed Tape** from the main menu, or press the  button on the toolbar. This will change the pipettes currently in the instrument head.

5 EXERCISE 2: SPECIFYING PIPETTE CHANGES

Load the 'Exercise 1' protocol saved from Exercise 1.

All the pipette changes are set using the transfer table on the **Protocol** tab.


5.1 Change Pipettes to Ensure No Cross-contamination in Source or Destination Plates

- Click on the **Protocol** tab.
- Change the Copy transfer to an Aliquot transfer in the transfer table.
- Adjust the Source column numbers to column 1 only (i.e. start at column 1 and end at column 1).
- Adjust the destination column numbers to columns 1 to 8 (i.e. start at column 1 and end at column 8).
- Set the transfer volume by typing 250 in to the Volume column.
- Set the Tip Changing column to ensure that there is no cross contamination in either the source or destination plates (i.e. select 'Always').
- The Dispense Type and Advanced columns may be adjusted if required.

The protocol should be similar to the one displayed below.

Transfer	Source						Destination						Liquid			
	Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced	
Aliquot		1	1				2	1	8			250 nL	Contact	Always	...	

5.2 Change Pipettes to Ensure No Cross-contamination in the Source Plate

- Use the **Copy Transfer** button () to copy the previous Aliquot transfer to the transfer table.

- Adjust the destination column numbers to columns 9 to 16 (i.e. start at column 9 and end at column 16).
- Set the Tip Changing column to ensure that there is no cross contamination in the source plates (i.e. select 'Multi-dispense').
- All other fields should be left as they were in the previous transfer.

The protocol should be similar to the one displayed below:

Number of tips used: 256

Transfer		Source					Destination					Liquid			
Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced	
Aliquot	1	1				2	1	8			250 nL	Contact	Always	...	
Aliquot	1	1				2	9	16			250 nL	Contact	Multi-dispense	...	

Source

Destination

Both

<< Park Left

Park Right >>

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

A B C D E F G H I J K L M N O P

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

A B C D E F G H I J K L M N O P

5.3 Change Pipettes when there is No Risk of Cross-contamination

For this transfer we are assuming that source liquid is common to all source wells and the destination plate is empty so there is no risk of cross-contamination. Here, pipette changes are optimised to use the minimum number of pipettes.

- Use the **Copy Transfer** button to copy the previous Aliquot transfer to the transfer table.
- Adjust the destination column numbers to columns 17 to 24 (i.e. start at column 17 and end at column 24).
- Assuming that there is no risk of cross-contamination, set the Tip Changing column to ensure that the minimum number of pipettes is used (i.e. select 'Between transfers').
- All other fields should be left as they were in the previous transfer.

The protocol should be similar to the one displayed below:

Number of tips used: 288

Transfer	Source					Destination					Liquid				
	Type	Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume	Dispense Type	Tip Changing	Advanced
Aliquot		1	1				2	1	8			250 nL	Contact	Always	...
Aliquot		1	1				2	9	16			250 nL	Contact	Multi-dispense	...
Aliquot	▶	1	1				2	17	24			250 nL	Contact	Between transfers	...

Source

Destination

Both

5.4 Examples of Pipette Changing

To demonstrate the effect that the pipette tip changing has on optimising pipette usage, suppose that we have an instrument with a 5 way deck and 4.5mm pitch tape loaded.

1. Create a protocol with generic 384 well plates loaded in every plate position.
2. Create a protocol consisting of two Copy transfers, one which copies all of position 1 to position 2 and one which copies all of position 1 to position 3, with transfer volume 100nL in each case and all other options identical.
3. Set Tip Changing to 'Always' for both transfers and press the **Test** button to see what mosquito will do. The sequence will be

```
Aspirate 100 nL from (P1, C1, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C1, R1, S1), well volume 0 µL
Change pipettes
```

```
Aspirate 100 nL from (P1, C2, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C2, R1, S1), well volume 0 µL
Change pipettes
```

```
Aspirate 100 nL from (P1, C3, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C3, R1, S1), well volume 0 µL
Change pipettes
```

...

