



A DIVISION OF SYNOPTICS LTD

ChromaZona Software User Guide

NOTICE TO USERS

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SAFETY INSTRUCTIONS

Safety Practices




Safety advice given in this manual is intended to supplement, not supersede, the normal safety codes in the User's country. The information provided does not cover every safety procedure that should be followed. Ultimately, maintenance of a safe laboratory environment is the responsibility of the User and the User's organisation.

Please consult all documentation supplied with the instrument the software is to run on / control before starting to work with the instrument. Carefully read the safety information in this document and in the other documentation supplied. When setting up the instrument or performing analysis or maintenance procedures, strictly follow the instructions provided.

Warning Notices

Within this User Guide WARNINGS are used to highlight information or instructions that **must** be followed in order to avoid personal injury to yourself or other people in the vicinity, e.g. switch off the mains voltage and remove the mains cord before cleaning.

WARNINGS appear as below:

 WARNING	Switch off the mains voltage and remove the mains cord before cleaning.
 WARNING	Ensure that all instrument Users read and understand the precautions listed below.
 WARNING	You are advised to post a copy of the precautions near to or on the instrument itself.

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OVERVIEW

The ChromaZona Software

ChromaZona is a software application which when used with the correct hardware instrument provides a combined system for automatically measuring antibiotic susceptibility, inhibition zones, MIC point and detecting colonies. The ChromaZona application runs on a Windows computer and is the means of operating the hardware - the instrument.

ChromaZona antibiotic susceptibility settings and measurements are calculated using EUCAST or CLSI guidelines.

ChromaZona Administrators can control which features of the program are available for different groups of Users, so some of the features described in this Manual will only be available to you if you have been granted access to them.

Once the combined system has been set up, all that has to be done to measure zones or count colonies on a series of plates is to put each plate in turn on the plate holder within the instrument and press measure. The measurement of each Plate takes no more than a few seconds and the results are shown instantly in a table.

ChromaZona allows Plate identifiers and dilutions to be entered manually, with an auto-incrementing plate identifier, or the entering of lists of plate identifiers and dilution series from a comma separated value (CSV) file or a Laboratory Information Management System (LIMS). If auto-incrementing Plate identifiers or a Plate list are used, ChromaZona will prompt the User for which Plate to insert next. The Plate identifiers and dilutions are then automatically assigned to the results and recorded. Plate identifiers and dilutions can also be read into ChromaZona using a Barcode Reader.

When measuring zones or MIC point, ChromaZona enables the user to:

- Accurately and quickly measure zone sizes or MIC point by applying sophisticated algorithms to measure the zones or bacterial gradient automatically
- The software makes appropriate allowance for any discs or wells present
- Automatically detects Mic Strips present on plates detailing type of MIC strip and ladder values

When detecting colonies, ChromaZona enables the User to:

- Distinguish between different types of colony according to their colour, size and/or shape, and to distinguish between colonies and debris or other artefacts.
- Apply sophisticated algorithms to distinguish touching colonies.
- Add or remove colonies manually after an automatic count - a completely manual count can also be performed, if required.
- Define 'exclude regions' on the Plate if there are any problem areas on an individual Plate where colonies cannot be distinguished for some reason - the missing area is then automatically allowed for in calculating the total count for the Plate.

All counting and measurement results are displayed and saved automatically, and any manual changes made to a result are marked against the individual result with comments and coded flags. By default, an image of the Plate is automatically saved with each result, and this can be reloaded at a later time for auditing. A full audit history can be viewed for any changes made to a result.

Once the User has completed the counts / measurements, a report can be compiled showing the results.

ChromaZona uses 'batches' to define the settings used to detect colonies or measure zones on Plates and to store the results of the measurements made using those settings. This means that before a User can use ChromaZona to take any measurements, there must be a batch open. This is done by opening an existing batch or by creating a new batch. A User can create a completely new batch or base a new batch on an existing one, either to use the same settings, or as a starting point for defining new settings. ChromaZona automatically saves results in the current batch, so once a batch design has been created and accepted, a User does not need to take any action to save it again.

Each batch contains the settings and results for all measurements within a batch.

ChromaZona Software Operating Environment

The ChromaZona software runs in a standard Windows environment and has an extensive array of features and can cover a wide range of applications. Zone measurement and colony counting are provided as standard. Other features include:

- All functions are accessed from a single window for each step
- Automatic imaging functions ensure perfect images without the need for any optimisation of contrast and sensitivity
- Automatic separation of touching colonies is included
- An advanced grid elimination function is included for filters and PETRIFILM™ plates
- Colonies can be marked with a choice of shapes, including cross, circle and square, for clear and precise visualisation
- An advanced polygonal exclusion tool is available to eliminate specific areas
- The system can be 'trained' to exclude writing on plates, debris, bubbles or other unwanted features
- Full editing functions for the adding or deleting of colonies and the manual measurement of zones are included
- Colonies as small as 0.043 mm can be detected, while zones can be measured accurately to 0.5 mm, with a theoretical detection limit of 0.1 mm
- A built-in database enables the sharing of results across individuals, laboratories and even facilities
- All results are automatically archived and can be printed
- All results are fully traceable with a full audit trail
- Compliant with the requirements of 21 CFR Part 11

ChromaZona Applications

As standard the ChromaZona application can process the following types of Plate:

- Antibiotic Susceptibility
- Chromogenic Media
- Inhibition Zone
- MIC Strip

Additional software modules can be added to the basic ChromaZona application to enable processing of the following types of Plate:

- Dilution Series
- Multi-sector Plate
- Multi-well
- OPKA
- Pour Plate
- SBA
- Spiral Dilution Series
- Spiral Plate

SOFTWARE INSTALLATION & USER ACCESS

Minimum System Requirements

The ChromaZona application can operate on the following Microsoft Windows Operating Systems:

Windows XP Professional SP3 (32 bit version only)
or
Windows 7 Professional

Windows 8

Windows 10

Note: Home versions of the Windows Operating Systems are not supported.

Software Installation

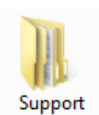
To install the ChromaZona application onto a PC:

1. Log onto the PC as an Administrator and close all running software
2. Insert the Synbiosis USB flash drive into a USB port on the PC
3. Open Windows Explorer and navigate to the Synbiosis USB drive
4. Open the ChromaZona (version) folder and run the Setup.exe program

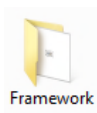
If the PC is running Windows XP, Windows Vista or Windows 7, the PC may require updating. If the following message is displayed; "This application needs Microsoft .Net Framework 4.0 full version which is not yet installed. This can be found in the Support directory.", then follow step 5., otherwise proceed to step 6.

5. Install Microsoft .Net Framework 4.0.

- (i) Open the **Support** folder



- (ii) Open the **Framework** folder



- (iii) Run SetupFramework.exe . Follow the on-screen instructions, performing a system restart if prompted.



- (iv) Run Setup.exe again and follow the on-screen instructions.



The ChromaZona application utilises Microsoft SQL Server. If this is not already present on the PC, the following message will be displayed; "This application requires SQL Server Express or SQL Express LocalDB, neither of which is yet installed on this computer. Do you wish to continue with the installation of ChromaZona software (Press OK) or cancel and install a database engine?". Press cancel to Install the server information or OK to continue with the ChromaZona software installation.

6. Install SQL Server.

- (i) Open the **Support** folder 

- (ii) Open the **Database** folder 

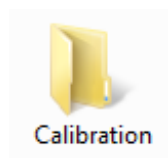
- (iii) Run SetupP3SqlServer.exe  and perform a PC restart on completion

Note: SQL LocalDB Express is designed for a single PC user. A single user should log onto the PC and, if there is more than one user running the ChromaZona software, they should enter their details within the ChromaZona software. **If more than one user is going to log onto Windows on the PC running ChromaZona software, you should install, and use, SQL Server Express.**

7. Once the SQL server has been installed Run the Setup.exe file again and follow the on-screen instructions (located on the flash drive)



8. Run Configuration Installation



- (i) Open calibration folder
(ii) Double click on Setup Calibration
(iii) Follow the onscreen instructions

Adding Users to ChromaZona

When ChromaZona SQL server is installed, user groups are created called ChromaZona Users. Every user of the software needs to be a member of the ChromaZona User group. For differing levels of access there are also advanced user and admin groups. Users must be added to the SQL server within the administrator account on the PC.

Adding users to SQL Server:

- (i) Access the computer control panel and select 'System and Security' then select 'Administrative Tools'
- (ii) Select 'Computer Management' by double clicking
- (iii) Within computer management locate Local Users and Groups from the left hand menu and click on it. Two sub menus of Users and Groups will appear
- (iv) Select Groups
- (v) Within the Groups menu three ChromaZona user groups are present:
 - a. ChromaZona Users
 - b. ChromaZona Advanced Users
 - c. ChromaZona Admins
- (vi) Double click on the ChromaZona Users group to open the ChromaZona User Properties window
- (vii) Select add to open the Select Users screen
- (viii) To add users enter their windows log in name and click check names
- (ix) Once all users have been added to this group click OK
- (x) Follow the same procedure to add users to the ChromaZona Advanced User and Admins Group

ACCESSING & EXITING THE SOFTWARE

Starting and Logging On to the ChromaZona Application

To start up and use ChromaZona:

1. Switch on and log on to Windows on your PC as normal
2. Switch on the instrument connected to your PC



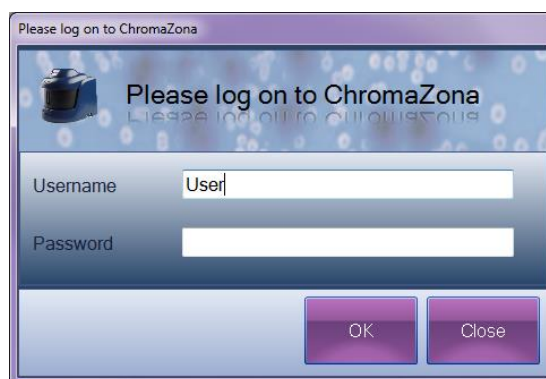
Select the  icon on the Desktop to start the ChromaZona application.

The ChromaZona application will launch and a Loading pop-up will be displayed:



LOADING POP-UP

Once the ChromaZona application has loaded the Log on dialogue box will appear.



LOG ON DIALOGUE BOX

Note: This dialogue box is also displayed when you log out from ChromaZona or if you do not perform an action within a pre-set period of time (default setting is 5 minutes). To continue using ChromaZona log on again.

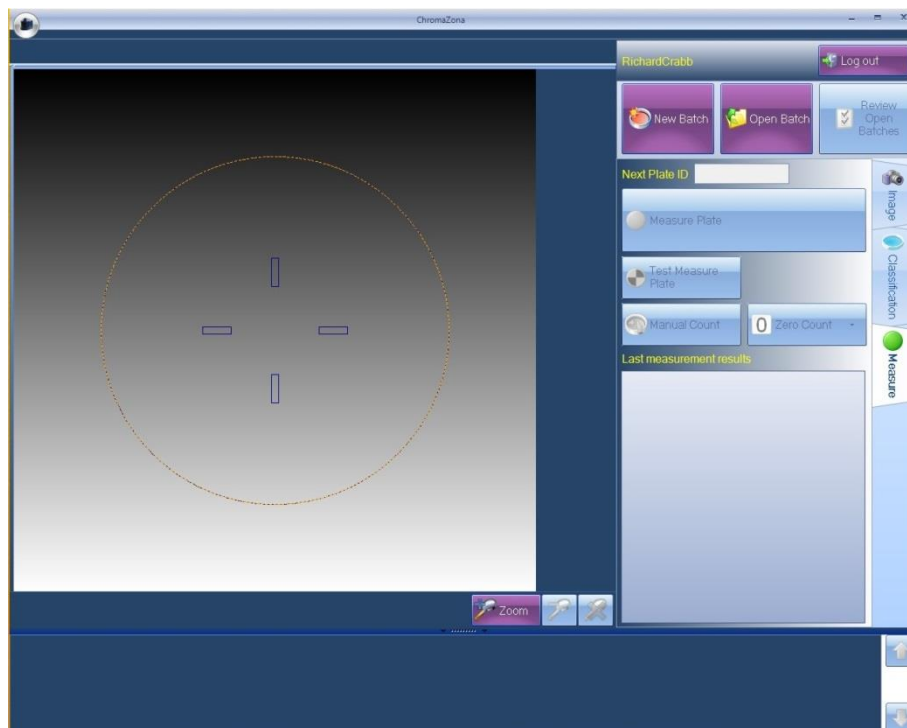
Your Username will be populated automatically from your Windows logon. Enter a valid Password, this should be the same as your Windows password.

ChromaZona checks that the Username / Password entered is a valid combination to log on to Windows on the PC, but does not check that it is the currently logged on User. This means that you can log on to ChromaZona while someone else is logged on to Windows on the PC.

Note: The username is the user windows account username and the password is the users window account password.

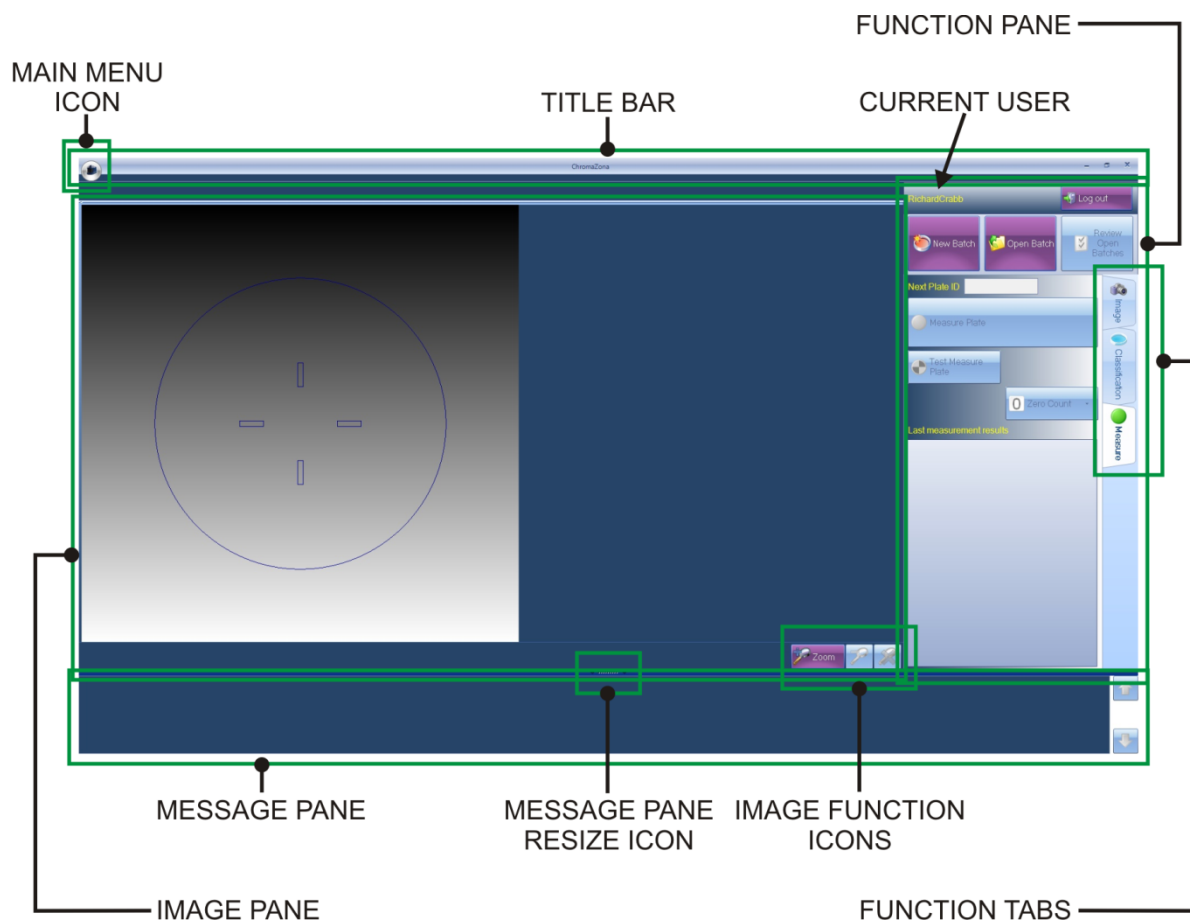
Select **OK**.

Once the ChromaZona application has started up the **Home** screen will appear.




ChromaZona HOME SCREEN

ChromaZona Home Screen Layout



ChromaZona HOME SCREEN LAYOUT

TITLE BAR

Displays the main menu icon,  icon. Used to access the main menu. On other screens within the program this button is used to return you to the Home screen.


Also displays the standard Windows program buttons , for minimise, maximise, and close.

IMAGE PANE

By default displays a target image or the live image.

In the bottom right hand corner of the Image Pane the currently available image function icons are displayed, e.g.



FUNCTION PANE

Buttons for selecting the various default main actions or functions that are available to the User from the current screen. These change depending on which function tab is selected. It also contains the name of the currently logged in User.

Function tabs are:

Image tab



Classification tab



Measure tab



Results tab



Configuration tab




Each tab has an associated set of User selectable buttons. The available selectable buttons change with options selected.

The function pane also displays brief sample results.


MESSAGE PANE

Displays input fields for inputting a Batch ID for a new Batch. Also displays the main sample results.

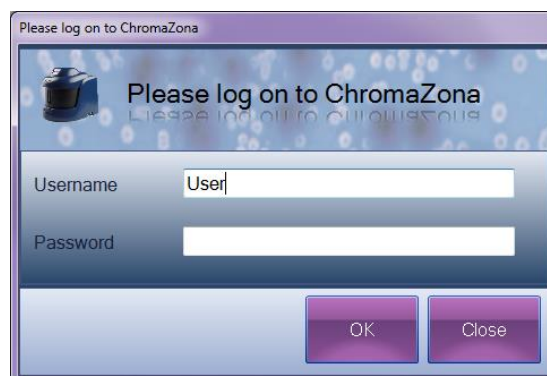
The size of the message pane can be increased / decreased using the draggable message pane resize icon .

Logging Out from the ChromaZona Application

To log out and exit from the ChromaZona application:

1. Select the **Log out** button  near the top right-hand corner of the ChromaZona application window.

If user authentication is enabled, the Log on dialogue box will appear.



LOG ON DIALOGUE BOX

2. Select the **Close** button.

The ChromaZona application closes, returning you to the Windows Desktop.

Note: This dialogue box is also displayed if you do not perform an action within a pre-set period of time (default setting is 5 minutes). To continue using ChromaZona, log on again.

