

QSoft Utilities

User Guide



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QSoft Imaging

Introduction

QSoft Imaging allows the camera operations to be tested without having to run any other QSoft application.

Run QSoft Imaging

To run QSoft Imaging - double-click the QSoft Imaging icon on the Windows® desktop.



Figure 1: QSoft Imaging Icon

If the icon is not present on the Windows desktop, it can be located from the **Start** menu under **Programs\Genetix**.

The screen below will be displayed.

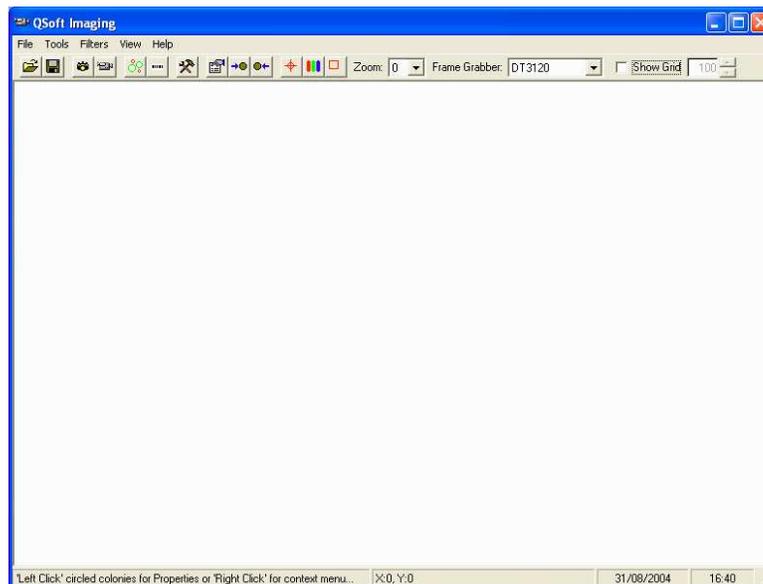


Figure 2: QSoft Imaging Screen

NOTE - Before using QSoft Imaging, select the correct frame grabber from the drop down list on the toolbar.

If an error message appears whilst trying to take a picture it is probably because the wrong frame grabber has been selected – check your hardware configuration or contact Genetix Customer Support.

If unsure about which frame grabber is installed in your PC, check this as follows:

- Run another QSoft application (such as Picking).
- From the **View** menu select **Configuration**.



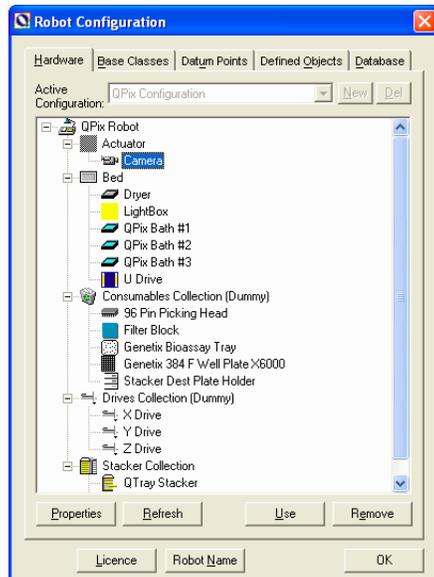


Figure 3: Hardware Configuration

- From the **Hardware** tab highlight **Camera** and click the **Properties** button.
- Scroll down the list of camera properties to the value alongside the **FrameGrabber** property.

The Menu Options

QSoft Imaging contains drop down menus that can be accessed by pointing with the mouse and clicking the left mouse button.

File Menu

The File menu contains facilities for file operations

Open – Allows an existing image file to be opened. A standard Windows file-open dialog will appear where the location and name of the image file can be specified.

Save As – Allows an image file to be saved. A standard Windows file-open dialog will appear where the location and name of the image file to be saved must be specified.

Tools Menu

Picture – The camera will take a still image.

Live Video – The camera will be put into live mode. The purpose of this is so that you can adjust the lens aperture and focus.

Locate Colonies – The image will be processed and good colonies will be outlined in green.

Note: Green is the default color for a selected colony. The color can be changed by selecting Colors from the View menu.



Filters

The options here allow manipulation of the image in order to improve colony finding.

If any of the filters are applied, 'Locate Colonies' will need to be selected to update the image.

View Menu

Tools

This option will display a dialog that allows the selection criteria and/or threshold values to be refined.