...

then

```
Aspirate 100 nL from (P1, C24, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C24, R1, S1), well volume 0 µL
Change pipettes
```

```
Aspirate 100 nL from (P1, C1, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C1, R1, S1), well volume 0 µL
Change pipettes
```

```
Aspirate 100 nL from (P1, C2, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C2, R1, S1), well volume 0 µL
Change pipettes
```

etc..

4. Set Tip Changing to Multi-Dispense in each case and press **Test** again. The sequence will be

```
Aspirate 200 nL from (P1, C1, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C1, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C1, R1, S1), well volume 0 µL
Change pipettes
```

```
Aspirate 200 nL from (P1, C2, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C2, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C2, R1, S1), well volume 0 µL
Change pipettes
```

```
Aspirate 200 nL from (P1, C3, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C3, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C3, R1, S1), well volume 0 µL
Change pipettes
```

etc..

5. Set Tip Changing to 'Between Transfers' in each case and press **Test** again. The sequence will be:

```
Aspirate 200 nL from (P1, C1, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C1, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C1, R1, S1), well volume 0 µL
Aspirate 200 nL from (P1, C2, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C2, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C2, R1, S1), well volume 0 µL
Aspirate 200 nL from (P1, C3, R1, S1), well volume 0 µL
Dispense 100 nL to (P2, C3, R1, S1), well volume 0 µL
Dispense 100 nL to (P3, C3, R1, S1), well volume 0 µL
```

etc..

6 EXERCISE 3: CHANGING A PIPETTE REEL

Mosquito will report that it is running low on tape by displaying the message ***** LOW TIPS ***** in the status bar together with the message 'Reel Low' in the log screen. When this message appears, there are enough tips to make 4 copies of a 384 well plate with 4.5mm-pitch tape, changing tips after every dispense, or one copy of a 1536 well plate.

To unload the old reel and load a new one, select **Options | New Tape** from the software main menu, or click on the **New Tape** icon .

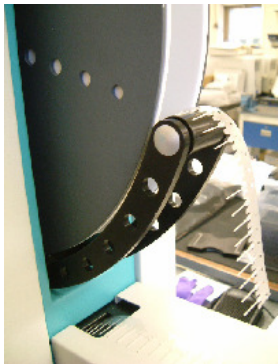
The steps to unload an old reel and reload a new one are described in the mosquito software. The process may be re-started or cancelled at any point.

6.1 Unload the Pipette Reel

To unload a pipette reel:



- Use scissors to cut the pipette tape above the instrument head.
- Press the **Purge** button to feed the remaining tape through the head until the head is clear.
- Lift the spool carrier up and out of the instrument. There are no clips to release.

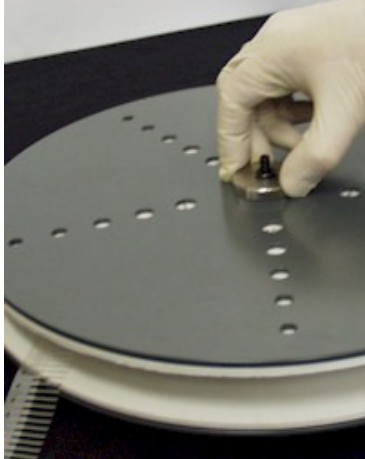


- 'Click in' the 'Dancer Arm' (the tape tensioner arm that the tape passes over immediately after leaving the spool) by pushing it downwards until it clicks.



6.2 Remove Old Pipette Tape

To remove old pipette tape:



- Unscrew the silver wheel holding the spool together.
- Lift off the top plate off the spool carrier.
- Lift out the old reel of pipette tips.