Exiting the ChromaZona Application Without Logging Out

To exit the ChromaZona application without logging out:

1. Select the main menu icon  to display the Main Menu.



ChromaZona MAIN MENU

2. Select the **Exit ChromaZona...** button.

The ChromaZona application closes, returning you to the Windows Desktop.

MAIN MENU SETTINGS

From the Home screen, select the Main Menu icon  to display the Main Menu.

The ChromaZona Main Menu is displayed.

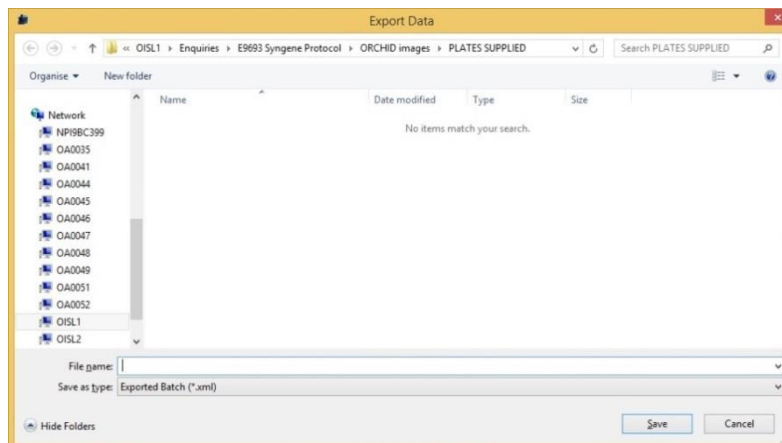


ChromaZona MAIN MENU

Exporting Data

With a Batch open, select the **Export Data as XML...** button.

The **Export Data** window is displayed.



EXPORT DATA WINDOW

- Enter a filename for the data file, in the File name field.
- Select a file format.
- Navigate to and specify a location for the file to be saved to.

Settings

Select the **Settings** button.

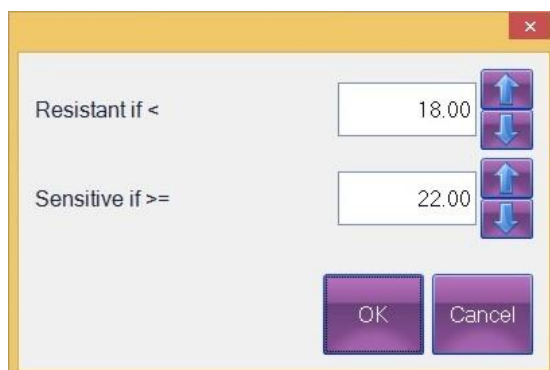
The **Settings** sub-menu is displayed.



SETTINGS SUB-MENU

Edit Default Antibiotic Susceptibility

The **Antibiotic Susceptibility Sensitivity** pop-up is displayed.



ANTIBIOTIC SUSCEPTIBILITY SENSITIVITY POP-UP

- Use the two controls to adjust the range of acceptable susceptibility markers.
- Adjust the values using the up / down arrows or enter values directly.

Enable Apply AST to all Plates button

This function is linked to the Antibiotic susceptibility application (please see page 90). When measurement settings are applied to a batch a tick box will appear asking if these settings are to be applied to all plates. If this tick box is checked it will not be shown again and further measurements will have these settings applied. If the user wishes to adjust these setting then Clicking **Disable Apply AST to all Plates** will result in the measurement settings becoming visible when further measurements occur for adjustment.





System Settings

The **Settings** pop-up is displayed.

A screenshot of the 'Settings' pop-up window. It has a yellow title bar with 'Settings' and a close button. The window contains several settings: 'Company Name' (text field with 'Synbiosis Ltd'), 'Save Images to' (drop-down menu), 'Image Path' (text field with 'C:\ProgramData\Synbiosis\ChromaZon' and a folder icon button), 'Export Path' (text field with 'C:\ProgramData\Synbiosis\ChromaZon' and a folder icon button), 'Min. Audit Reason Length' (text field with '3' and up/down arrow buttons), 'Authentication Method' (drop-down menu with 'Local User Groups'), 'Time Out (Minutes)' (text field with '20' and up/down arrow buttons), 'Display Exponential Numb...' (checkbox, checked), 'Flip Scanner Image' (checkbox, checked), and 'Show X plates (0 = All)' (text field with '50' and up/down arrow buttons). At the bottom are three buttons: 'Reset' (blue with a circular arrow), 'Save' (green with a checkmark), and 'Cancel' (red with an X).

SETTINGS POP-UP

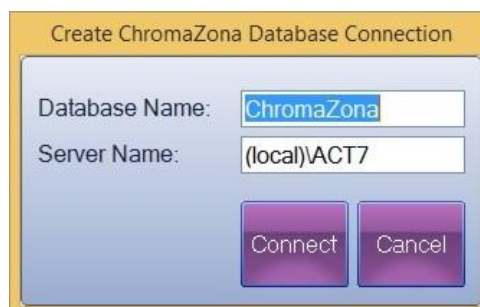
- In the **Company Name** field enter the name of your company or organisation. This will be used in reports generated by ChromaZona.
- In the **Save Images to** field use the drop-down to select whether or not to save files when you make a measurement. The options are:
 - Save images to
 - Do not save images
- In the **Image Path** field accept the default, as installed, location for saving images to, or enter a new location, either directly or select the  button to open a standard Windows browser.

- In the **Export Path** field accept the default, as installed, location for saving batch details to, or enter a new location, either directly or select the  button to open a standard Windows browser
- In the **Min. Audit Reason Length** field set the minimum number of characters that must be entered when giving a reason for a change. Adjust the value using the up / down arrows or enter a value directly
- In the **Authentication Method** field select the required Authentication Method from the drop-down list. The options are:
 - **None**
In this case there is no access control and anybody will be able to access and use the ChromaZona application without having to log on to the application.
Audit trails will record that changes were made by the User logged in to Windows at the time the changes were made.
 - **Windows**
In this case Users have to log on to ChromaZona using their Windows user name and password.
All Users will have access to all ChromaZona functions.
Audit trails will record that changes were made by the User who was logged in to ChromaZona at the time the changes were made.
User names and passwords for all Users who are going to use the ChromaZona application must be added to Windows.
 - **Local User Groups**
In this case Users must be members of a ChromaZona Local User Group on the PC.
Users have to log on to ChromaZona using their Windows user name and password.
Members of different ChromaZona groups can be given different access rights to some ChromaZona functions.
Users may be required to enter reasons for changes they make to Batches or results.
Audit trails will record which ChromaZona User made any changes.
User names and passwords for all Users who are going to use the ChromaZona installation must be added to Windows.
User groups must be defined on the PC.
Permissions for each User group must be defined.
 - **Network User Groups**
As for Local User Groups except that User groups are defined at the network level.
- In the **Time Out (Minutes)** field set a time out period, in minutes. When in use, if the application is not used for the time period set the current User will be logged out and the **Please log on to ChromaZona** dialogue box will be displayed. In order to use the application either the former current User, or another User, must log on. Type a number directly into the field or use the arrow buttons to increase / decrease the number
 - If the **Authentication Method** field is set to **None** the **Time Out (Machine)** function is disabled

- If the **Authentication Method** field is set to **Windows, Local User Groups** or **Network User Groups** the **Time Out (Machine)** function is enabled
- If the **Authentication Method** field is set to **Windows, Local User Groups** or **Network User Groups** and the **Time Out (Minutes)** function is not required, set the **Time Out (Minutes)** time to 0
- Check the **Display Exponential Numb...** checkbox to display results in exponential format
- Check the **Flip Scanner Image** checkbox to flip displayed scanner images from left to right. This corrects for the fact that Plates are typically scanned from below and the display is therefore left to right inverted
- In the **Show X plates (0 = All)** field choose how many Plates to include in the Results Table. Type the number directly into the field or use the arrow buttons to increase / decrease the number
- Select the **Reset** button to reset all settings to the values they had when the dialogue box opened, but leaving the box open
- Select the **Save** button to save the entered settings
- Select the **Cancel** button to close the dialogue box without saving any changes made

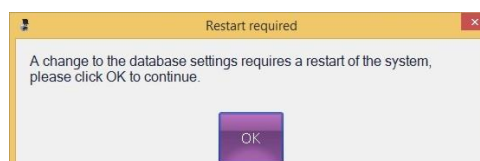
Edit Database Location

The **Create ChromaZona Database Connection** pop-up is displayed.



CREATE ChromaZona DATABASE CONNECTION POP-UP

- In the **Database Name:** field enter the name of the database you want to edit.
- In the **Server Name:** field enter the new location name for the database
- Select the **Connect** button to make the changes
- The **Restart required** pop-up appears. This requires you to select the **OK** button to proceed. The application will shut itself down, restart, and you will be required to log on again



RESTART REQUIRED POP-UP

- Select the **Cancel** button to cancel the database changes

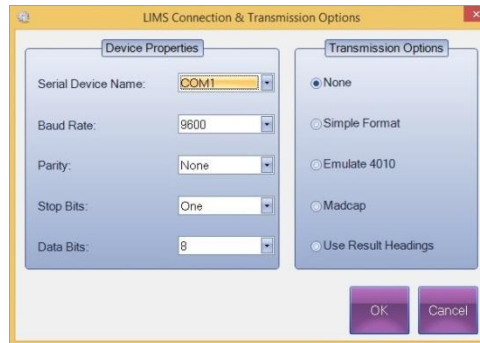
Calibration

DO NOT adjust any of the device or software calibration settings as this can lead to inaccurate measurements.

If calibration issues do occur see page 114 - Operator Support.

LIMS Connection Properties

The **LIMS Connection & Transmission Options** dialogue box is displayed.



LIMS CONNECTION AND TRANSMISSION OPTIONS DIALOGUE

- Consult the documentation for the LIMS you are using and select the appropriate settings in the fields on the dialogue box
- Select the **OK** button to save the entered settings and close the dialogue box
- Select the **Cancel** button to close the dialogue box without saving any settings entered

Edit User Permissions

The **Edit User Permissions** dialogue box is displayed.

EDIT USER PERMISSIONS - ChromaZona
Admins TAB

The screenshot shows the 'Edit User Permissions' dialog box with the 'ProtoCOL Admins' tab selected. The 'Administrator Permissions' section lists 17 items, all of which are checked. The 'Requires Audit Reason' section has a single checkbox, which is also checked. At the bottom, there are 'Save' and 'Cancel' buttons.

Administrator Permissions	Requires Audit Reason
<input checked="" type="checkbox"/> Archive Batch	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> Calibrate Colour	
<input checked="" type="checkbox"/> Calibrate Marker Position	
<input checked="" type="checkbox"/> Calibrate XY	
<input checked="" type="checkbox"/> Create Batch	
<input checked="" type="checkbox"/> Delete Batch	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> Edit Default Antibiotic Values	
<input checked="" type="checkbox"/> Edit LIMS Transmission Parameters	
<input checked="" type="checkbox"/> Edit MIC strip results	
<input checked="" type="checkbox"/> Edit Results	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> Edit Results via Statistics	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> Edit Spiral Plate Properties	
<input checked="" type="checkbox"/> Edit System Settings	
<input checked="" type="checkbox"/> Import Image	
<input checked="" type="checkbox"/> View History	
<input checked="" type="checkbox"/> View Reports	

EDIT USER PERMISSIONS - ChromaZona
Advanced Users TAB

The screenshot shows the 'Edit User Permissions' dialog box with the 'ProtoCOL Advanced Users' tab selected. The 'Advanced User Permissions' section lists 17 items, all of which are checked. At the bottom, there are 'Save' and 'Cancel' buttons.

Advanced User Permissions
<input checked="" type="checkbox"/> Archive Batch
<input checked="" type="checkbox"/> Calibrate Colour
<input checked="" type="checkbox"/> Calibrate Marker Position
<input checked="" type="checkbox"/> Calibrate XY
<input checked="" type="checkbox"/> Create Batch
<input checked="" type="checkbox"/> Delete Batch
<input checked="" type="checkbox"/> Edit Default Antibiotic Values
<input checked="" type="checkbox"/> Edit LIMS Transmission Parameters
<input checked="" type="checkbox"/> Edit MIC strip results
<input checked="" type="checkbox"/> Edit Results
<input checked="" type="checkbox"/> Edit Results via Statistics
<input checked="" type="checkbox"/> Edit Spiral Plate Properties
<input checked="" type="checkbox"/> Edit System Settings
<input checked="" type="checkbox"/> Import Image
<input checked="" type="checkbox"/> View History
<input checked="" type="checkbox"/> View Reports

The screenshot shows the 'Edit User Permissions' dialog box with the 'ProtoCOL Users' tab selected. The 'Basic User Permissions' section lists 17 items, all of which are checked. At the bottom, there are 'Save' and 'Cancel' buttons.

Basic User Permissions
<input checked="" type="checkbox"/> Archive Batch
<input checked="" type="checkbox"/> Calibrate Colour
<input checked="" type="checkbox"/> Calibrate Marker Position
<input checked="" type="checkbox"/> Calibrate XY
<input checked="" type="checkbox"/> Create Batch
<input checked="" type="checkbox"/> Delete Batch
<input checked="" type="checkbox"/> Edit Default Antibiotic Values
<input checked="" type="checkbox"/> Edit LIMS Transmission Parameters
<input checked="" type="checkbox"/> Edit MIC strip results
<input checked="" type="checkbox"/> Edit Results
<input checked="" type="checkbox"/> Edit Results via Statistics
<input checked="" type="checkbox"/> Edit Spiral Plate Properties
<input checked="" type="checkbox"/> Edit System Settings
<input checked="" type="checkbox"/> Import Image
<input checked="" type="checkbox"/> View History
<input checked="" type="checkbox"/> View Reports

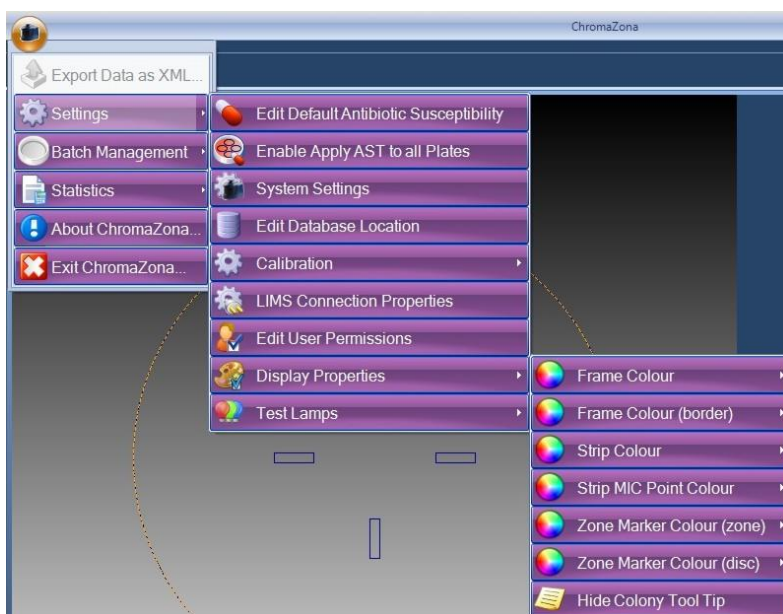
EDIT USER PERMISSIONS - ChromaZona Users TAB

The dialogue box has separate tabs for each of the three classes of ChromaZona users; Administrators (Admins), Advanced Users, and Users. The selection boxes on the three tabs are identical except for the addition of a **Requires Audit Reason** selection box on the **Admins** tab.

- On the **Admins** tab select the **Requires Audit Reason** selection box against each of the **Permission** types applicable. Once selected, this option requires the two User types to enter a reason for making changes to the types of function against which the **Requires Audit Reason** selection box is selected. A dialogue box will be displayed when a User makes an applicable change and they will not be able to proceed unless they enter a reason for the change
- On each tab select the **Permissions** selection boxes for each function that each type of user is permitted to carry out
- Select the **Save** button to save the entered settings and close the dialogue box
- Select the **Cancel** button to close the dialogue box without saving any settings entered

Display Properties

The **Display Properties** sub-menu is displayed.



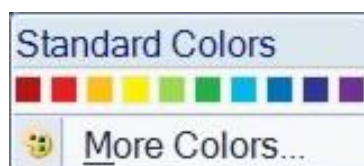
DISPLAY PROPERTIES SUB-MENU

- The options that appear on the **Display Properties** sub-menu enable changes to be made to the appearance of display elements within ChromaZona

(Continued on next page)

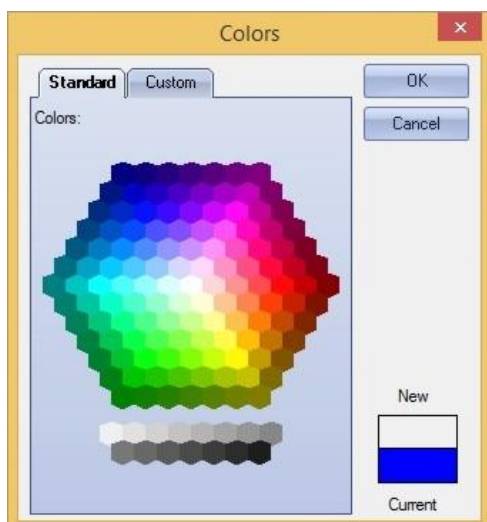
Sub-menu Category	Changes
Frame Colour	The individual zone detection area.
Frame Colour (border)	The graticule.
Strip Colour	The outline of the MIC strip.
Strip MIC Point Colour	A detected MIC point.
Zone Marker Colour (zone)	A detected zone.
Zone Marker Colour (disc)	A detected disc or well.
Hide Colony Tool Tip	A simple choice of whether or not to display the Colony Tool Tip. Choice is between Hide Colony Tool Tip and Show Colony Tool Tip. If set to Hide Colony Tool Tip, as the cursor is hovered over a colony it does not display a colony area marker.