Criteria

These variables define the size, proximity and shape of the colonies that will be picked.

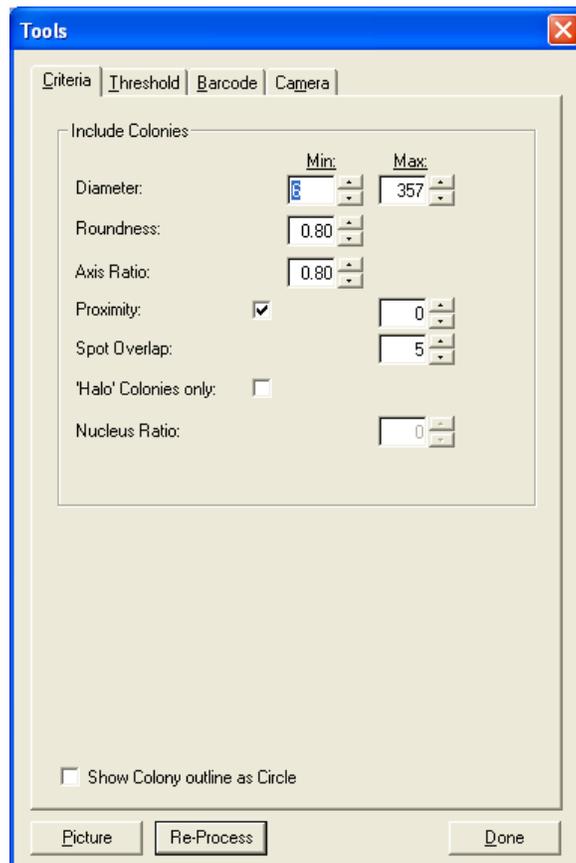


Figure 4: Tools Criteria

Diameter – Set the minimum and maximum area (in pixels) of the colonies.

Roundness – Set the minimum roundness of the colonies. This is a ratio of the circumference of a perfect circle (taken from the measured radius of the colony) divided by the actual circumference of the colony. For a perfect circle, the value would be 1.00. A typical value is between 0.80 and 0.95. Only colonies with a roundness greater than that set will be picked.



Axis Ratio – The axis ratio is found by dividing the shortest axis by the longest axis. For a perfect circle (or square) the value would be 1.00. Typical values would be between 0.60 and 0.90. Only colonies with an axis ratio greater than the set value will be picked.

Proximity – The facility has two settings, the first is a checkbox to turn it on or off, and the second allows the setting (in pixels) to be varied, when enabled.

If the distance between the outer edges of two colonies (based on them being perfect circles, derived from their maximum radii), is smaller than the value set for the proximity detection, they will both be rejected as being too close.

Spot Overlap – Because the total image of the tray is made up of 35 frames, in order not to miss any colonies, the frames have to overlap by a small amount.

There may be occasions when an individual colony occupies one of these overlap areas, and so could be identified more than once. If it were present on the very corner of a frame, it might be "seen" in up to three other frames, making a total of four identified "good" colonies. However, the software is designed to recognize instances such as these and pick multiple identified colonies only once. There are however slight problems with exactly matching a colony seen in one frame to the same colony seen on an adjoining frame, so this setting allows for a certain amount of mismatch between the frames. Typically this is set to about 20 pixels.

Halo Colonies Only – If this option is checked, only halo colonies will be picked.

Nucleus Ratio – If halo picking is enabled, the nucleus ratio may be used to set the percentage ratio between colony and halo area. Below this ratio, a colony will be rejected.

Threshold

A Threshold value is a grey value, between 0 and 255 (0 being the value for black and 255 for white) which enables the software to distinguish between the background and foreground features of an image. To distinguish between agar and colonies, the range of grey levels that represent the agar (background) should be excluded from the image. The range of grey levels that represent the colonies should be higher than the threshold range.

Where the image data is uncomplicated, it is advisable to use the automatic thresholding. This is quicker and simpler to set up.