6.3 Load New Pipette Tape

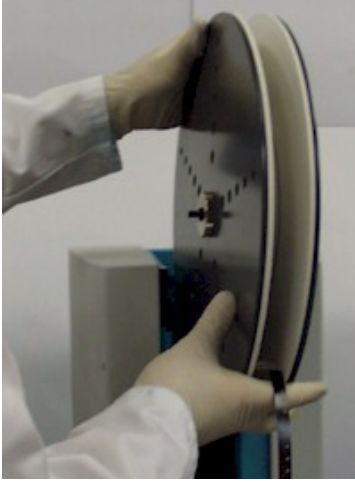
To load new pipette tape:



- Put a new reel of pipette tape on the base plate.
- Rotate the reel so the location pad on the base plate fits into the cut-out on the reel and the two plates lie snugly together.
- Check the tape sits with the pipettes pointing upwards.
- Replace the top plate.
- Replace and tighten the silver wheel to fix the top plate in position.
- Fit a new pipette tape leader, if one is not already fitted (see Section 6.6).

6.4 Reload the Pipette Reel

To reload the pipette reel:



- Ensure the silver wheel is on the left of the spool as you face the instrument.
- Check the pipette tape leader is accessible from the front of the instrument.
- Slide the reel carrier pins in to the slots at the top of the instrument.
- Check the pipette tips point to the left. Reload the reel if they point to the right.

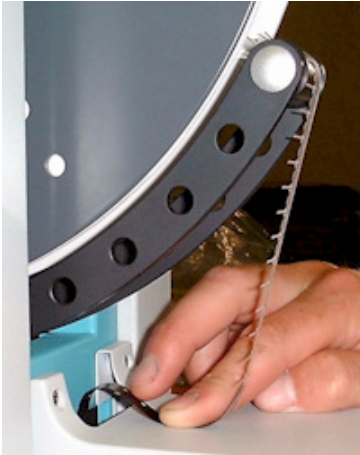
6.5 Prime Tape in the Instrument Head

To feed pipette tape into the instrument head:



- Unclick the dancer arm by pushing it down until it clicks again and returns to its normal position.





- Pass the pipette tape over the tensioner arm so there is a small loop of free pipette tape at the front of the instrument.
- To feed more tape through, push down on the tensioner arm. Tape cannot be pulled through by hand.
- Feed the black leader tape into the slot on top of the instrument head, keeping it to the right hand side.
- Feed the leader tape in until it reaches a dead end, then push a small loop of tape against the slot (see picture).
- After a few moments the instrument will wind on the pipette tape. If it does not, re-feed the leader tape into the slot and try again.

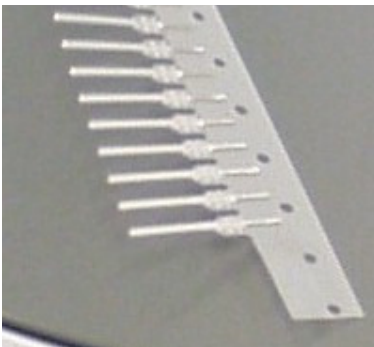


- In the **Load New Tape** software window, specify the pitch of the pipette tape loaded (4.5mm for 384-pitch or 9mm for 96-pitch tape).
- Look along the plate deck and check that pipettes are loaded correctly and ready to run (see picture).
- If the pipette tape is only partially loaded, follow the instructions in Section 4.4 to wind through more pipette tape. If the tape is stuck, check the recovery details given in Section 8.2.3.

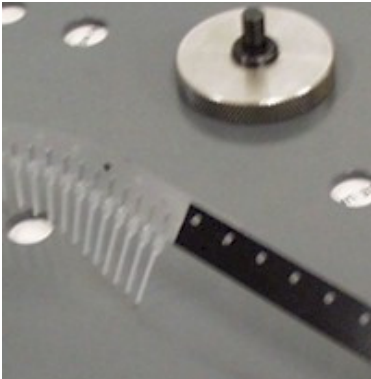
The mosquito instrument is now primed with tips and ready for use.