- Selecting one of the first six options displays the **Standard Colours** pop-up

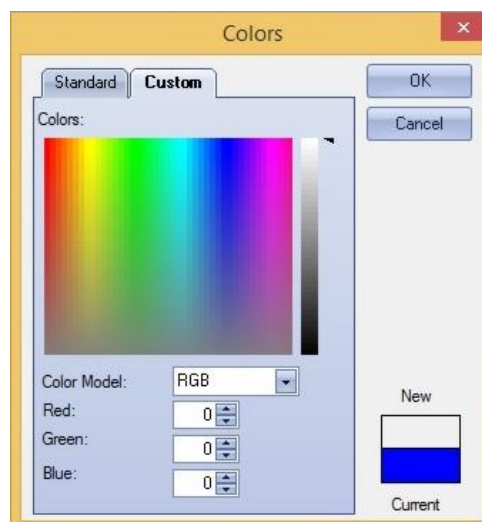


STANDARD COLORS POP-UP

- Select one of the standard colour blocks or select **More Colors...** to display the **Colors** pop-up



COLORS POP-UP - STANDARD

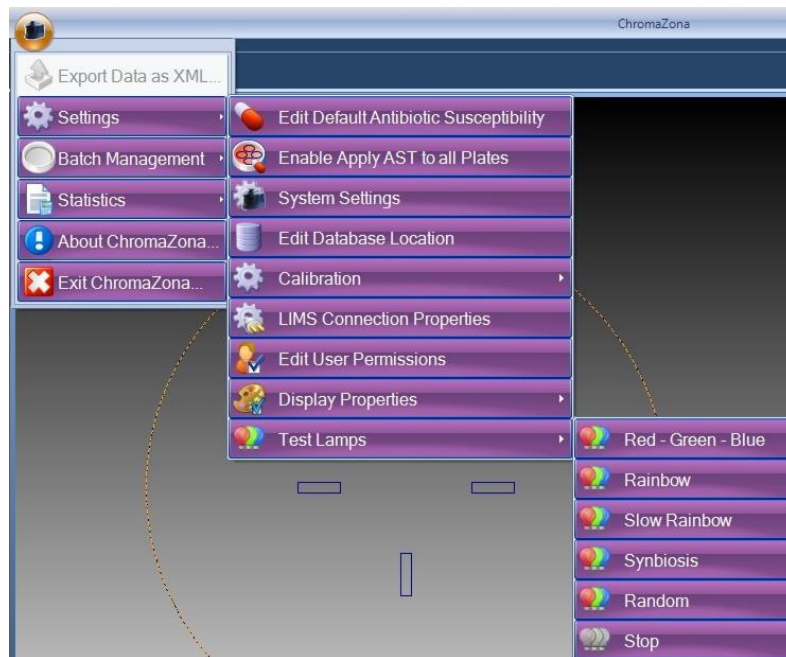


COLORS POP-UP - CUSTOM

- The **Standard** tab provides a range of coloured cells, select a colour cell and then select the OK button, to change the colour of the selected display element
- The **Custom** tab provides a much wider range of colour selections; select a colour point on the display or enter the exact colour reference using the RGB colour space

Test Lamps

The **Test Lamps** sub-menu is displayed.



TEST LAMPS SUB-MENU

- To test the lights in the instrument connected to the PC, select an option from the sub-menu. The lights will display in the relevant pattern
- To stop the light display, re-select **Test Lamps** and select **Stop** from the sub-menu

Batch Management

Select the **Batch Management** button.

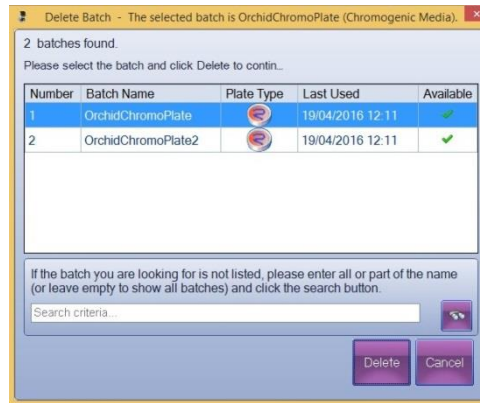
The **Batch Management** sub-menu is displayed.



BATCH MANAGEMENT SUB-MENU

- (a) Select the **Delete Batch** button

The **Delete Batch** pop-up is displayed.

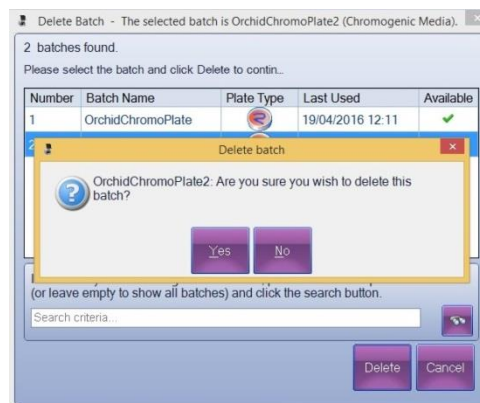


DELETE BATCH POP-UP

- Select the unwanted Batch from the list

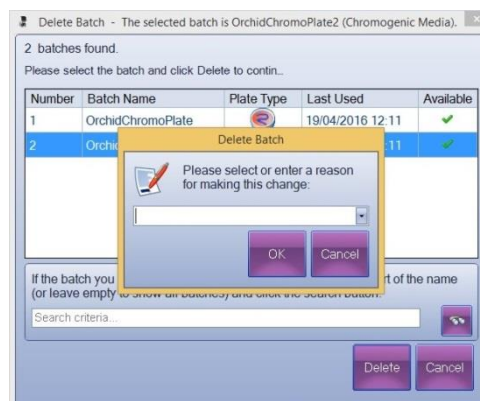
Note: The Batch you want to delete must be closed; an open Batch cannot be deleted.

- If the Batch to be deleted is not in the list, select the search button and search for the Batch
- Once a Batch has been selected, select the **Delete** button to delete it from the list
- An 'Are you sure' pop-up appears



DELETE BATCH - ARE YOU SURE - POP-UP

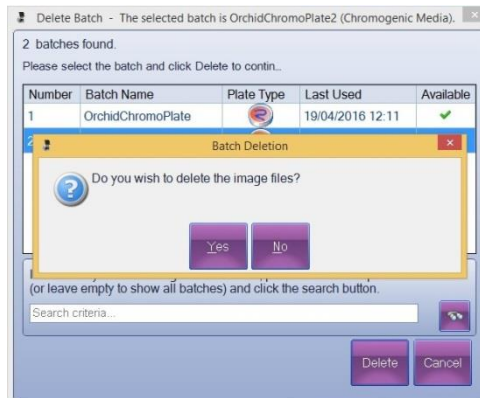
- Select the **Yes** button to continue with the Batch deletion
- The **Delete Batch - Reason** pop-up appears



DELETE BATCH - REASON POP-UP

- Enter a reason for deleting the Batch, either by direct text entry, or select from the drop-down list. The drop-down list will only be populated if previous Batch deletion reasons have been entered
- Select the **OK** button

The **Batch Deletion - Images** pop-up appears.



BATCH DELETION - IMAGES POP-UP

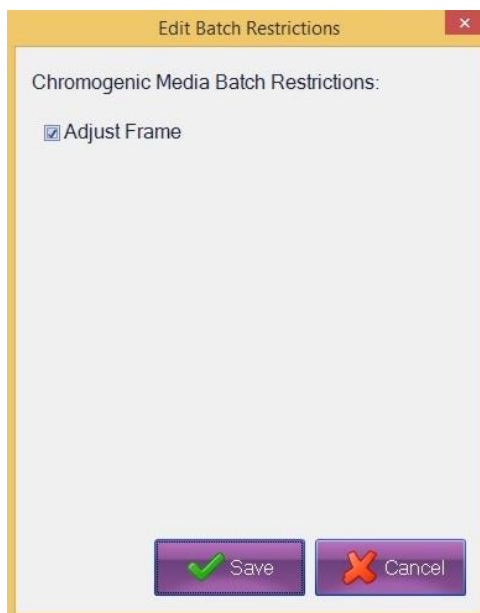
- Select the **Yes** button to delete all of the images associated with the Batch

The **Delete Batch** pop-up is displayed; the selected Batch is now deleted from the list.

Note: The Batch can no longer be viewed in ChromaZona, but it is not actually removed from the ChromaZona database and can be viewed there if required for audit purposes.

- (b) Select the **Manage Restrictions** button

The **Edit Batch Restrictions** pop-up is displayed.



EDIT BATCH RESTRICTIONS POP-UP

- Batch Restrictions enable some Batch properties to be protected against accidental changes. Batch Restrictions can be changed at any time; in particular they can be changed before or after the Batch has been accepted

- Batch Restrictions have a particular use with Multiwell, SBA and OPKA batches as they enable you to choose between moving the whole frame or individual sector sub-frames
- The parameters in the pop-up depend on the type of Batch selected
- Check the checkboxes against all parameters that Users will be allowed to change when they are working with the Batch in Measurement mode. Unchecked parameters will be locked so that Users cannot change them accidentally
- Select the **Save** button to confirm the settings and close the pop-up

(c) Select the **Batch Details** button

The **Open Batch** pop-up is displayed.

Open Batch - The selected batch is OrchidChromoPlate (ChromogenicMedia).

Title	Value
Batch Name	OrchidChromoPlate
Batch ID	16
Plate Type	Chromogenic Media
Is based on Batch	False
Image Source	Imported Image
Use Barcode	False

Copy Select All Cancel

OPEN BATCH POP-UP-1

Open Batch - The selected batch is OrchidChromoPlate (ChromogenicMedia).

Title	Value
Upper Count Limit	0
Lower Count Limit	0
Exclude from Mean	False
Plate Diameter	90
Plate Width	90
Plate Height	90

Copy Select All Cancel

OPEN BATCH POP-UP-2

Open Batch - The selected batch is OrchidChromoPlate (ChromogenicMedia).

Title	Value
Sample Volume	1
Spiral Threshold	0
Spiral Volume	100 microlitres
Pour Plate Dimension Units	Millimetre
Pour Plate Volume Units	Millilitre
Restrict Max Colony Size	False

Copy Select All Cancel

OPEN BATCH POP-UP-3

Open Batch - The selected batch is OrchidChromoPlate (ChromogenicMedia).

Title	Value
Pour Plate Volume Units	Millilitre
Restrict Max Colony Size	False
Exposure Time	0.2
Sensitivity is Auto	False
Small Particle Rejection On	True
Split Colonies	False

Copy Select All Cancel

OPEN BATCH POP-UP-4

- This pop-up allows the copying of Batch parameters, these can then be pasted into an external application, eg Word or Excel.
- Selection can be made using the standard keyboard / mouse functions:
 - Single left click to select a single parameter
 - Ctrl plus left click to select another single parameter
 - Single left click to select the first single parameter followed by Shift plus left click to select a range

- Select parameters, then select the Copy button. The selected and copied parameters can now be pasted into an external application, e.g. Word, where the parameters are inserted as a two-column table

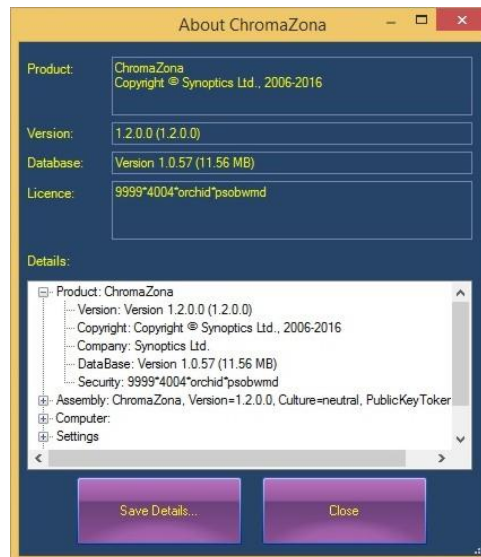
(d) Select the **Show Light and Dark Control** button

- A simple choice of whether or not to display the **Light Colonies Only / Dark Colonies Only** function on the **Classification** tab. Choice is between Show Light and Dark Control and Hide Light and Dark Control

About ChromaZona

- Select the **About ChromaZona...** button.

The **About ChromaZona** pop-up is displayed.



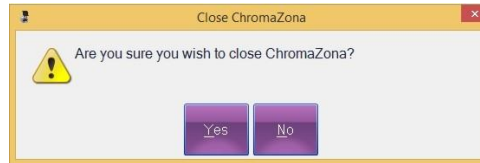
ABOUT CHROMAZONA POP-UP

- Use the horizontal and vertical scroll bars to view the full extent of the data entries
- Expand the entries by selecting the + boxes
- Reduce the entries by selecting the - boxes
- The **About ChromaZona** pop-up can be enlarged by grabbing and dragging the pop-up borders
- Data can be saved to an external file by selecting the **Save Details...** button. This opens a standard Windows Save As dialogue box

Exiting the Software

Select the **Exit ChromaZona...** button.

The **Close ChromaZona** pop-up is displayed.



CLOSE CHROMAZONA POP-UP

- Select the **No** button to close the pop-up and return to ChromaZona.
- Select the **Yes** button to immediately close the ChromaZona application.

ChromaZona Software Applications

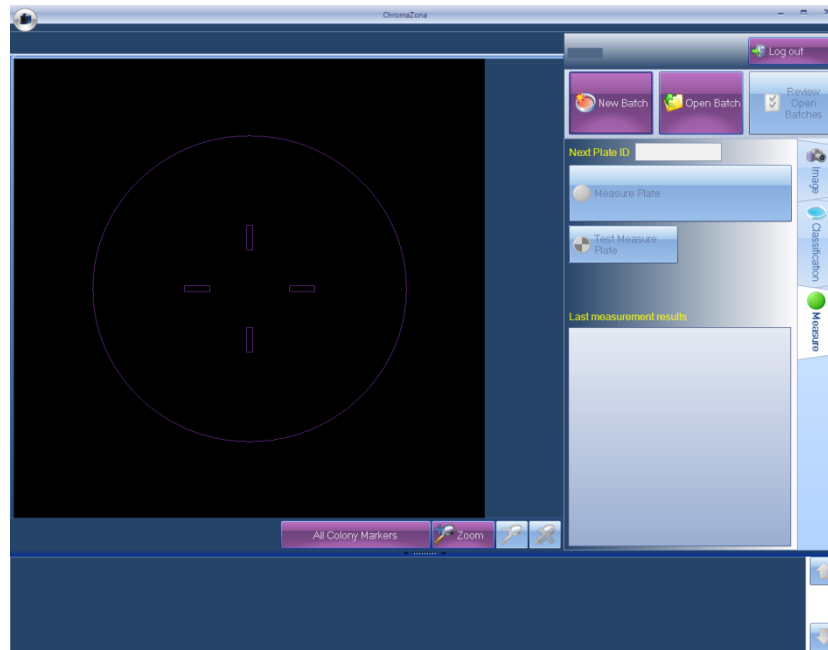
APPLICATION	DESCRIPTION
Chromogenic Media	Application module designed to make the analysis of chromogenic agars standardised, accurate and simple for the end user.
Dilution Series	Application module designed to count plates in a dilution series.
Multi-Sector Plate	Application module to enable counting of individual elements of a plate.
Multi-well Plate	Application module to allow counting of up to 120 wells on an individual plate.
OPKA (Opsonophagocytic Killing Assay)	Application module designed for the reading of OPKA assay plates.
Pour Plate	Application module designed to read colonies on an agar plate. Within the pour plate batch numerous classification options can be applied for plate measurement such a colour, shape and size. Plates with gridded membrane features can also be analysed.
SBA (Serum Bactericidal Assay)	Application module designed for the reading of SBA assay plates.
Spiral Dilution Series	Application module designed to count spiral plates in a spiral dilution series.
Spiral Plates	Application module designed to count spiral plates created from WASP and Eddy Jet spiral platers.
Antibiotic Susceptibility	Application module to read bacterial growth inhibition zones. Software utilises unique algorithm to precisely calculate the diameter of zones created. All measurements apply EUCAST or CLSI guidelines and expert rules to determine antibiotic susceptibility.
Inhibition Zone	Application module designed to read zones of inhibition, such as single radial immunodiffusions. Software utilises unique algorithm to precisely calculate the diameter of zones created.
MIC Strip	Application module designed to read bacterial growth inhibition zones created by MICE strips from Thermofisher.

IMAGE FUNCTIONS

Capturing an image

1. Start up the instrument and log on to the ChromaZona application

The **Home Screen** appears.



HOME SCREEN

2. Insert the Plate Holder in the instrument that gives the best contrast between the colony and the background.
3. Place the Plate onto the Plate Holder.
4. Select the **Image** tab



The **Image Controls** appear.



IMAGE CONTROLS

5. Check the **Live Image** checkbox

The live image of the Plate appears on the screen, appearing as it would if the currently set exposure time is used to capture the image.

6. Adjust the exposure of the image by increasing or decreasing the exposure time. Exposure time can be adjusted using either the arrow buttons or the slider. The new setting will be indicated between the arrow buttons



decrease exposure by 100 ms



decrease exposure by 10 ms



increase exposure by 10 ms



increase exposure by 100 ms

7. Select the **Capture Image** button

The instrument uses the new exposure setting to capture an image, which is displayed on the screen.

8. Check the displayed image

9. Create a new Batch, depending on which options have been installed, Batches can be created for the following Plate types:

- Antibiotic Susceptibility
- Chromogenic Media Plate
- Dilution Series
- Inhibition Zone
- MIC Strip
- Multi-Sector Plate
- Multi-well
- OPKA Plate
- Pour Plate
- SBA Plate
- Spiral Plate




Zooming




By default, images are scaled to fit the Image Pane in the ChromaZona window. Zoom controls are provided for:

Zoom in 

Zoom out 

Zoom to original size 

Initially only the zoom in function is available, with the zoom out and zoom to original size functions greyed out and unavailable;   .

Once the zoom in function has been used, the zoom level (scaling factor) is indicated on the zoom in button, and the zoom out and zoom to original size functions become available;   .

The zoom controls are also displayed with the image displayed in dialogue boxes – used in exactly the same way as in the main ChromaZona window.

Importing an Image

A previously captured and saved image can be imported into ChromaZona from a file.

1. Select the **Image** tab  to display the image controls

The **Image Controls** appear.



IMAGE CONTROLS

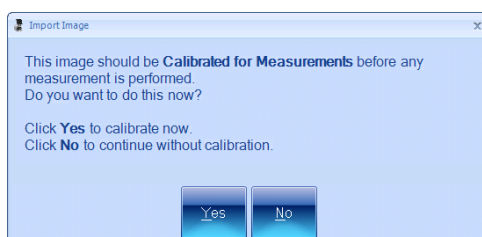
2. Select the **Import Image from file...** button



This displays a standard Windows Open dialogue box.

3. Navigate to the folder holding the required image and select the required image
4. Select **Open** to open the file and load it into the ChromaZona window

- The following **Import Image** warning is displayed



CALIBRATE THE IMAGE WARNING

- Refer to Calibrating an Image

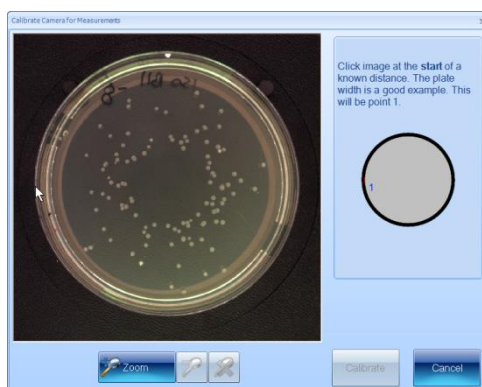
Calibrating an Image

When importing an image from a file, capturing an image from an external camera or scanner, or trying to perform a measurement from an uncalibrated imported image, the **Import Image** warning dialogue box is displayed, refer to Importing an Image.