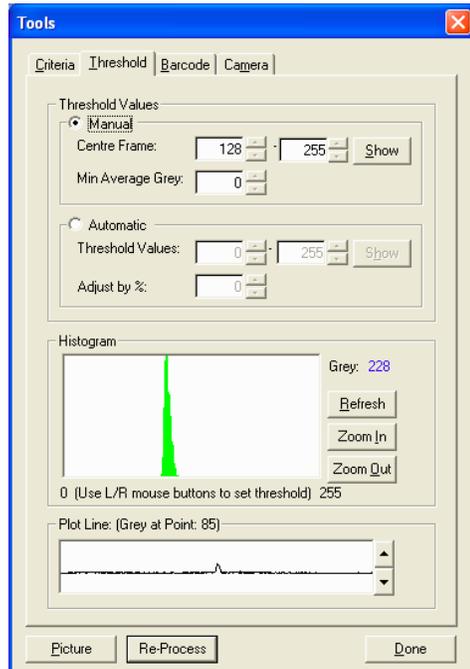


Figure 5: Tools Threshold



Manual

Center Frame - These two values are used to set the minimum and maximum threshold for the background at the center of the **QTray**, which is to be picked. The lower and upper limits for center frame threshold define a range of pixel grey levels that will be considered “background” or agar.

For normal “White” or “Blue” colony picking, the lower threshold must be set to zero, and the upper threshold should be varied to correctly exclude background pixels. Typically, the upper threshold will be a value between 160 and 220. Its limit is 255.

For “phage” picking, the lower center frame threshold should be varied, and the upper threshold should be set to 255.

Automatic

Threshold Values – Software-calculated threshold values based on each frame.

Adjust by % - A global parameter which allows the automatic threshold to be fine-tuned. This parameter allows the upper calculated automatic threshold value to be sharpened or softened, the typical range is +10% to -10%.

Show – When this button is clicked the screen will differentiate between the thresholds by turning the background to black.

Now, the threshold values can be “fine-tuned”. The image screen now has a horizontal red line running across the center of the image. By adjusting the values for **Center Frame**, the threshold values on the screen will change.

When picking **White** or **Blue/White** colonies, the ideal is to have a black background and white colonies, with good definition between the two.

Once the thresholds have been set click on the **Picture** button to ensure that the new threshold values are correct. The robot will take a new picture and display the results on the screen. Colonies circled in green are “good” (if default colors are set) and those circled in red are “bad” (assuming default colors are used). Review these results; if they are acceptable continue by clicking the OK button, if not repeat the thresholding process.

Histogram

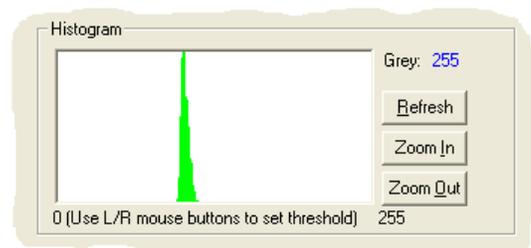


Figure 6: Threshold - Histogram

The Histogram shows the volume of grey pixels in the image by value. The left of the Histogram represents black pixels whilst the right of the histogram represents white pixels.

Typically you would expect to see one large peak in the histogram, which represents the grey scale of the agar in the image.

If there is a large population of colonies you may see a second peak in the histogram, which represents the grey scale of the colony data.

The histogram can be used for setting the Manual Threshold parameters. Click, using the left mouse, in the Histogram to set the Min threshold level and click, using the right mouse button, to set the Max threshold level.

For typical E-coli plates the Min threshold should be set to zero (0) and the Max threshold can be set by Right clicking just to the right of the first major peak in the Histogram.

Refresh – Occasionally, when re-taking images in the Test Image screen the histogram data may not update the Histogram window. In this case clicking the Refresh option button will correct this.

Zoom in/Zoom out – If there is little color contrast between the Colonies and the Agar the Histogram data will appear very flat and no obvious peaks are displayed. In this instance, use the Zoom In/Out options to alter the scale of the graph to better identify the peaks.

Plot Line

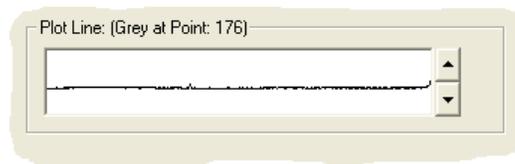


Figure 7: Threshold - Plot Line

The graph in the Plot Line window represents the red line that pans up and down the image window. Click on the scroll buttons to move the red line and as it passes over colonies peaks will appear which describe the intensity of each colony.

Barcode

This tab is displayed if barcodes are read using the camera.