6.6 Attaching a New Tape Leader

A tape leader is provided on new reels of pipette tape. Tape that has jammed or been removed can be fitted with a new leader as follows:



Cut the pipette tape near a hole and trim enough pipettes from the tape to expose approximately 24mm (1") of backing tape.




Align the holes on the pipette tape and the leader tape and stick the leader in position using the double-sided adhesive pad on one end of the leader.

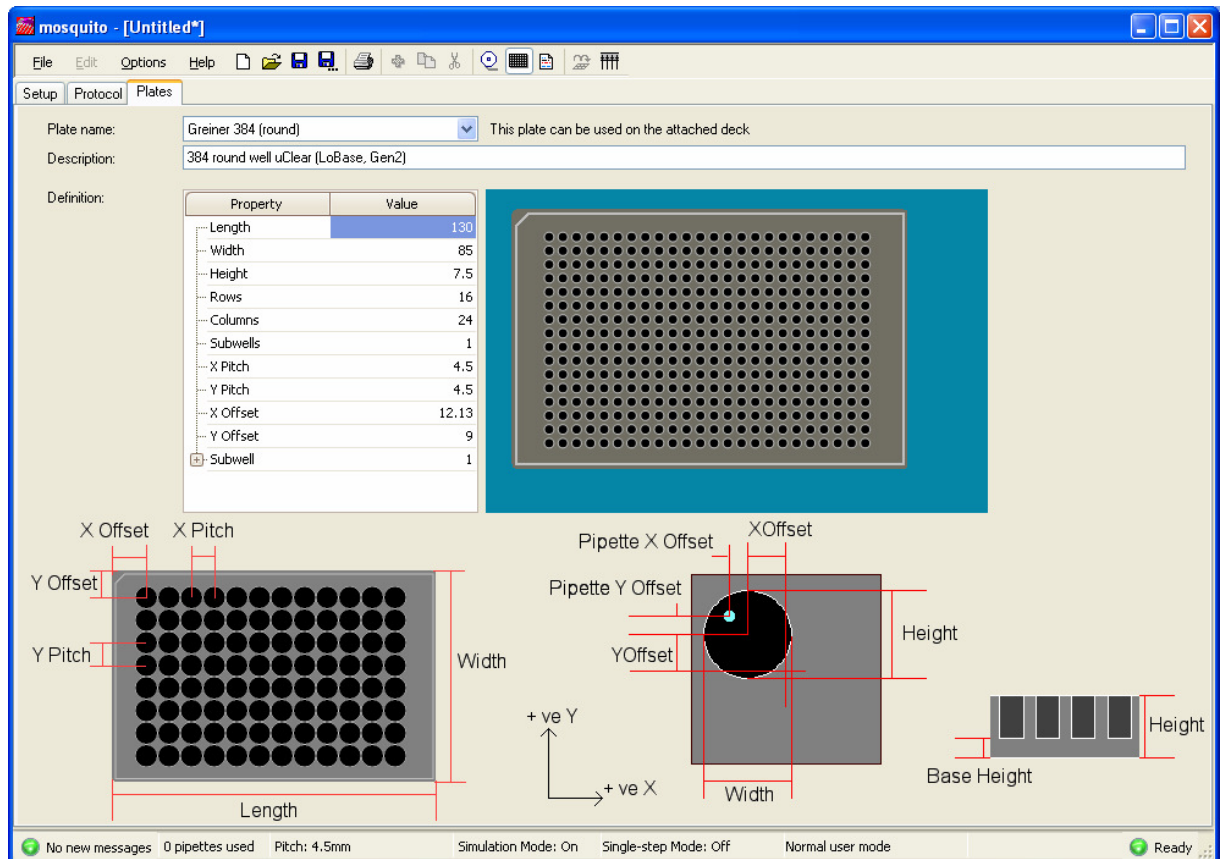
Note: do not reuse leader tape. Always use a new leader.