In order to perform an accurate calibration it is necessary to know the actual size of some feature in the image, eg the dimensions of the plate itself. To calibrate an image:

1. Select **Yes** in the **Import Image** warning dialogue box to commence the calibration process

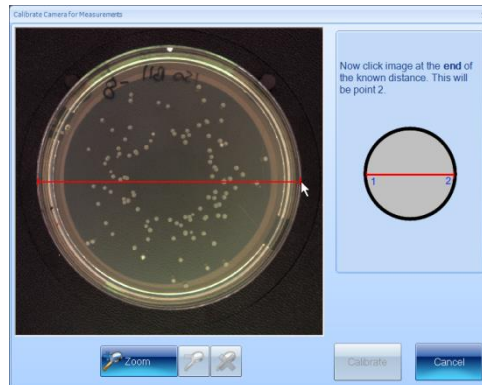
This displays the **Calibrate Camera for Measurements** dialogue box.



CALIBRATE CAMERA FOR MEASUREMENTS-1

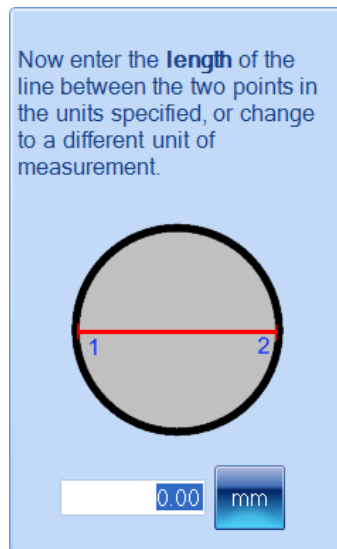
5. Choose a feature in the image, eg the plate itself, and select a horizontal or vertical edge. Click / tap to mark the selected point. This is represented by point 1 on **CALIBRATE CAMERA FOR MEASUREMENTS-1**

6. Drag the pointer to the other end of the feature, as this is done a line will be drawn back to point 1. Click / tap to mark the selected point. This is represented by point 2 on **CALIBRATE CAMERA FOR MEASUREMENTS-2**.



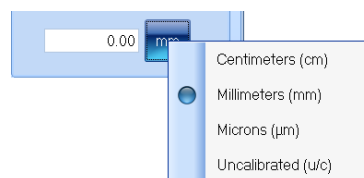
CALIBRATE CAMERA FOR MEASUREMENTS-2

This displays the **calibration edit** box.



CALIBRATION EDIT BOX

- The units associated with the measurement can be changed, if required, by selecting the **Units** button:



CALIBRATION UNITS

- Select the unit required.
7. Enter the length of the known feature into the measurement box
 8. The **Calibrate** button is now available. Select the **Calibrate** button to set the calibration and return to the main ChromaZona window

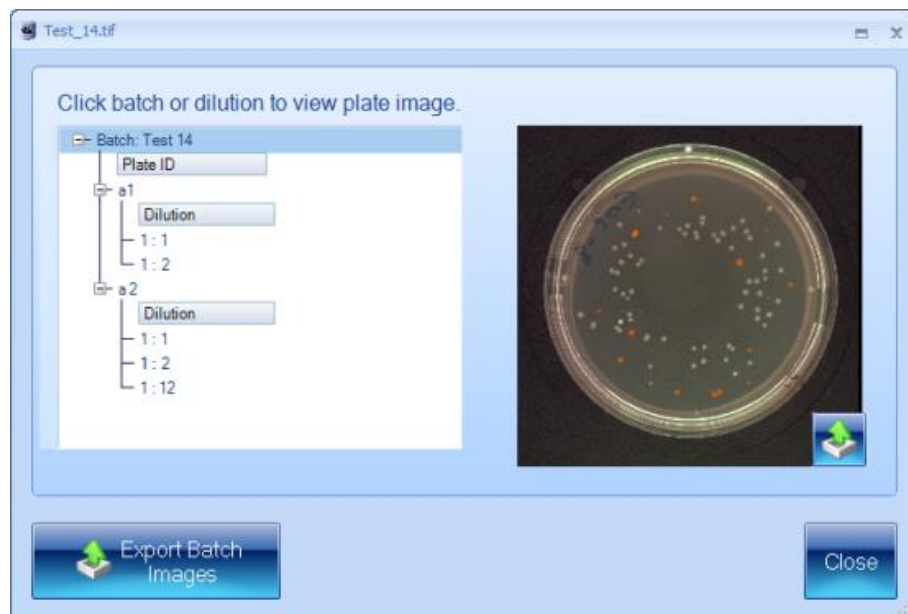
Viewing Batch Images

By default, when a new Batch is created, the image used to set it up is saved with the Batch, and then, once the batch design is accepted, each time a measurement is taken in Measurement mode, the resulting image is also saved with the results in the Batch.

To view the Batch setup image and any images used for measurements:

1. Select the **Image** tab  to display the image controls. Since there is an open Batch, the **View Batch Images** button on the **Image Controls** is active
2. Select the **View Batch Images** button 

This displays a **batch images** dialogue box. The title of the dialogue box will be the name of the Batch whose images are being viewed.



BATCH IMAGES DIALOGUE BOX

- The left hand pane shows the structure of the images in the selected Batch
- The right hand pane shows the selected image, when first opened the Batch setup image is displayed
- The Batch setup image is represented by the top line, the Batch identifier
- Measurement images are next, listed in order of their plate identifiers, and in the case of a dilution series, in order of dilutions
- To view the Batch setup image, select the Batch identifier, ie Batch: Test 14
- To view a measurement image, select the plate identifier, ie Plate ID, or in the case of a dilution series, the dilution line within the plate identifier entry, ie Dilution

Exporting an Image

The current captured image in the ChromaZona window can be exported to a file:

1. Select the **Image** tab  to display the image controls

The **Image Controls** appear.



IMAGE CONTROLS

9. Select the **Export Image to file...** button



This displays a standard Windows Save-as dialogue box

10. Select an image format from the **Save as type** drop-down list
11. Select a destination folder
12. Enter a file name for the image
13. Select **Save** to save the file

Export All Images from a Batch

1. Select the **Export Batch Images** button




This displays a Windows Browse for Folder dialogue box

2. Select the required destination folder for the exported images. If necessary, select **Make New Folder** to add a new folder to the selected folder, and type in a name for the new folder
3. Select **OK** to save the images from the Batch to the selected folder

- The Batch setup image is saved using the name of the Batch
- The individual measurement images are saved using the image identifier - if the same identifier has been used for more than one measurement, e.g. in a dilution series Batch, a number suffix will be added to the name, i.e. _2 is added to the second image name, _3 is added to the third image name, etc.
- An XML file containing information about the images is also saved in the folder

Export a Single Image from a Batch

1. Select the required image to be exported
2. Select the  button
This displays a standard Windows Save As dialogue box
3. In the Save As dialogue box, select a folder and enter a filename for the saved image
4. Select **Save** to save the selected image
5. Select **Close** to close the dialogue box and return to the main ChromaZona window

Selecting an Alternative Imaging Device

The ChromaZona application allows the use of an external imaging device, either a camera or a scanner, connected via a USB port. If using a scanner it must be a TWAIN device and its drivers must be loaded on the controlling PC. To choose the imaging device:

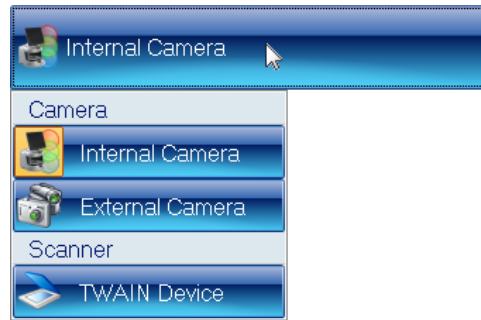
6. Select the **Image** tab  to display the image controls

The **Image Controls** appear. By default the Internal Camera in the ChromaZona is selected



IMAGE CONTROLS

7. To use an external camera or a scanner, select the **Internal Camera** button and select the required device from the drop-down list



IMAGING DEVICE SELECTION

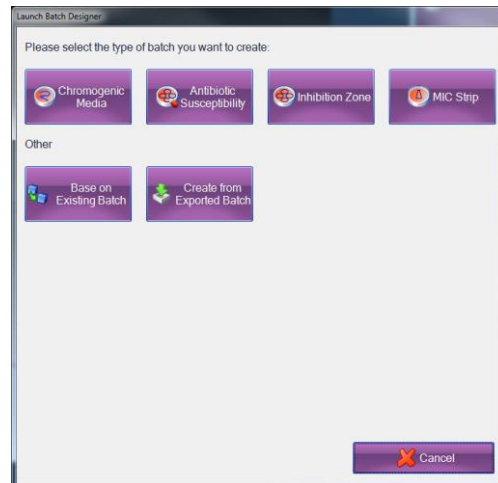
- Ensure that ChromaZona has been configured to use the intended external camera or scanner
- If more than one TWAIN scanner is connected more than one TWAIN device will be listed, select the required device
- If using a TWAIN scanner, ensure that the scanner is connected to the PC and switched on
- Refer to the documentation supplied with the scanner for how to control it
- If using an external camera, ensure that the camera is connected to the PC and switched on
- When using an external camera with ChromaZona, check the **Live Image** checkbox on the **Image Controls** tab to display a live image from the external camera. Refer to the separate documentation available on using ChromaZona with an external camera

PLATE CONFIGURATION

Plate configuration is used to set the plate parameters.

1. On the **Home Screen** select the **New Batch** button

The **Launch Batch Designer** pop-up appears.



LAUNCH BATCH DESIGNER POP-UP

2. Select the **Plate Configuration** button

The **Plate Configuration** pop-up appears.

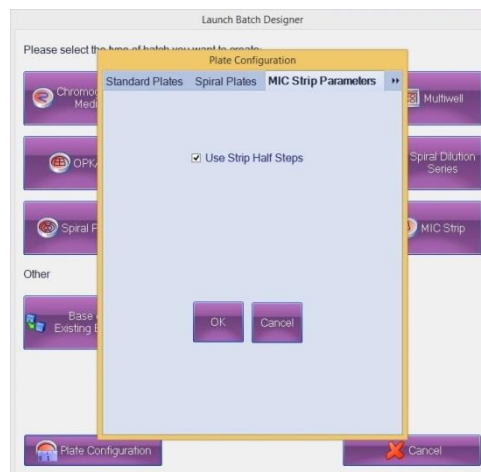


PLATE CONFIGURATION POP-UP

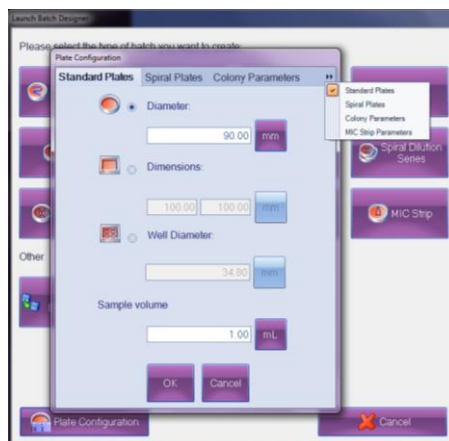
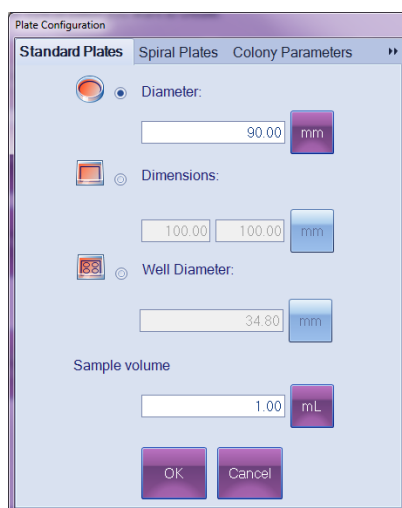


PLATE CONFIGURATION POP-UP - 2

3. Select the >> icon to view the different configuration screens. This can also be done by clicking on the screen names in the top bar
4. Select the **Standard Plates** tab

A modified **Plate Configuration** pop-up appears.

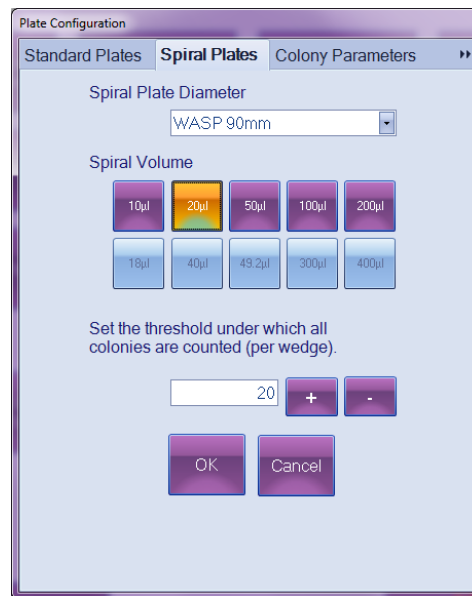


STANDARD PLATES PLATE CONFIGURATION POP-UP

Select the required parameters:

- Select **Diameter** if using circular Plates. Enter a Plate size. Change the units by selecting the **mm** button. Select millimetres or centimetres
- Select **Dimensions** if using rectangular Plates. Enter the Plate dimensions. Change the units by selecting the **mm** button. Select millimetres or centimetres
- Select **Well Diameter** if using Plates with ready cut wells. Enter the Well diameter. Change the units by selecting the **mm** button. Select millimetres or centimetres
- Enter the **Sample volume**. Change the units by selecting the **mL** button. Select microlitres or millilitres
- Select the **OK** button

5. Select the **Spiral Plates** tab

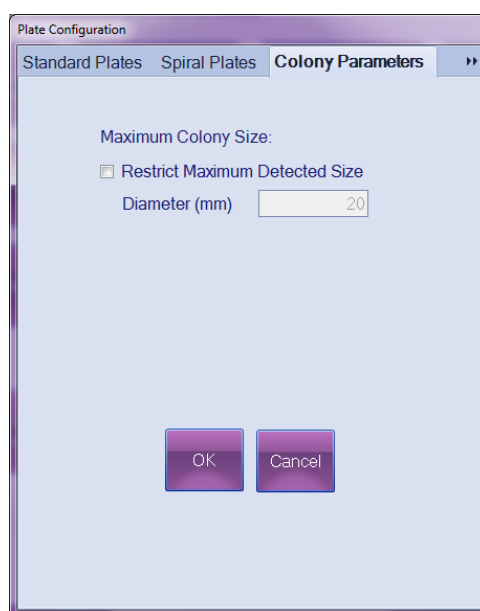


SPIRAL PLATES PLATE CONFIGURATION POP-UP

Select the required parameters:

- Select which **Spiral Plate Diameter** is being used. Change which spiral plater is selected by pressing down on the drop down menu
- Select the **Spiral Volume**. Volume is selected from options given, these vary depending upon which spiral plater is being used
- Select the Count Threshold. Adjust using the + or – buttons
- Select the **OK** button

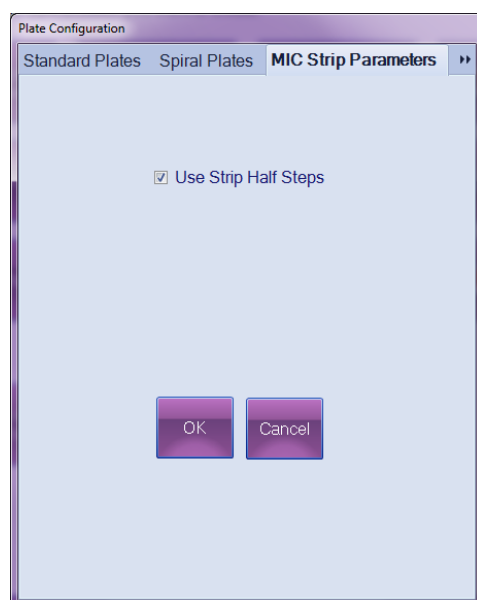
6. Select the **Colony Parameters** tab



COLONY PARAMETERS PLATE CONFIGURATION POP-UP

Select to restrict the maximum detected colony size in mm.

7. Select the **MIC Strip Parameters** tab



MIC STRIP PARAMETERS PLATE CONFIGURATION POP-UP

PLATE COUNT

Total plate count

On the Home screen, select the **Total Plate Count** button.

The **Colonies** drop-down appears.



SECTOR TYPE DROP-DOWN

On the **Colonies** drop-down:

- (a) Select **Light Colonies Only** or **Dark Colonies Only**
- (b) Adjust the **Settings** using the slider or left / right arrow controls
 - Adjust Sensitivity
 - Adjust Reject Small Particles
- (c) The software can automatically separate touching colonies, if this option is required; select the **Split Touching Colonies** checkbox
- (d) If the **Split Touching Colonies** is selected, use the **Splitter Type** drop-down to select a splitting parameter - Standard or Fine

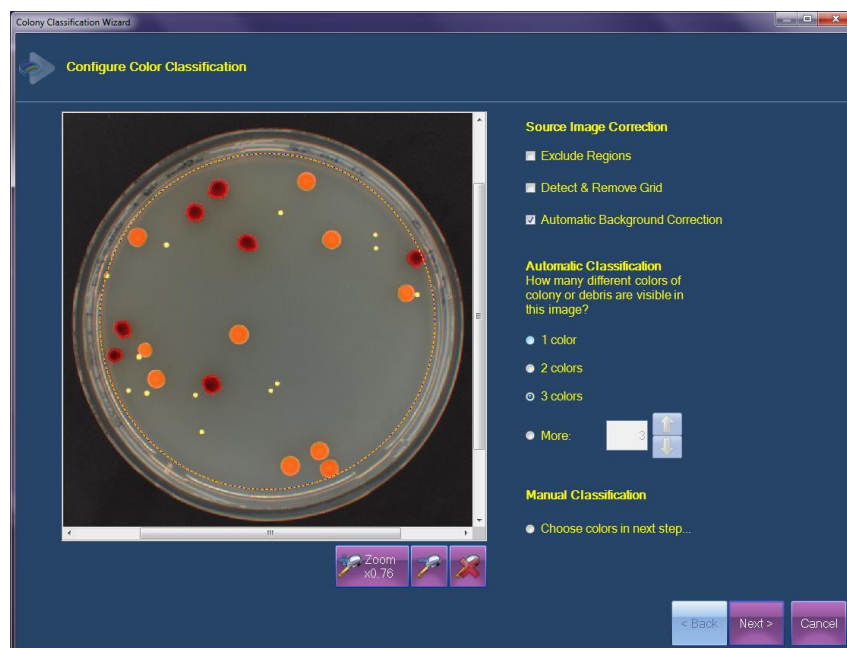
Colour Classification - Automatic

The colour classification option can be used to differentiate between colonies based on the colony colour. Automatic colour classification involves the software distinguishing between different colony colours and automatically grouping these colonies based on similar colour.