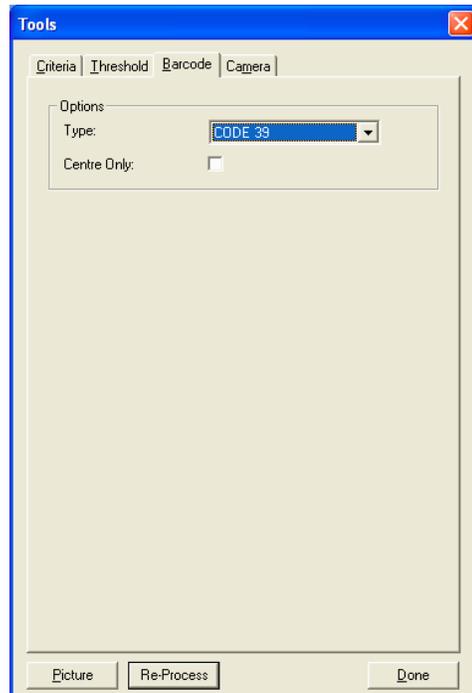


Figure 8: Tools Barcode

Type – Select the barcode type from the drop-down list.

Center Only – Where more than one barcode may be present check this option to ensure that the one nearest to the center is read.

Camera

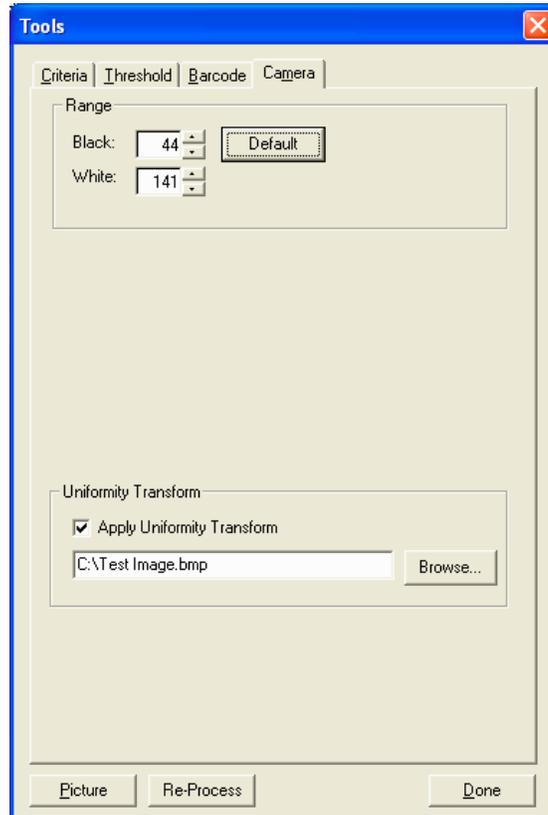


Figure 9: Tools Camera

Set the required brightness and contrast values here – the default values are determined by which frame grabber is in use.

Apply Uniformity Transform – This is used for correcting certain anomalies in images taken by a digital camera.

Properties

Displays information about the currently selected colony – see example below.

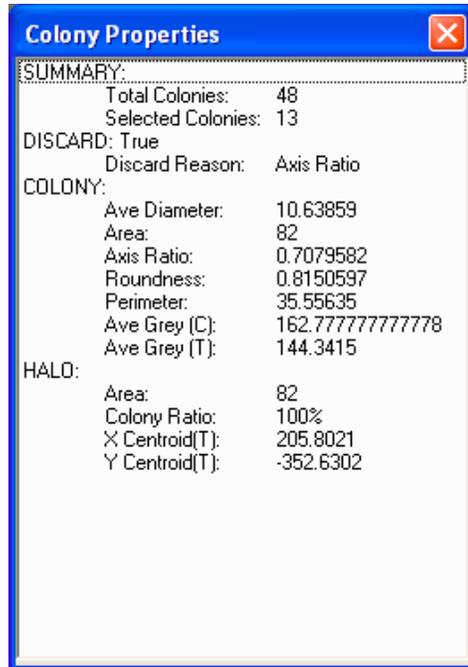


Figure 10: Colony Properties

Crosshair

The red crosshair symbol represents the center of the camera. Jog the camera by moving the drives by hand to improve accuracy.

Colors

Colonies are outlined according to a color code associated with the discard reason. Select this option to change these colors.

Toolbar

Displays or hides the toolbar.

Status Bar

Displays or hides the status bar.