7 EXERCISE 4: VIEWING PLATE DETAILS

7.1 Open the Plate View

The **Plates** tab contains a drop-down list of plate names (types) which the user can specify in a deck position. These plate types contain the data required for accurate aspiration and dispenses.



- Access the **Plates** tab by clicking on the **Plates** icon  or by selecting **Options | Plates** from the main menu.
- Select a plate name to display details of the plate layout and dimensions, and details of the well volume.



7.2 Check a Plate's Details from the Setup Tab

- Click on the **Setup** tab to bring it to the front.
- Drag a plate type onto a deck position.
- Double-click on the plate to be taken directly to the definition of that plate.

7.3 Print a Plate Files

- Access the **Plates** tab by clicking on the **Plates** icon  or by selecting **Options | Plates** from the main menu.
- Select **File | Print** or press the Print  button on the toolbar to print out the current plate in text form.

Note: the **Plates** window must be open in order to print a plate file.

8 ERROR HANDLING & RECOVERY

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



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.

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.

8.1 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

Telephone: +44 (0) 1763 266708
Fax: +44 (0) 1763 266755

MERA
P.O. Box 167
759 191/2 Street
Chetek, WI 54728
USA

Telephone: 800 776 7158
Email: meradispatch@meraserv.net

Email: mosquitosupport@ttplabtech.com

Website: <http://www.ttplabtech.com>

Availability:

8:30am-5:30pm UK Time Monday-Friday.

Availability:

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.

8.2 Troubleshooting

8.2.1 No Connection Between mosquito and Computer

No connection between the PC or laptop and the mosquito instrument 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 the mosquito instrument is receiving power by checking that mains power is switched on at the socket, the cables are correctly connected (see Section 1.3.1) and the fan at the back of the instrument is running.
- Power the instrument off and on again, then restart the mosquito software. If there is still no connection contact a service representative (see Section 8.1)

8.2.2 Error Appears on PC Screen

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




This error is most commonly caused if the laptop is set to run off a battery. If it tries to hibernate or standby it is prevented from doing so by mosquito's CAN interface.

- Check that the laptop is not operating on batteries and that power cables are on and plugged in.
- To rectify the error, click **OK**, and, if necessary, power off the instrument and restart the laptop before reconnecting.

8.2.3 Pipette Tape Fails to Load

If the pipette tape doesn't wind on at all:

- Check whether there is any noise:

- If yes, wait and start the tape feed process again as the tape leader has missed the sprocket. To reload pipette tape, a new leader tape is required. See Section 6.6.
- If not, try withdrawing and then re-feeding the leader tape, as it has not triggered the sensor.
- ❑ If the tape has caught but pipettes are only partially loaded, wind on the tape manually. Select **Options | Feed Tape** from the main menu, or press the  button on the toolbar. This will change the pipettes currently in the instrument head.
- ❑ If the index winds in but the leader tape doesn't emerge from the pipetting head and there is a juddering noise, the leader tape has jammed in the head. See Section 8.2.4 below.

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.