- (a) On the Home screen, select the **Colour Classification** button

Note: If there is no current image, an image will be captured automatically when the **Colour Classification** button is selected.

The **Colony Classification Wizard** appears, opening on the **Configure Colour Classification** page.



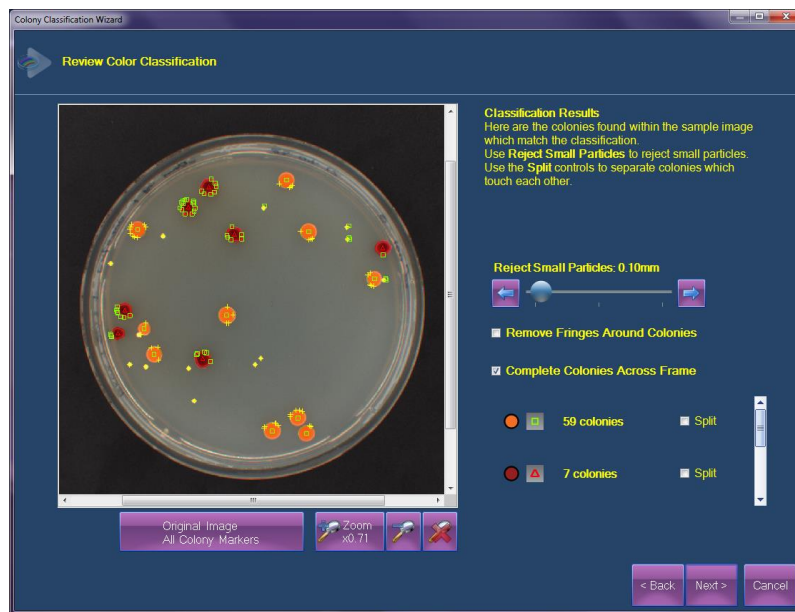
CONFIGURE COLOUR CLASSIFICATION WIZARD-1

- (b) Ensure the graticule in the Image Pane covers the area that will be used for colour classification; this can be resized by selecting and dragging it, to make it larger or smaller. Adjust the graticule until it sits just inside the circular rings which define the edge / physical structure of the Plate. This will remove these rings from the colour classification process
- (c) Use the **Automatic Classification** settings to carry out an automatic colour classification of the colonies on the Plate

Using the checkboxes, set the number of colony colours that need to be counted:

- If the Plate has colonies which are all one colour, select the **1 colour** checkbox
 - If the Plate has colonies of more than one colour, but only an overall colony count is required, select the **1 colour** checkbox
 - If the Plate has colonies of more than one colour, and separate counts are required for each colour select the relevant checkbox; **2 colours**, **3 colours**, or **More**. If **More** is selected, enter the number of colours directly into the field or use the up / down arrow buttons to enter the number
 - **Automatic Classification** may have problems if there are two or more colours of colonies and there is debris which needs to be distinguished from the colonies, if this occurs use **Manual Classification**
- (d) Once the number of colours has been selected, select the **Next>** button. The software will now analyse the image and present the results in the **Review Colour Classification** page

The **Review Colour Classification** page is displayed.

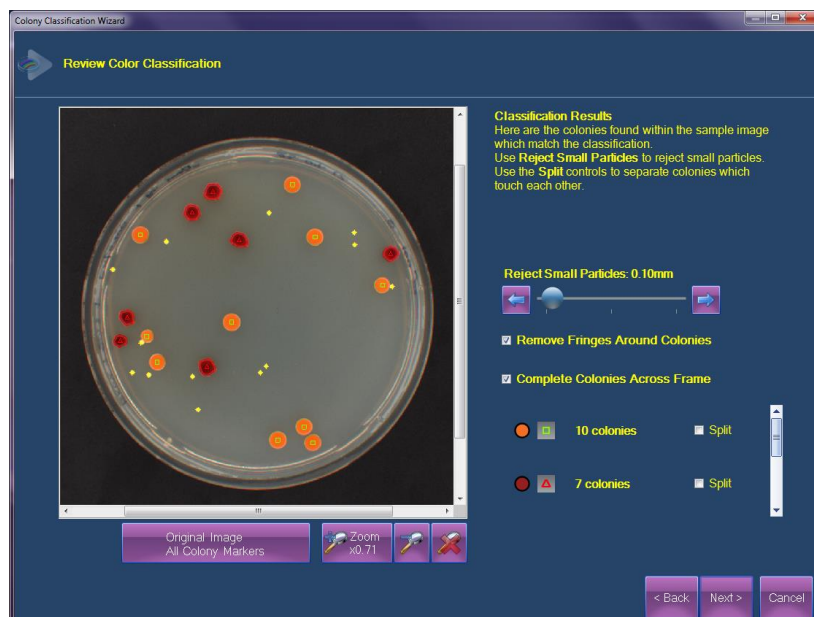


REVIEW COLOUR CLASSIFICATION WIZARD

Note: The **Review Colour Classification** page is displayed after carrying out an automatic or a manual colour classification process. This page displays the results based on the selections made previously.

- Examine the detected colonies and image carefully to check that:
 - Colonies are being detected successfully
 - Different colours of colonies are being distinguished successfully
 - Colonies are distinguished from debris with a different colour

Note: The software will analyse each colony pixel by pixel to determine which colour classification they belong in. Due to this, colonies that have a gradual colour change may be displayed as multiple colonies. To avoid this, check the **Remove Fringes Around Colonies** box.



REVIEW COLOUR
CLASSIFICATION WIZARD
– SHOWING REMOVE
FRINGES AROUND
COLONIES FUNCTION

- (e) Ensure the software has identified all colonies present

Note: It may be easier to see the results if the image is zoomed, use the Zoom Controls under the image to zoom in / out.

- (f) Use the **Reject Small Particles** slider to remove the effect of small coloured regions of the image background that may be wrongly interpreted as colonies. Adjust the slider to set the level of size filtering used
- If small debris particles are wrongly detected as colonies, try increasing the **Reject Small Particles** setting
 - If small colonies are being wrongly rejected, try decreasing the **Reject Small Particles** setting
 - Adjust the slider directly or use the left / right arrow buttons



REJECT SMALL PARTICLES SETTINGS

- (g) Use the **Complete Colonies Across Frame** setting to decide whether or not to include in the count colonies that the Plate frame passes through
- To include the affected colonies, select the checkbox
 - To reject the affected colonies, leave the checkbox unchecked
- (h) Use the **Split** setting for each colony colour to prevent overlapping colonies from being counted as a single colony. To split overlapping colonies, select the **Split** checkbox next to each colony type

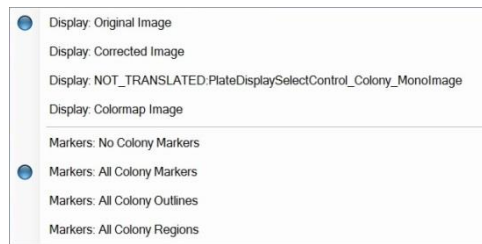


SPLIT NOT SELECTED



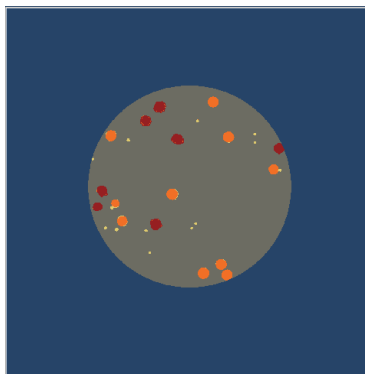
SPLIT SELECTED

- (i) The image display can be changed by selecting the **Original Image All Colony Markers** button and selecting options from the drop-down, default settings illustrated. Select a suitable combination of options; each of the **Marker** options can be applied to each of the **Display** options

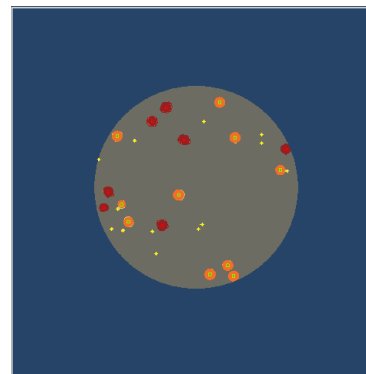


ORIGINAL IMAGE ALL COLONY MARKERS DROP-DOWN

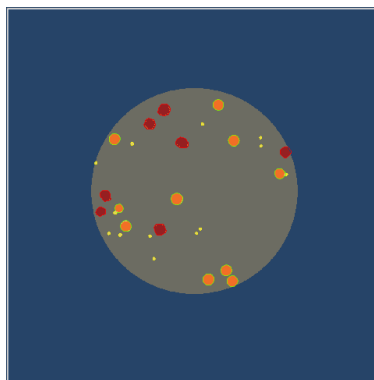
- **Colormap Image:** displays the image using contrasting colours to show which areas of the image have been detected as background and which as foreground features
- **Original Image:** displays the original uncorrected image
- **Corrected Image:** displays the image with the specified background correction.
- **No Colony Markers:** colonies are unmarked
- **All Colony Markers:** each detected colony is identified with a symbol showing the type of colony (as specified on the Colour Classification page), with a key to the colony symbols displayed to the right of the image
- **All Colony Outlines:** the boundary of each detected colony is marked in the colour of the symbol representing the colony type
- **All Colony Regions:** the area of each detected colony is marked in the colour of the symbol representing the colony type



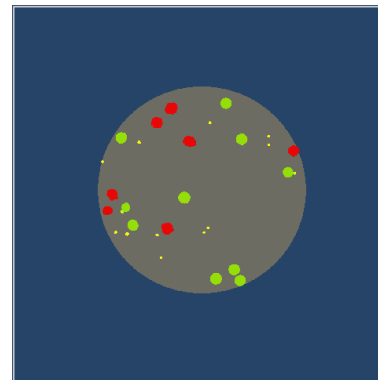
DISPLAY: COLORMAP IMAGE
MARKER: NO COLONY MARKERS



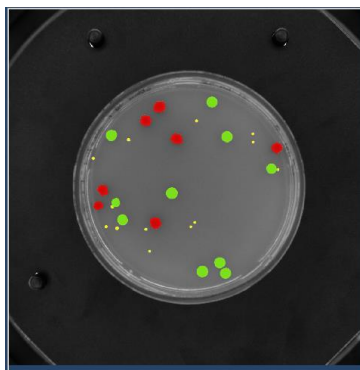
DISPLAY: COLORMAP IMAGE
MARKER: ALL COLONY MARKERS



DISPLAY: COLORMAP IMAGE
MARKER: ALL COLONY OUTLINES



DISPLAY: COLORMAP IMAGE
MARKER: ALL COLONY REGIONS



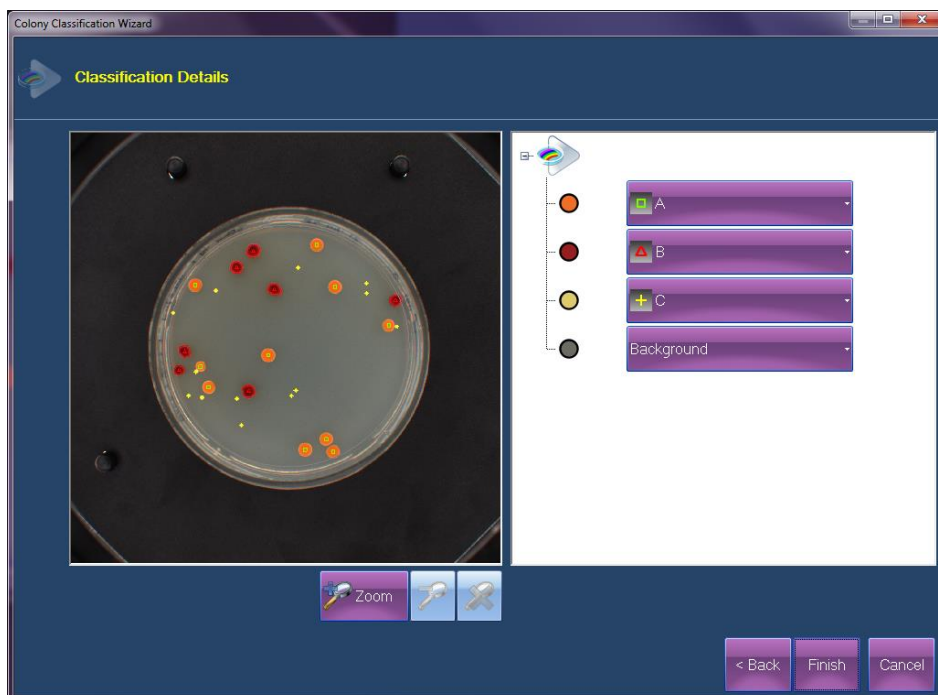
DISPLAY: NOT_TRANSLATED: PlateDisplaySelectControl_Colony_MonoImage
 MARKER: ALL COLONY REGIONS

(j) If colours are being wrongly classified, e.g. if colonies are not being detected because they are being treated as background, or if colonies and debris with different colours are not being distinguished accurately:

- If an automatic colour classification has been performed, select the **Back** button until the **Configure Colour Classification** page is displayed and try increasing or decreasing the number of colours selected, or try a manual colour classification
- If an automatic background correction has been performed, select the **Back** button until the **Configure Colour Classification** page is displayed and select manual background correction by de-selecting the **Automatic Background Correction** checkbox

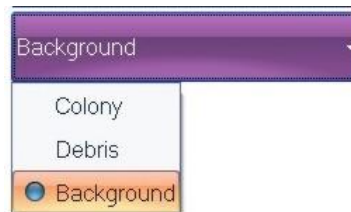
(k) If the colour classification is correct, select the **Next>** button

The **Classification Details** page is displayed.



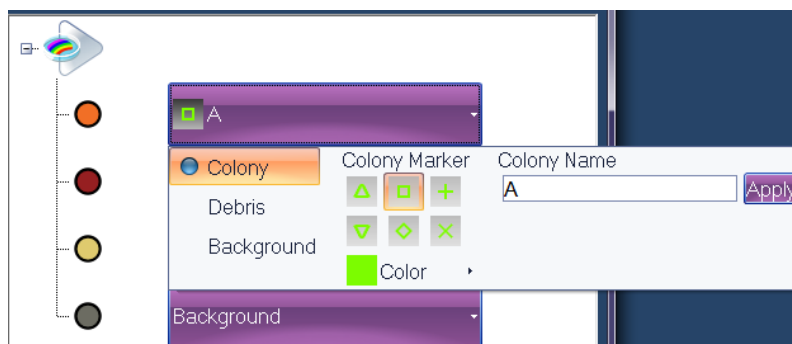
COLONY CLASSIFICATION DETAILS WIZARD

- This page is used to specify whether the detected colours correspond to colonies, debris or background, and if there is more than one type of colony colour and what organisms they correspond to. It is also used to change the classification details for a colony type produced by a colour classification
- The left hand pane displays the image with the colony markers superimposed
- The right hand pane shows the classified colours in the image in a tree structure and identifies whether they represent colonies, debris or background (not all options may be present in an image)
- Selecting any of the colour classification buttons allows the classification to be amended
- Select the **Background** button. A simple classification sub-menu is displayed



SIMPLE BACKGROUND CLASSIFICATION SUB-MENU

- To change a colony marker or the colony marker colour select the drop down menu and adjust



CLASSIFICATION SUB-MENU WITH CLASSIFICATION DETAILS PANEL

- Use the **Colony Marker** options to select a marker shape
 - Use the **Colour** option to display and select a standard colour for the marker shape selected
 - If required, enter a name for the colony in the **Colony Name** field. By default ChromaZona assigns letters of the alphabet to distinguish colony types if more than one type is detected. The name of the organism forming the colonies could be used as the colony name
 - Select the **Apply** button to save any changes made
- (I) Select the **Finish** button to complete the colour classification process and return to the main ChromaZona window

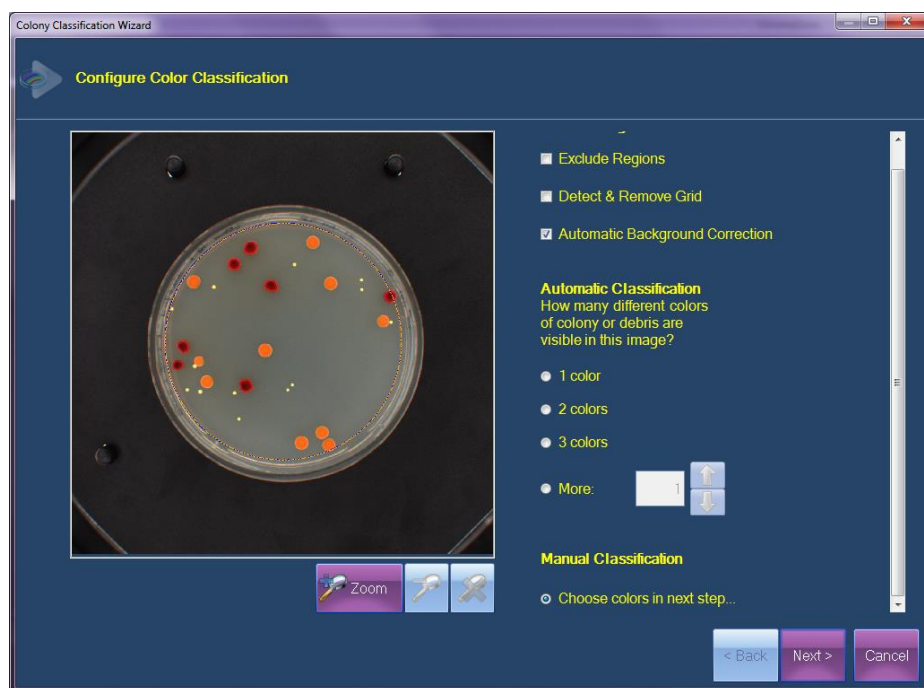
Colour Classification - Manual

The colour classification option can be used to differentiate between colonies based on the colony colour. Manual colour classification involves the user selecting the colony colours to be found by the software to group colonies together based on similar colour.

- (a) On the Home screen, select the **Colour Classification** button

Note: If there is no current image, an image will be captured automatically when the **Colour Classification** button is selected.