Other Toolbar Options

Define ROI – Allows an area that represents valid background data to be defined. Use this option when an image contains obstacles such as the edge of a Petri dish or bioassay tray. For detailed information about Threshold Region of Interest, see the Picking manual.

Zoom - The zoom control allows digital magnification of the image.

Grid – Checking this box displays a grid over the image which can be helpful when aligning the camera/CCD in Test Image mode. The grid size can be altered (in steps of 10 pixels) as required using the up and down arrows.

QSoft IO Manager

Introduction

This utility allows all of the mechanical inputs and outputs on the robot to be viewed and to switch outputs on or off as required.

Run QSoft IO Manager

When the IO Manager utility is installed, this icon should be displayed on the Windows desktop.



Figure 11: QSoft IO Manager Icon

Double-click the QSoft IO Manager icon.

If the icon is not present on the Windows desktop, it can be located from the **Start** menu under **Programs\Genetix**.

The first time the utility is run a series of prompts will appear to determine which robot you are using.



Figure 12: Robot Type Message

When the prompt shows the robot type, click the **Yes** button.

If **No** is the answer to all of the prompts, the message below will be displayed.



Figure 13: Robot Not Selected Message

Click the **Yes** button to cycle through the robot types again or click **No** to exit QSoft IO Manager without selecting a robot type.

The QSoft IO Manager screen will look similar to the one shown below.

The names of each output and input are listed alongside the I/O address.

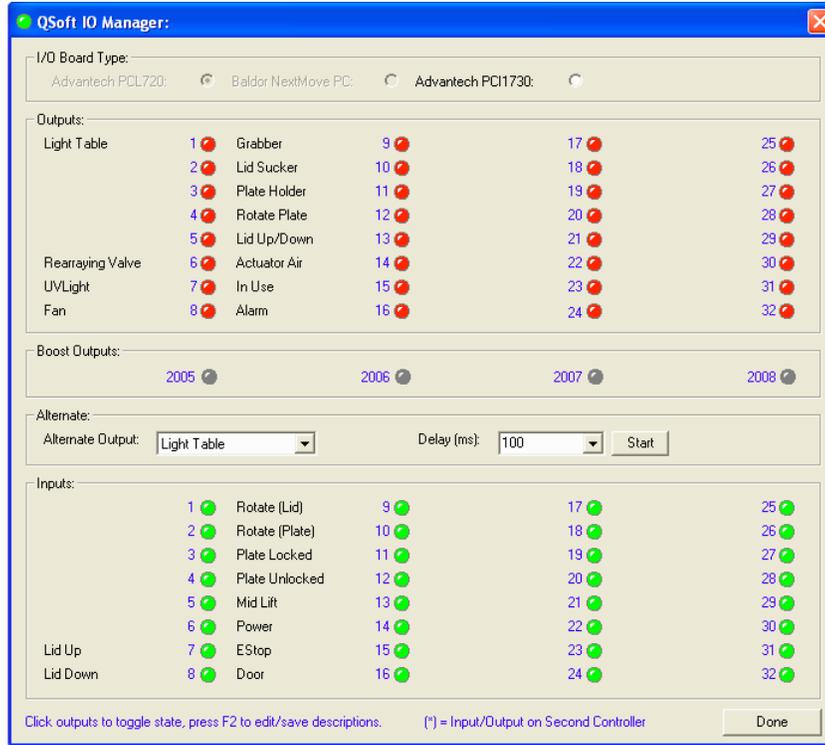


Figure 14: QSoft IO Manager

I/O Board Type – To determine which board(s) are installed on your PC:

- Run another QSoft application (such as Picking).
- From the **View** menu select **Configuration**.