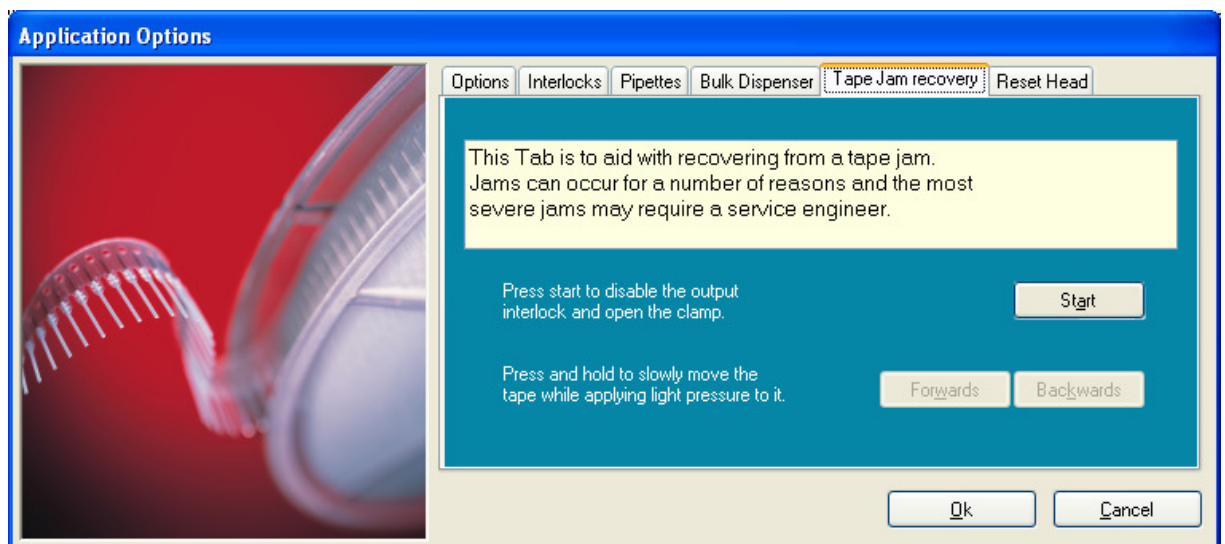
8.2.4 Clearing a Tape Jam

(NOTE: You need to be in Calibration mode to see this tab.)

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

- ❑ Select **Options | Application Preferences** from the main menu and click on the **Tape Jam Recovery** tab.

The following screen is displayed:



- ❑ 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 tape does not come free easily, contact a service engineer for advice (see Section 8.1). It may be necessary to remove the head cover and clear the head.

8.2.5 Pipette Tips Produce Erratic Drops on Dry Surfaces.

- Check the volume in the source wells using the **Setup** tab. There may be inadequate liquid in the source wells.

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 8.1).

8.2.6 Pipette 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.

9 APPENDIX A: 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.

 10 APPENDIX B: MOSQUITO OPERATOR TRAINING RECORD

Trainer name	
---------------------	--

Trainee name	
Trainee company	

Standard Operator Training Course Components	Completed	Date
Overview of mosquito instrument		
Exercise 1: Create a Protocol		
Exercise 2: Specifying Pipette Changes		
Exercise 3: Changing a Pipette Reel		
Exercise 4: Viewing Plate Details		
Recovery procedures		
Contacting Customer Support		

I certify that the above named trainee has completed the Operator Training course.

Trainer's signature:

11 APPENDIX C: HISTORY

Revision	Date	Comments
Draft A	22 January 2004	CEM First issue of document
Draft B	5 March 2004	SRC Added tip changing option "Never"
Draft C	6 October 2004	SRC Updated for software version 2.5.1.42
Draft D	22 November 2004	SRC Updated for software version 2.5.1.44
Draft E	21 January 2005	SRC Updated for software version 2.5.1.45
Draft F	12 May 2005	SRC Updated for software version 2.6.1.47
Draft G	17 June 2005	ESJT Updated for software version 2.6.1.48
Draft H	29 September 2005	ESJT Updated for software version 2.7.49
Draft I	8 March 2006	SRC Updated for software versions 2.7.50 & 2.7.51
Draft J	19 October 2006	PGS Updated for software version 2.8.52
Draft K	20 April 2007	SRC Updated for software version 2.8.55
Draft L	1 May 2007	SRC Updated for software version 2.8.56
Draft M	9 October 2007	PGS Updated for software version 2.8.57
Draft N	25 September 2008	PJB Updated for software version 3.0.0
Draft O	12 March 2009	PJB Updated for software version 3.0.1
Draft P	21 July 2009	PJB Updated for software version 3.2.0