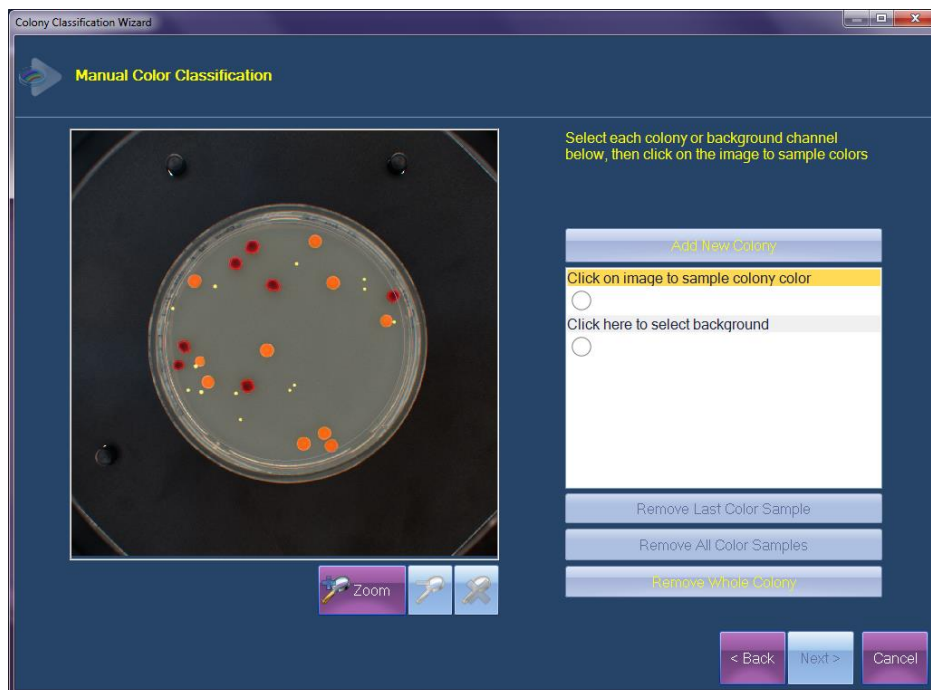
The **Colony Classification Wizard** appears, opening on the **Configure Colour Classification** page



CONFIGURE COLOUR CLASSIFICATION WIZARD

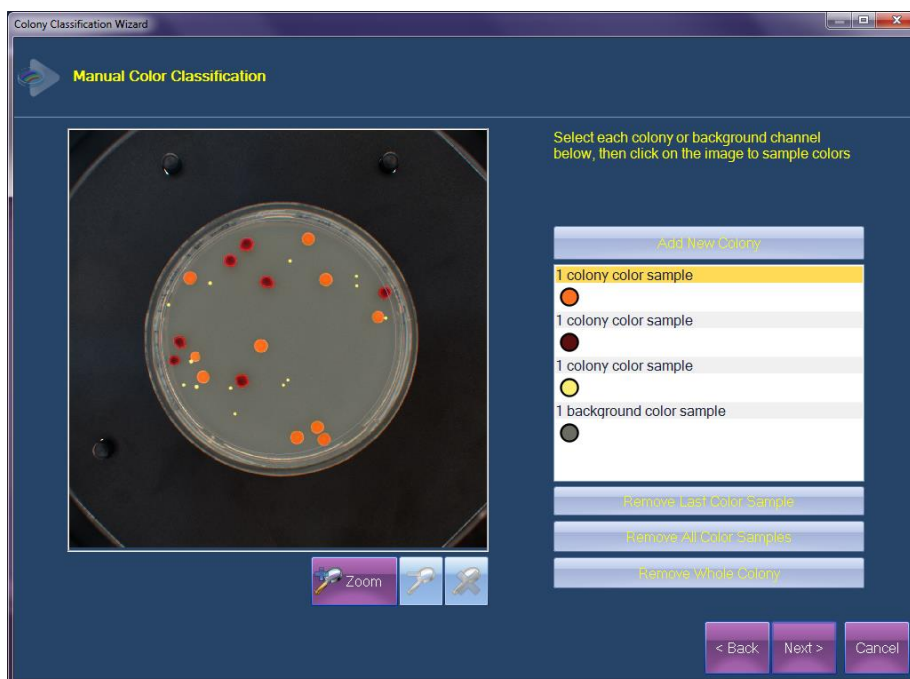
- (b) Ensure the graticule in the Image Pane covers the area that will be used for colour classification; this can be resized by selecting and dragging it, to make it larger or smaller. Adjust the graticule until it sits just inside the circular rings which define the edge / physical structure of the Plate. This will remove these rings from the colour classification process
- (c) Under **Manual Classification** select **Choose Colours in Next Step....** to carry out a manual classification of the colonies on the Plate

The **Manual Colour Classification** page is displayed.



MANUAL COLOUR CLASSIFICATION WIZARD - 1

- (d) Use the **Manual Colour Classification** settings to select the colony colours and the background colour
- Select the colony colour by selecting the clear circle and then selecting the centre of the corresponding colony. Multiple colour samples can be taken to help aid the software in distinguishing different colony colours; this is particularly useful for plates which may have a slight lighting difference from one side of the plate to the other
 - To add another colony select the **Add New Colony** button
 - To remove the last sample colour taken for a colony or background select **Remove Last Colour Sample**
 - To remove all the colour samples taken, select **Remove All Colour Samples**



MANUAL COLOUR CLASSIFICATION WIZARD – EXAMPLE OF COLOUR SELECTION

- (e) Once the number of colours has been selected, select the **Next>** button. The software will now analyse the image and present the results in the **Review Colour Classification** page

The **Review Colour Classification** page is displayed.

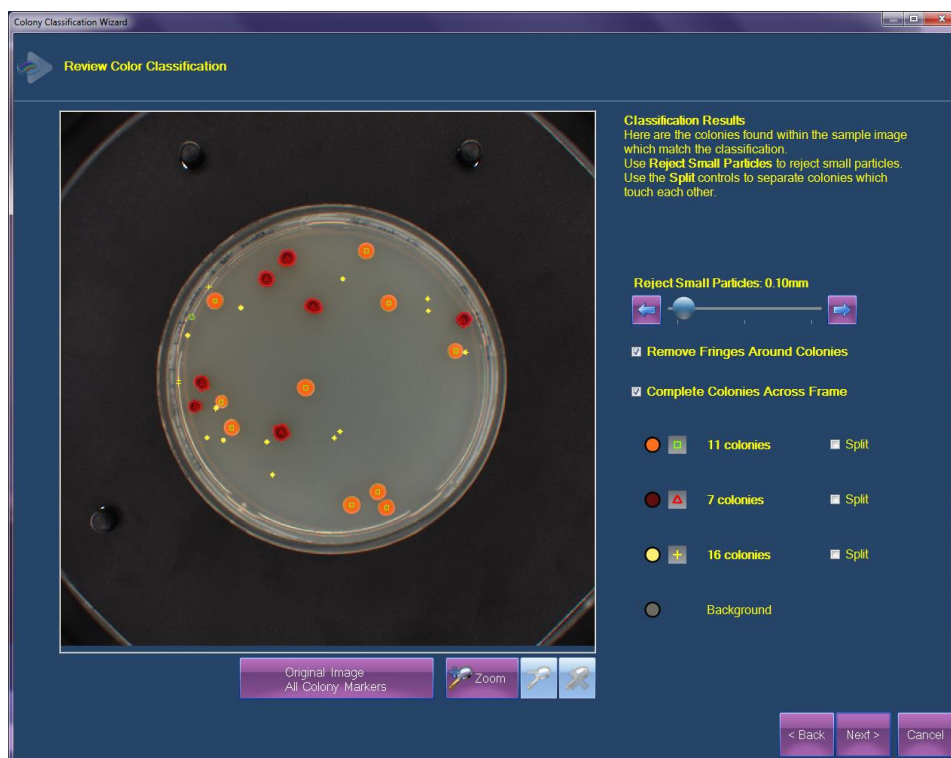


REVIEW COLOUR CLASSIFICATION WIZARD

Note: The **Review Colour Classification** page is displayed after carrying out a manual or an automatic colour classification process. This page displays the results based on the selections made previously.

- Examine the detected colonies and image carefully to check that:
 - Colonies are being detected successfully
 - Different colours of colonies are being distinguished successfully
 - Colonies are distinguished from debris with a different colour

Note: The software will analyse each colony pixel by pixel to determine which colour classification they belong in. Due to this, colonies that have a gradual colour change may be displayed as multiple colonies. To avoid this, check the **Remove Fringes Around Colonies** box.



REVIEW COLOUR CLASSIFICATION WIZARD – SHOWING REMOVE FRINGES AROUND COLONIES FUNCTION

- (f) Ensure the software has identified all colonies present

Note: It may be easier to see the results if the image is zoomed, use the Zoom Controls under the image to zoom in / out.

- (g) Use the **Reject Small Particles** slider to remove the effect of small coloured regions of the image background that may be wrongly interpreted as colonies. Adjust the slider to set the level of size filtering used
- If small debris particles are wrongly detected as colonies, try increasing the **Reject Small Particles** setting
 - If small colonies are being wrongly rejected, try decreasing the **Reject Small Particles** setting
 - Adjust the slider directly or use the left / right arrow buttons



REJECT SMALL PARTICLES SETTINGS

- (h) Use the **Complete Colonies Across Frame** setting to decide whether or not to include in the count colonies that the Plate frame passes through
- To include the affected colonies, select the checkbox
 - To reject the affected colonies, leave the checkbox unchecked
- (i) Use the **Split** setting for each colony colour to prevent overlapping colonies from being counted as a single colony. To split overlapping colonies, select the **Split** checkbox next to each colony type



SPLIT NOT SELECTED



SPLIT SELECTED

- (j) The image display can be changed by selecting the **Original Image All Colony Markers** button and selecting options from the drop-down, default settings illustrated. Select a suitable combination of options; each of the **Marker** options can be applied to each of the **Display** options
- (k) If the colour classification is correct, select the **Next>** button

The **Classification Details** page is displayed (see pg 64 for information about this window).

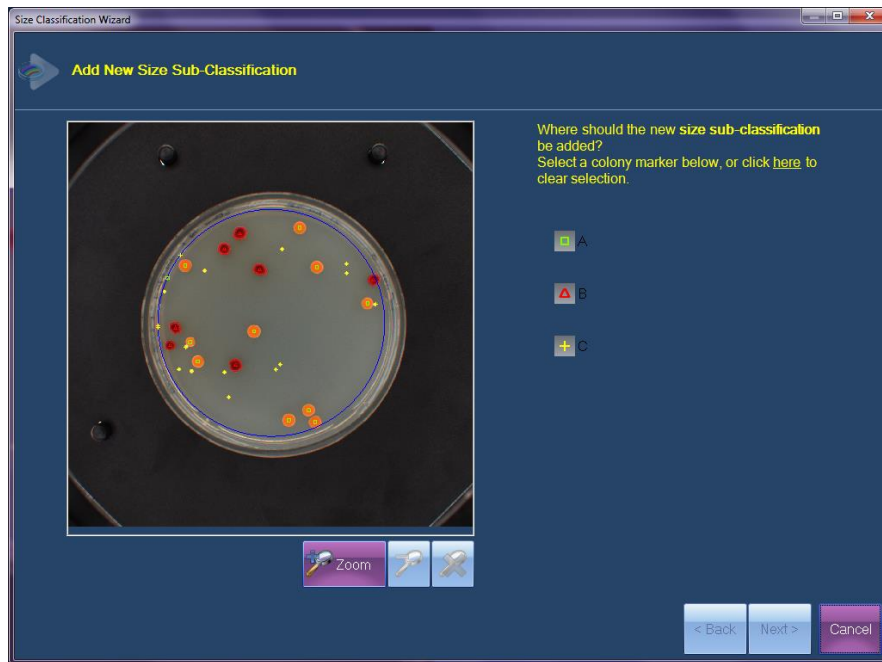
- (l) Select the **Finish** button to complete the colour classification process and return to the main ChromaZona window

Size Sub-Classification

Size classification is used to distinguish between different sizes of a particular colony type, or to set maximum and/or minimum size limits for colonies of that type to be counted.

- (a) To add a size classification to the colony classification, select the **Add Size Classification** button once Colour Classification has been carried out

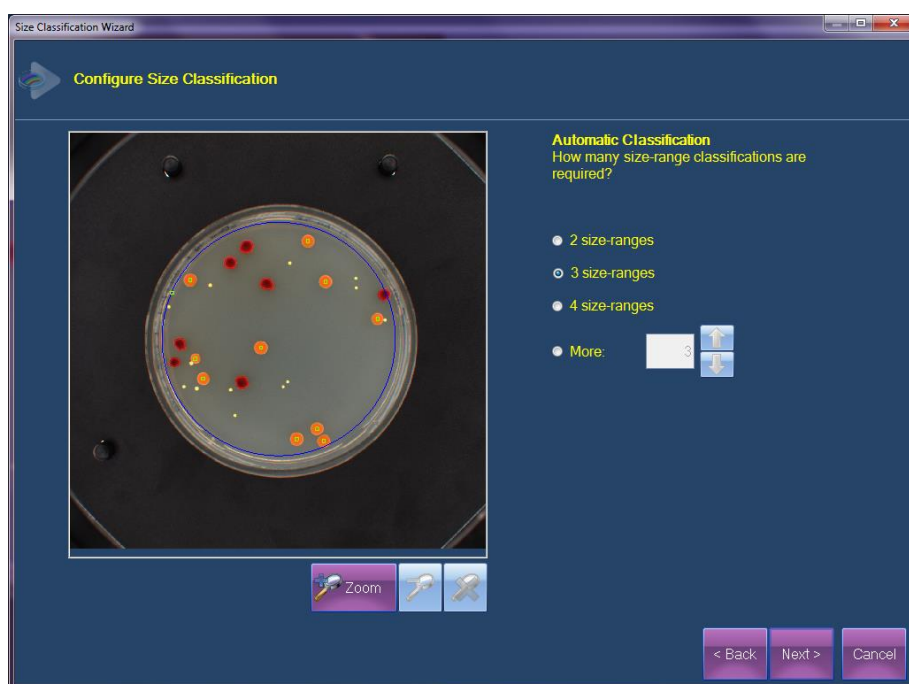
The **Size Classification Wizard** is displayed, opening on the **Add New Size Sub-Classification** page.



SIZE CLASSIFICATION WIZARD-ADD NEW SIZE SUB-CLASSIFICATION

- (b) The first step is to decide where to add the sub-classification, i.e. to which colony type. If there is only one colony type detected this step will be ignored and the Wizard will go straight to the **Configure Size Classification** page
- (c) Select the colony type that is to be size classified, e.g. select the **B** colony
- (d) Select the **Next >** button

The **Configure Size Classification** page is displayed.



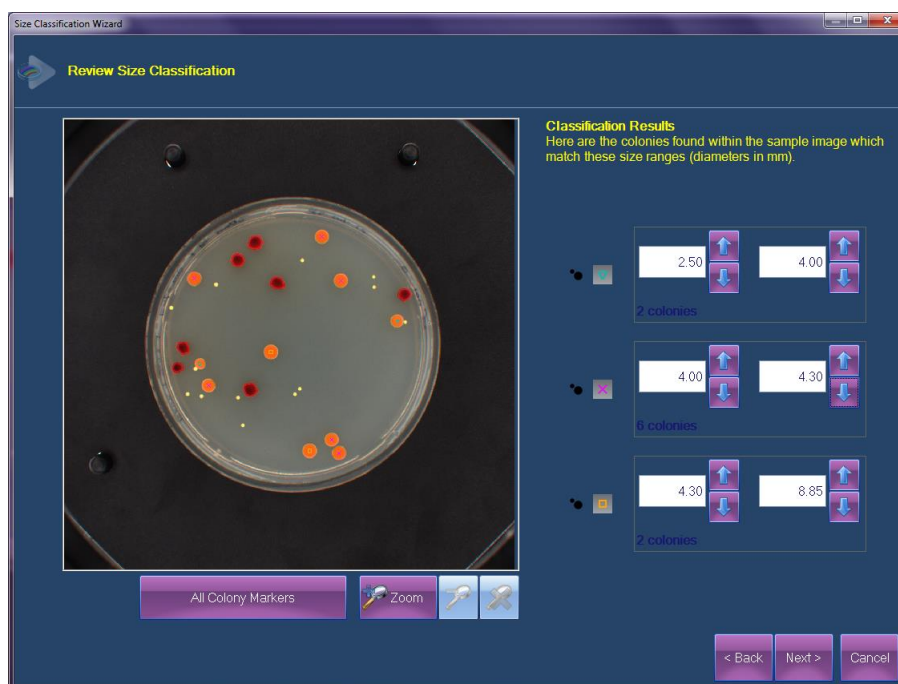
SIZE CLASSIFICATION WIZARD-CONFIGURE SIZE CLASSIFICATION

(e) Select the number of size classifications required:

- Select one of the pre-set **size-ranges** checkboxes
- Select the **More** checkbox and then enter the number of sizes required directly or using the up / down arrows
- For example, select the **2 size-ranges** checkbox

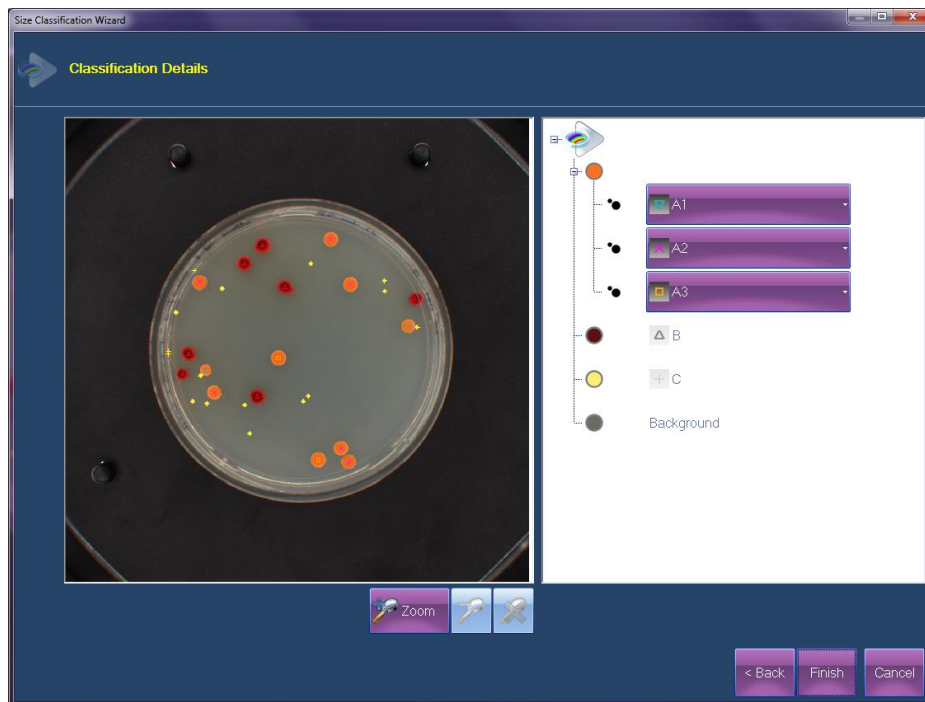
(f) Select the **Next** button to carry out the size classification process

The **Review Size Classification** page is displayed.