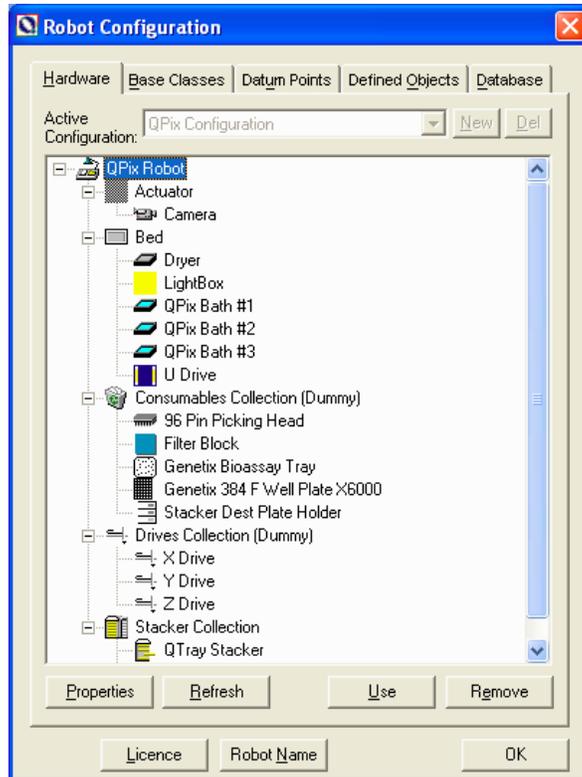


Figure 15: Hardware Configuration

- From the Hardware tab highlight the Robot name and click the Properties button.
- Scroll down the list of camera properties to the value alongside the IOController property.

Note: A second IOController might be installed if the robot has stackers or additional hardware options. The property name for this is IOController2.

Outputs – You can control the outputs by clicking the required indicator light. The indicator light shows red when the I/O is **off** and green when the I/O is **on**.

Boost Outputs – These outputs are for calibration purposes and only to be used by a Genetix engineer.

Alternate – Set options in this section in order to alternate the state of an output between on and off.

Alternate Output – Select from the list which output is to be tested.

Delay (ms) – Select the time delay from the drop-down list. The output will remain in each state (on and off) for the specified time (100 ms – 1 second)

Start/Stop – Click the Start button, the output will alternate between on and off states. Click stop to end test.

Inputs - You cannot control the inputs, these are for information only.

The labels can be edited if necessary. Press the **F2** key, text windows will open for each label. Press **F2** again to save the edited descriptions.

QSoft Restacker

Introduction

The QSoft Restacker utility allows the WellPlate Stackers and/or the QTray stackers (if fitted) to be operated without having to run any other QSoft application.

This can be used for re-ordering plates after running another QSoft application or for reading barcodes on plates and trays in preparation for an application run.

QSoft Restacker will send plates from the feed stack to the robot bed, read the barcodes (if this option has been selected) and then, after a short pause, each plate is sent to the return stack.

When all plates have been restacked QTrays are fed out on to the bed one at a time, the barcodes are read (if this option has been selected), each tray is then returned and the stack is positioned ready for the next pair of trays to be loaded.

ReStacker Software

When the ReStacker utility is installed, this icon should be displayed on the Windows desktop.



Figure 16: QSoft Restacker Icon

Double-click the ReStacker icon.

If the icon is not present on the Windows desktop, it can be located from the **Start** menu under **Programs\Genetix**.

Configuration and Diagnostics

These buttons are described in detail in the robot manual.

WellPlate Stacker

QSoft will check how many plate stackers are configured for the robot and how many stacks are on each stacker. Each stack is represented on the form by a check box.



Figure 17: WellPlate Stacker Options

WellPlateStack *n* - Check the box alongside each stack to be restacked. Stack 1 is always nearest the front of the robot.

Number of Plates Loaded – Enter the number of plates contained in the selected stack.

Copy – If all stacks to be restacked contain the same number of plates, click the **Copy** button to copy the settings from the first stack to the other stacks.

Plate – Ensure that the correct plate is selected from the drop-down list.

Import – If Read Barcodes (below) is checked, Import will display a dialog to allow you to locate a barcode list text file.

Read Barcodes – If this box is checked the barcodes buttons of selected stacks are enabled.

Barcodes – Click this button to enter barcodes for the current stack or to display a list of barcodes allocated to the current stack.



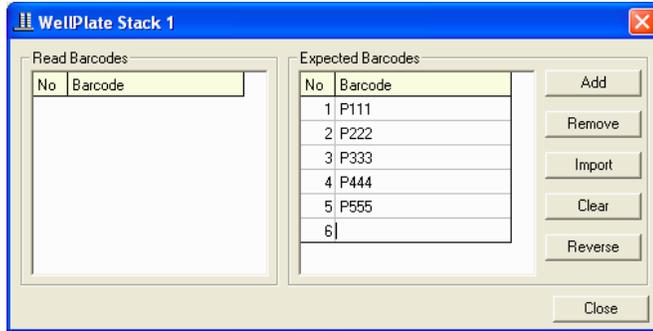


Figure 18: WellPlate Stack Barcode Dialog

Add – Presents a new blank line to type in the next barcode.