SIZE CLASSIFICATION WIZARD-REVIEW SIZE CLASSIFICATION

- (g) Check the marked colonies on the image to see if the size classification result using the automatic size classification process is satisfactory
- Zoom the image to make it easier to view
 - The colony markers beside each size range field indicate on the displayed image the colonies which fall into the different size ranges. The number of colonies classified within each size range is also indicated
 - If necessary, adjust the size ranges, either by entering a figure directly or using the up / down arrows. As the upper limit in a lower range is changed, the lower limit in the next higher range will be changed to match, so that adjacent ranges do not have any size overlap
 - The image display changes to reflect the adjustments made to the size ranges. The number of classified colonies in each size range also changes in the size range fields
- (h) When satisfied with the size classification process, select the **Next** button to display the **Classification Details** page. This has changed to reflect the new size sub-classification added



CLASSIFICATION DETAILS - ADDED SIZE CLASSIFICATION

- (i) The new colony sub-classification buttons can be used in the same way as for colour classification to specify whether each size sub-classification corresponds to colonies or debris. Size sub-classifications cannot be identified as background
- (j) In addition, colony markers can be changed and a name can be entered
- (k) If happy that the size sub-classification process is as good as possible, select the **Finish** button to complete the size sub-classification process and return to the main screen

Shape Sub-Classification

Shape classification is used to classify different shapes of colony as different types or classes of colony, or to distinguish between colonies and unevenly shaped debris.

- (a) To add a shape classification to the colony classification, select the **Add Shape Classification** button once Colour Classification has been carried out

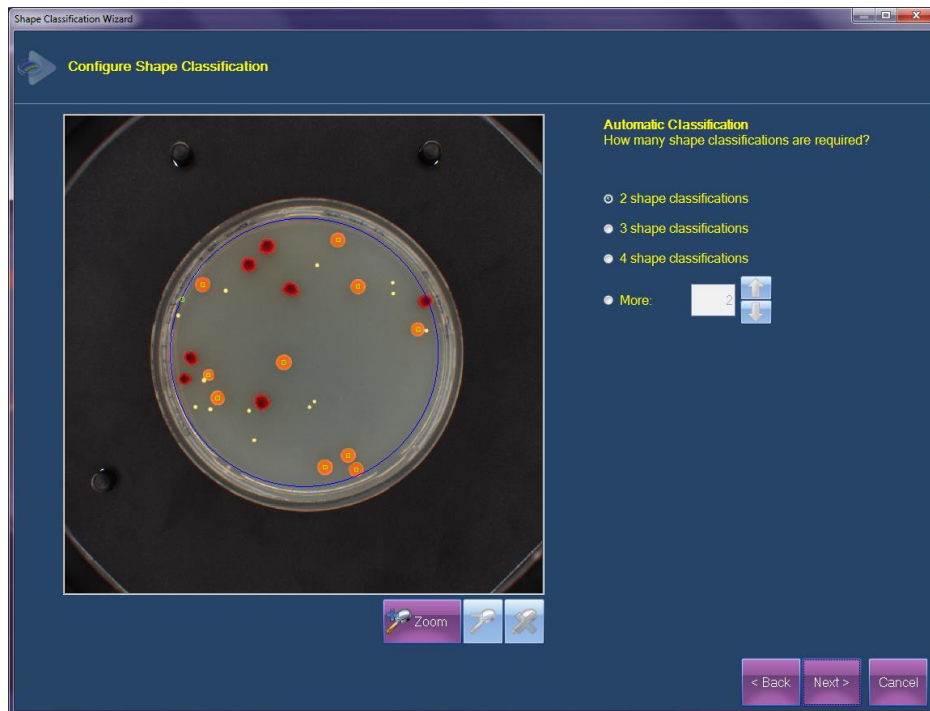
The **Shape Classification Wizard** is displayed, opening on the **Add New Shape Sub-Classification** page.



SHAPE CLASSIFICATION WIZARD-ADD NEW SHAPE SUB-CLASSIFICATION

- (b) The first step is to decide where to add the sub-classification, i.e. to which colony type. If there is only one colony type detected this step will be ignored and the Wizard will go straight to the **Configure Shape Classification** page
- (c) Select the colony type that is to be shape classified, e.g. select the **C** colony
- (d) Select the **Next** button

The **Configure Shape Classification** page is displayed.



SHAPE CLASSIFICATION WIZARD-CONFIGURE SHAPE CLASSIFICATION

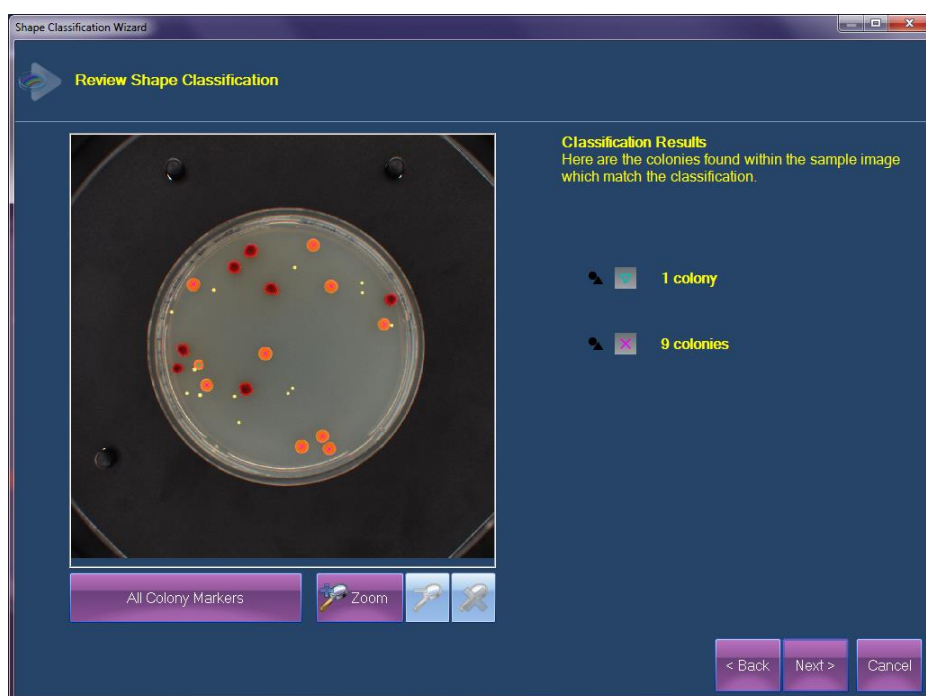
(e) Select the number of shape classifications required:

- Select one of the pre-set **shape classifications** checkboxes
- Select the **More** checkbox and then enter the number of shapes required directly or using the up / down arrows
- For example, select the **2 shape classifications** checkbox

(f) Select the **Next** button to carry out the shape classification process

(Continued on next page)

The **Review Shape Classification** page is displayed.



SHAPE CLASSIFICATION WIZARD-REVIEW SHAPE CLASSIFICATION

- (g) Check the marked colonies on the image to see if the shape classification result using the automatic shape classification process is satisfactory
- Zoom the image to make it easier to view
 - The colony markers indicate on the displayed image the colonies which fall into the different shapes. The number of colonies classified within each shape is also indicated
 - If the results are not satisfactory, select the **Back** button to return to the **Configure Shape Classification** page, and try selecting a different number of shape sub-classifications
- (h) When satisfied with the shape classification process, select the **Next** button to display the **Classification Details** page. This has changed to reflect the new shape sub-classification added



ADDED SHAPE CLASSIFICATION

- (i) The new colony sub-classification buttons can be used in the same way as for colour classification to specify whether each shape sub-classification corresponds to colonies, debris or background
- (j) In addition, colony markers can be changed and a name can be entered
- (k) If happy that the shape sub-classification process is as good as possible, select the **Finish** button to complete the shape sub-classification process and return to the main screen

USING PLATE NAMING SOURCES – EXTERNAL & MANUAL

By default, in the **Plate ID** field the **Auto-Increment** checkbox is checked. The ChromaZona software then takes the base Plate ID entered previously and adds an auto-indexing digit element to the end of it, or, if the base Plate ID already includes a digit element, it will automatically index this element. If two or more digit elements are included in the base Plate ID, the software auto-indexes the right most element.

BASE PLATE ID	FIRST PLATE IDENTIFIER	SECOND PLATE IDENTIFIER	THIRD PLATE IDENTIFIER
abc	abc1	abc2	abc3
abc18	abc19	abc20	abc21
abc18def	abc19def	abc20def	abc21def
66abc18def	66abc19def	66abc20def	66abc21def
SpiralPlate01	SpiralPlate02	SpiralPlate03	SpiralPlate04

Barcode Reader

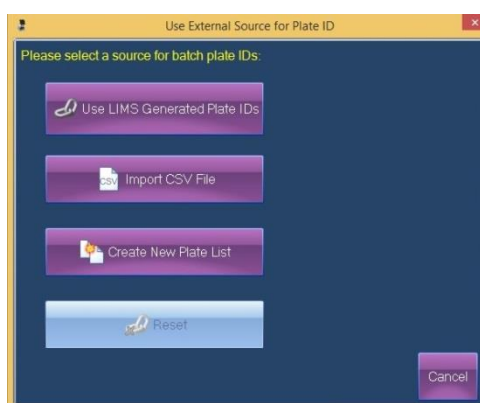
A Barcode Reader can be used to read in data from externally generated barcodes attached to the Plates to be imaged. In this case, uncheck the **Auto-Increment** checkbox and check the **Use Barcodes** checkbox. The Barcode Reader can be used to read in Plate identifier or identifier plus dilution information from an external source of Plate identifiers. It can also be used to read in manual Plate identifiers, in barcode form. Make sure that a Barcode Reader is attached to the PC.

Plate identifier and dilution information can be read in from an external source, either:

- A Laboratory Information Management System (LIMS)
- A comma separated values (CSV) file
- A Plate list created using ChromaZona

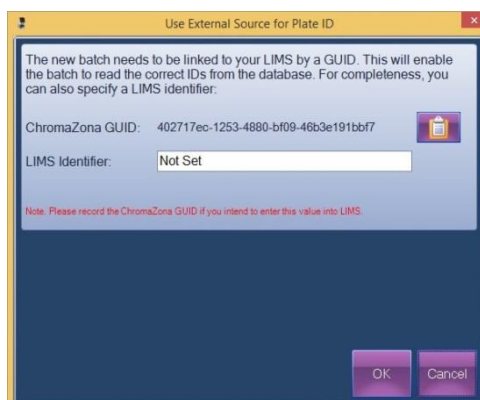
Reading from a LIMS system

- (a) In the **Plate List** field, select the **Configure** button. The **Use External Source for Plate ID** pop-up appears



USE EXTERNAL SOURCE FOR PLATE ID POP-UP

- (b) To use a LIMS, select the **Use LIMS Generated Plate IDs** button
- (c) A modified **Use External Source for Plate ID** pop-up appears




LIMS USE EXTERNAL SOURCE FOR PLATE ID POP-UP

- (d) In order for the Batch to read the correct information from the LIMS, the Batch must be linked to the LIMS. This is done using a Globally Unique Identifier (GUID). A GUID is generated by the ChromaZona software. To fully identify the plates in the Batch, the GUID needs to be added to the LIMS sourced ID in the ChromaZona database. The ChromaZona and the LIMS database tables can be linked by entering a LIMS Identifier. The details of how to add the **LIMS Identifier** and the information required depend on the LIMS
- (e) As an example, the following SQL code could be used to add the Plate Identifier 'Plate 1' which has a dilution of '1:10' to the ChromaZona database for a Batch that has been assigned the GUID 'a94d6efd-1fe1-4285-a14f-0cb77e680363':

```
INSERT INTO Lims_plates (PlateID, GUID)
VALUES ('1:10','Plate1',' a94d6efd-1fe1-4285-a14f-0cb77e680363')
```

If a LIMS Identifier is added:

```
INSERT INTO Lims_plates (Dilution, PlateID, GUID, LimsID)
VALUES ('1:10','Plate1',' a94d6efd-1fe1-4285-a14f-0cb77e680363','xfs')
```

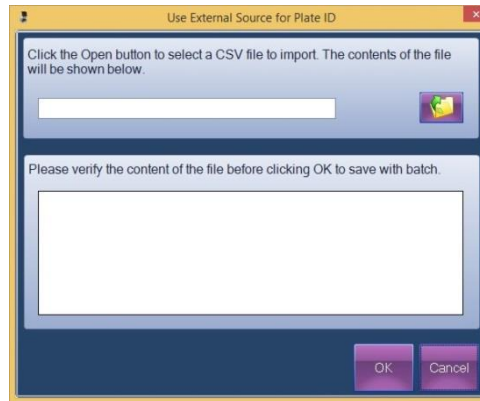
- (f) The GUID can be copied to the Clipboard by selecting the  button.
- (g) The LIMS Identifier should be entered into the **LIMS Identifier** field.
- (h) Select the **OK** button.
- (i) The **Plate List** changes to reflect that a LIMS has been selected as the source of external Plate IDs.




LIMS SELECTED AS EXTERNAL SOURCE OF PLATE IDENTIFIERS

Reading from a CVS file

- (a) To use a CSV file, select the **Import CSV File** button.
- (b) A modified **Use External Source for Plate ID** pop-up appears.



CSV FILE USE EXTERNAL SOURCE FOR PLATE ID POP-UP

- (c) Select the open  button to navigate to and select an external CSV file
- (d) Select **Open** to open the CSV file
- (e) The contents of the selected (Plate Identifiers) CSV file will be displayed in the preview pane in the lower portion of the pop-up
- (f) Check the contents of the preview pane and if correct select the **OK** button
- (g) Repeat the steps to load a (Dilutions) CSV file
- (h) The Plate Identifiers and associated Dilutions will be displayed in the preview pane
- (i) Check the contents of the preview pane and if correct, select the **OK** button
- (j) The **Plate List** changes to reflect that a CSV file has been selected as the source of external Plate IDs



CSV FILE SELECTED AS EXTERNAL SOURCE OF PLATE IDENTIFIERS


Reading From a Manually Created List

- (a) To create a new list of Plates manually, select the Create New Plate List button
- (b) A modified **Use External Source for Plate ID** pop-up appears.

MANUAL PLATE LIST USE EXTERNAL SOURCE FOR PLATE ID POP-UP


- (c) This pop-up is used to manually create a custom Plate list. Each Plate will be given a unique identifier and a dilution. Identifiers do not have to include any sequential elements and dilutions can be different for each Plate
- (d) Enter an identifier for the first Plate in the long field in the upper part of the pop-up.

- (e) Enter the dilution ratio for the first Plate using the two ratio number operators in the upper part of the pop-up. In the first field the ratio number is entered directly, though normally the default value of 1 will be acceptable. In the second field enter the ratio number directly or use the + and - buttons to enter the required value.

Note: The left hand box represents the sample proportion, the right hand box represents the total volume. A ratio of 1:10 means 1 part sample in a total volume of 10 parts. Therefore the left hand number must be smaller than the right hand number. Once the identifier and dilution ratio have been entered select the  button. The entered Plate identifier and dilution information will appear in the **Plate ID list** in the lower part of the pop-up.

- (f) Enter the identifier and dilution information for the rest of the Plates in the list

EXAMPLE OF A MANUAL PLATE LIST

- (g) Plates can be removed from the list by selecting them in the list and selecting the  button.
- (h) Check the contents of the **Plate ID list** and if correct, select the **OK** button.
- (i) The **Plate List** changes to reflect that a CSV file has been selected as the source of external Plate IDs.



EXTERNAL FILE SELECTED AS EXTERNAL SOURCE OF PLATE IDENTIFIERS

Removing an External Source

To cancel the use of an external source for the Plate identifiers and dilutions, in the **Plate List** field, select the **Configure** button and then the **Reset** button on the **Use External Source for Plate ID** pop-up.

BATCH CREATION AND USE

Introduction

As stated in the Overview, the ChromaZona application uses a Batch to define and contain a set of instrument settings, and to store the results of the measurements made using those settings, when making measurements on a series of plates that have the same types of colony / zone, use the same medium and are of the same type. This simplifies the process of running repeated measurements.

Before a User can take any measurements, there must be a Batch open in the application. This is done by opening an existing Batch or by creating a new Batch. A new Batch can be created by defining a completely new set of settings or can be created by modifying the settings of an existing Batch. Once defined, the new Batch must be accepted (saved) and can then be used to take measurements.

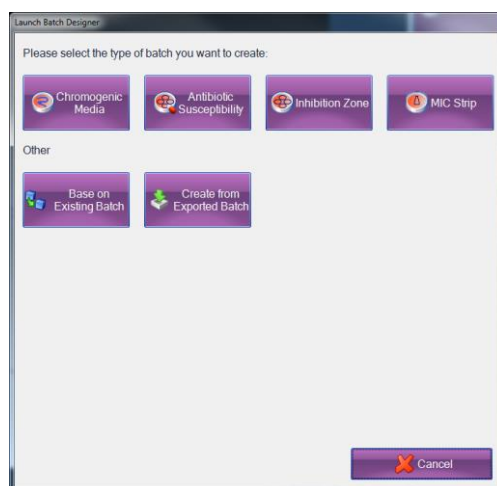
Each Batch contains the settings and results for detecting and counting / measuring the colonies / zones on a series of plates that have the same types of colony / zone, use the same medium and are of the same type.

ChromaZona automatically saves the results of any measurements in the currently open Batch.