Remove – Will delete the selected barcode.

Import – Will display a dialog allowing a barcode list text file to be located.

Clear – Will clear all barcodes in the current list.

Reverse – Will reverse the order of the barcode list.

QTray Stacker

QSoft will check whether a tray stacker is configured for the robot. QTray stackers always have two stacks. Each stack is represented on the form by a check box.

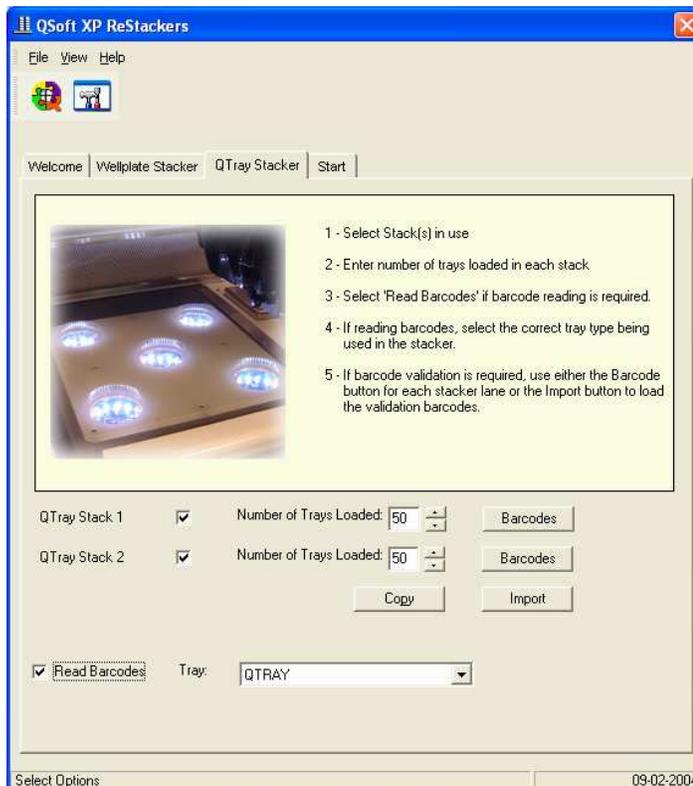


Figure 19: QTray Stacker Options



QTray Stack *n* - Check the box alongside each stack to be restacked. Stack 1 is always on the right-hand side when facing the front of the robot.

Number of Trays Loaded – Enter the number of trays contained in the selected stack.

Copy – If all stacks to be restacked contain the same number of plates, click the **Copy** button to copy the settings from the first stack to the other stacks.

Tray – Ensure that the correct tray is selected from the drop-down list.

Import – If Read Barcodes (below) is checked, Import will display a dialog to allow you to locate a barcode list text file.

Read Barcodes – If this box is checked the barcodes buttons of selected stacks are enabled.

Barcodes – Click this button to enter barcodes for the current stack or to display a list of barcodes allocated to the current stack.

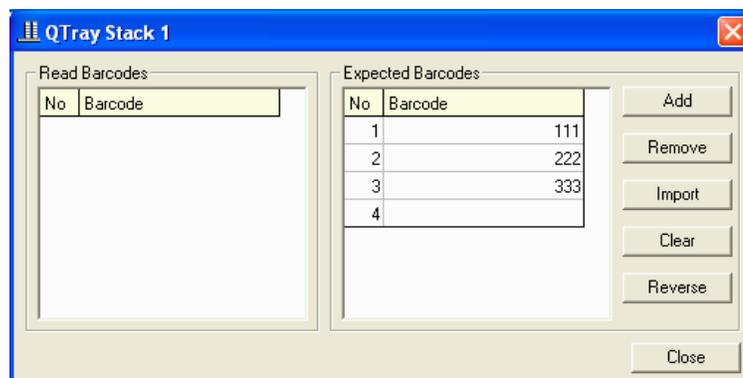


Figure 20: QTray Stack Barcode Dialog

Add – Presents a new blank line to type in the next barcode.

Remove – Will delete the selected barcode.

Import – Will display a dialog allowing a barcode list text file to be located.

Clear – Will clear all barcodes in the current list.

Reverse – Will reverse the order of the barcode list.

Start

When all the required options have been set click the **Start** button.



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