Creating and using a Chromogenic Media Batch

1. On the **Home Screen** select the **New Batch** button.

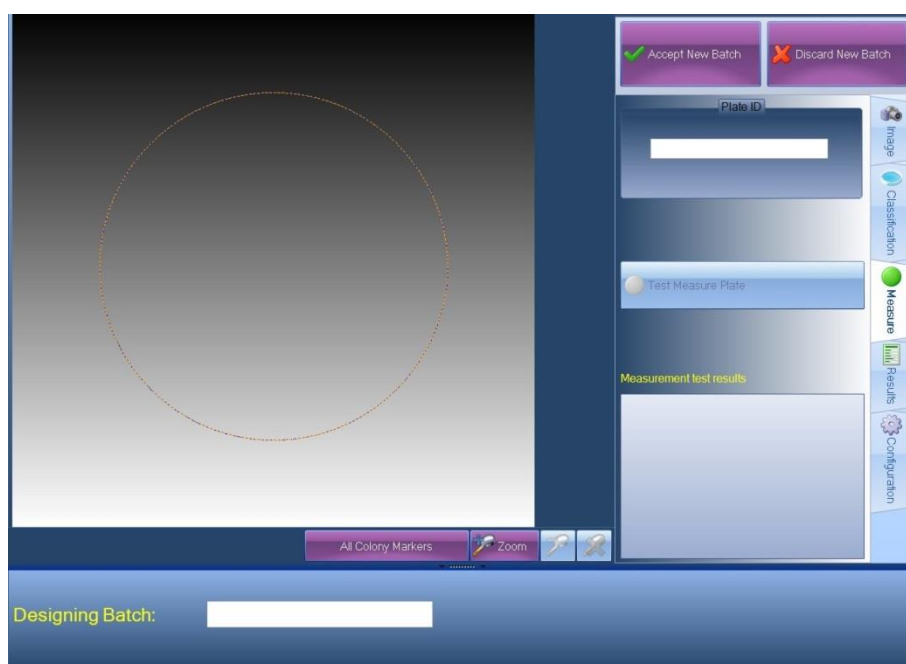
The **Launch Batch Designer** pop-up appears.



LAUNCH BATCH DESIGNER POP-UP

2. Select the **Plate Configuration** button and adjust the parameters to the required plate types
3. On the **Launch Batch Designer** pop-up select the **Chromogenic Media** button.

The **Chromogenic Media Home Screen** appears.



CHROMOGENIC MEDIA HOME SCREEN

4. In the **Designing Batch:** field enter a name for the new Batch
5. In the **Plate ID** field, enter a Plate ID for the first Plate in the new Batch. If using a barcode reader, select the **Configuration** tab and check the barcode checkbox. If using a LIMS or CVS file system select configure and then select which system you are using (see pg 66, USING AN EXTERNAL PLATE NAMING SOURCE)



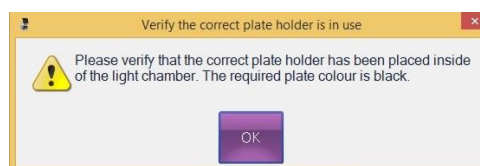
CHROMOGENIC MEDIA EXTERNAL PLATE LIST SELECTED

6. Select the **Results** tab



RESULTS TAB

7. Use the checkboxes beside each parameter to determine which parameters appear in the Results Table. The order of the parameters in the list is the order in which the parameters will appear in the Results Table
8. To change the order of the parameters in the list, and therefore also the order in which the parameters appear in the Results Table, select a parameter and use the up/down arrow buttons at the bottom of the pane to move the parameter up or down in the list
9. Select the **Accept New Batch** button
10. A dialogue box appears requesting confirmation that the correct Plate Holder is installed



CORRECT PLATE HOLDER DIALOGUE BOX

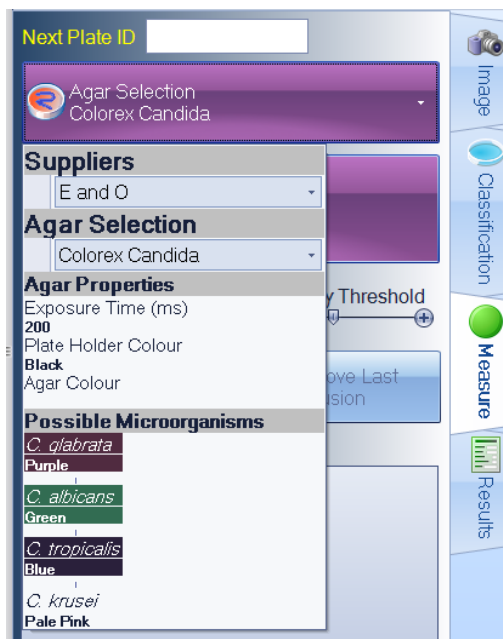
11. If the correct Plate Holder is in the instrument, select the **OK** button
12. The **Measure** tab opens



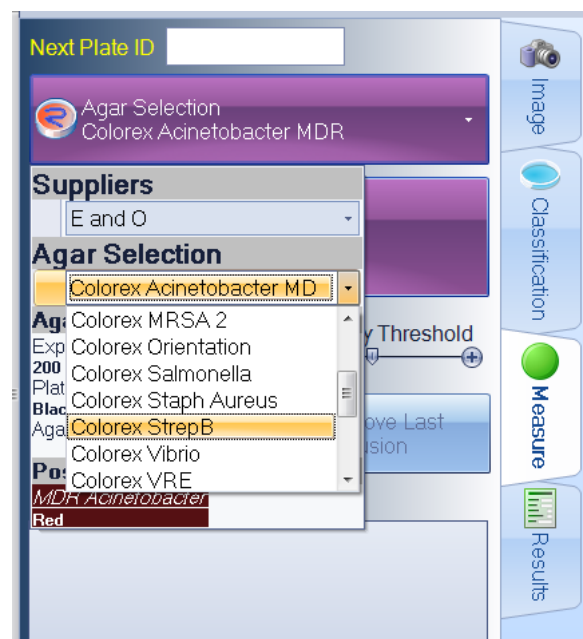
MEASURE TAB

13. Select the **Agar Selection** button.

14. The **Agar Selection** menu appears.



AGAR SELECTION MENU



EXAMPLE AGAR SELECTIONS

15. Select a supplier using the **Suppliers** drop-down

The supplier options are:

- CHROMagar
- E and O
- HARDY
- Thermo Scientific

Select an agar type using the **Agar Selection** drop-down. The selection varies, depending on the supplier selected:

CHROMagar	E and O	HARDY	Thermo Scientific
Acinetobacter	Colorex Acinetobacter MD	HardyCHROM O157	Brilliance Candida
E. coli	Colorex Candida	HardyCHROM Candida	Brilliance CRE
E. sakazakii	Colorex ESBL	HardyCHROM CRE	Brilliance UTI
ECC	Colorex KPC	HardyCHROM ECC	Brilliance UTI Clarity
ESBL	Colorex Listeria	HardyCHROM ESBL	--
KPC	Colorex MRSA 2	HardyCHROM HUrBi	--
Listeria	Colorex Orientation	HardyCHROM Listeria	--
O157	Colorex Salmonella	HardyCHROM MRSA	--
Orientation	Colorex Staph Aureus	HardyCHROM MRSA Bi	--
Pseudomonas	Colorex StrepB	HardyCHROM Sakazakii	--
Rambach	Colorex Vibrio	HardyCHROM Salmonella	--
Salmonella	Colorex VRE	HardyCHROM SS	--
Staph Aureus	Colorex O157	HardyCHROM Staph	--
STEC	Colorex O157 CT	HardyCHROM UTI	--
StrepB	Primary UTI	HardyCHROM Vibrio	--
--	Primary UTI Opaque	--	--
--	TBX	--	--

16. Select the **Image** tab.

17. Select an image source.

- To capture a live image, insert a plate and select the **Capture Image** button.
- If there is no Plate in the Light Chamber or if there is no camera available (internal or external) a **No Image** dialogue box is displayed. If there is no camera available or an external image is to be used select import an image from file (see pg. 34, IMAGE FUNCTIONS)



NO IMAGE DIALOGUE BOX

- To calibrate the camera to the imported image, select the **Yes** button on the **Import Image** pop-up and follow the instructions. Refer to **Calibrating an Image** in IMAGE FUNCTIONS.

18. Select the **Measure** tab.

19. Select the **Test Measure Plate** button to perform a test count using the parameters specified.

- Check the image to make sure that the colonies have been correctly detected and distinguished from the background and any debris on the plate.
- The results are shown in the **Last measurement results** field.

20. Adjust the **Probability Threshold** slider to ensure detection of all colonies.




PROBABILITY THRESHOLD

21. If one or more areas of the Plate have a problem, e.g. a label or other artefact, one or more exclude regions can be created. Exclude regions are defined using the **Exclude Regions** function (see pg. 105, MANUAL EDITING OF PLATES)

22. Select the **Measure Plate** button and then add exclusion regions if required



MEASURE PLATE SELECTED

(b) Adjust the size of the message pane by dragging the message pane resize icon  until all of the results are visible.

(c) Select a result in the results table. The display changes - the **Edit/Review** tab is added.



EDIT/REVIEW TAB ADDED

23. Selecting a single detected colony type in the Results field changes the image display. Only the relevant colony type is highlighted on the image, the highlights are removed from the other colony types.



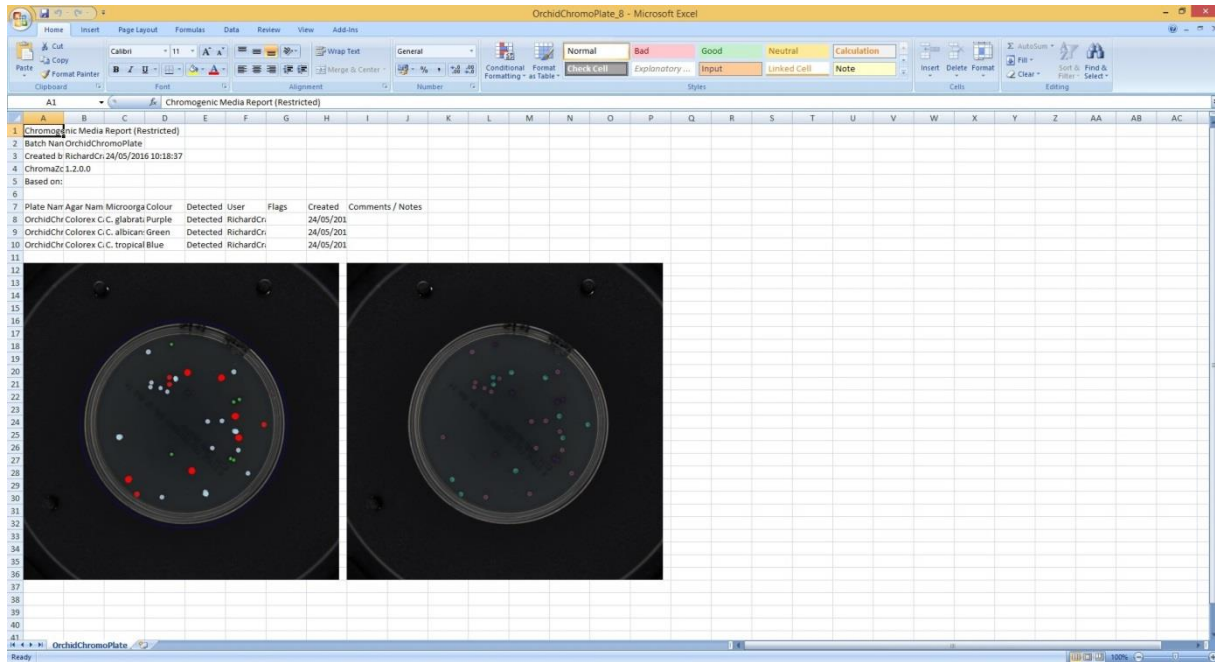
SINGLE COLONY TYPE SELECTED

24. To print out a copy of the Plate details, select the **Print Plate Report** button below the Plate image, and select **Print Plate Report** from the drop-down.



PRINT PLATE REPORT DROP-DOWN

- (j) ChromaZona opens Microsoft Excel and populates cells with the Plate Report details. An example is shown below.

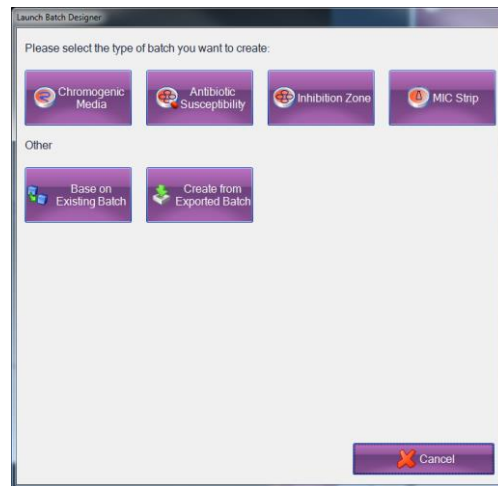


EXAMPLE PLATE REPORT

Creating and using an Antibiotic Susceptibility Batch

1. On the **Home Screen** select the **New Batch** button.

The **Launch Batch Designer** pop-up appears.



LAUNCH BATCH DESIGNER POP-UP

2. Select the **Plate Configuration** button and adjust the parameters to the required plate types
3. On the **Launch Batch Designer** pop-up select the **Antibiotic Susceptibility** button

The **Antibiotic Susceptibility** home screen will appear



ANTIBIOTIC SUSCEPTIBILITY HOME SCREEN

4. In the **Designing Batch:** field enter a name for the new Batch

5. In the **Plate ID** field, enter a Plate ID for the first Plate in the new Batch. If using a barcode reader, select the **Configuration** tab and check the barcode checkbox. If using a LIMS or CVS file system select configure and then select which system you are using (see pg 66, USING AN EXTERNAL PLATE NAMING SOURCE)
6. Select the **Image** tab.
7. Select an image source.
 - (a) To capture a live image, insert a plate and select the **Capture Image** button.
 - (d) If there is no Plate in the Light Chamber or if there is no camera available (internal or external) a **No Image** dialogue box is displayed. If there is no camera available or an external image is to be used select import an image from file (see pg 34, IMAGE FUNCTIONS)



NO IMAGE DIALOGUE BOX

- (e) To calibrate the camera to the imported image, select the **Yes** button on the **Import Image** pop-up and follow the instructions. Refer to **Calibrating an Image** in **IMAGE FUNCTIONS**.
8. Select the **Zone Classification** button

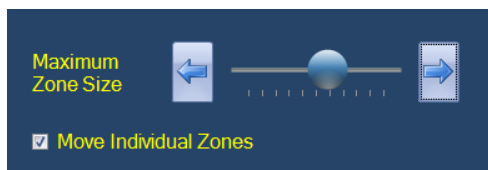
The **Configure Zone Frame** window opens.



CONFIGURE ZONE FRAME WINDOW

9. Select the layout and number of zones required for the plate being measured

Note: Once the number of zones has been selected for the batch, that will be the number measured in further measurements. For plates with more or less zones than the created batch, a new batch would need to be created.



ZONE EDITING FUNCTIONS

10. Adjust the placement of the zones so the letter for each zone lies directly above the disc/well. Move Individual Zones by clicking on the Move Individual Zones button
11. Increase or decrease the Zone size using the **Maximum Zone Size** adjustable slider. Ensure the zone size is larger than the zone present so the software can correctly measure the zone size.
12. Once all zones have been placed, click **Next >**

The **Configure Type of Analysis** screen will appear.



CONFIGURE TYPE ANALYSIS WINDOW

13. Select if the plate contains Disc/ Wells in each zone or if there are no Disc/ wells present
14. If there are Disc/ Wells present, the size needs to be set so the software can read the zones present. Disc/ Well size can be entered manually or by using the up/ down arrows. The software will automatically position a disc diameter over one of the Disc/ Wells present on the plate, this can be moved to be more centred by using the mouse and clicking on the centre of the circle

Note: Ensure the Disc/ Well diameter is larger than the Disc/ Well on the image to aid the software in calculating the zone diameter.

15. Once the Disc/ Well size is set, click **Next >**

The **Identify Zone Colours** screen will appear



IDENTIFY ZONE COLOURS WINDOW

16. Select the Disc, Zone/Edge and background colour:

- Select the Disc Colour by selecting the clear circle and then selecting the centre of one of the discs present
- Select the Zone/Edge colour by selecting the clear circle and then selecting anywhere within one of the zones present on the plate being measured
- Select the background colour by selecting the clear circle and then selecting anywhere on the background colony growth

Note: Multiple colour samples can be taken to help aid the software in distinguishing different colony colours. However, this is only recommended if the software encounters issues identifying zones. Normal procedure is to sample one colour to identify each area. Multiple colour samples can be particularly useful for plates which may have a slight lighting difference from one side of the plate to the other.

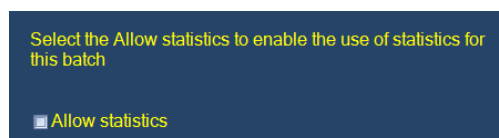
(Continued on next page)



IDENTIFY ZONE COLOURS WINDOW WITH COLOUR EXAMPLES

17. Once the colours for each section have been selected, click **Next >**

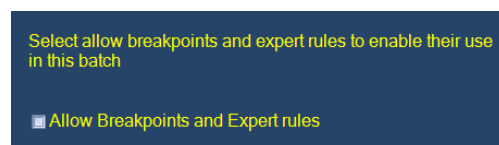
The **Configure Statistics** screen appears.



ALLOW STATISTICS CHECKBOX

18. If using a statistics package to analyse results, select the **Allow Statistics** checkbox and then click **Next >**

The **Configure Breakpoints and Expert Rules** screen will appear.



ALLOW BREAKPOINTS AND EXPERT RULES CHECKBOX

19. To apply standard breakpoints and expert rules select the **Allow Breakpoints and Expert Rules** checkbox, then click **Next >**

The software will analyse the image and open the **Review Zone Measurement** window



REVIEW ZONE MEASUREMENT WINDOW

20. Ensure the measured zones are aligned correctly and then click **Finish**

The batch home screen will appear with the measure tab highlighted.

21. Click on the **Measure** tab

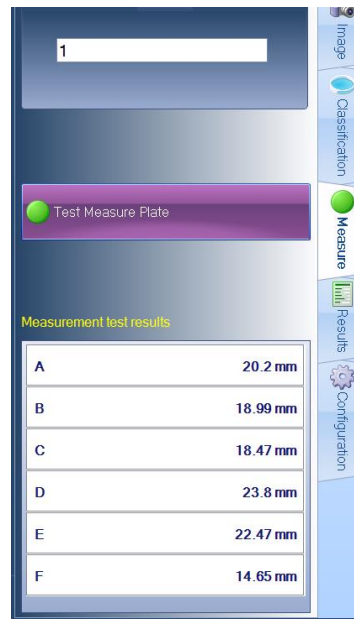
The **Measure** screen will appear.



MEASURE SCREEN

22. Click on **Test Measure Plate**

Note: A test measure of the plate must be performed before the software will allow the new batch to be accepted.



The screenshot shows a software window with a sidebar on the right containing icons for Image, Classification, Measure, Results, and Configuration. The main area displays a 'Test Measure Plate' button and a table of measurement test results.

Measurement test results	
A	20.2 mm
B	18.99 mm
C	18.47 mm
D	23.8 mm
E	22.47 mm
F	14.65 mm

MEASUREMENT TEST RESULTS TABLE

23. Once a test measure has been performed, click **Accept New Batch**

The batch will be saved and measurements can begin.

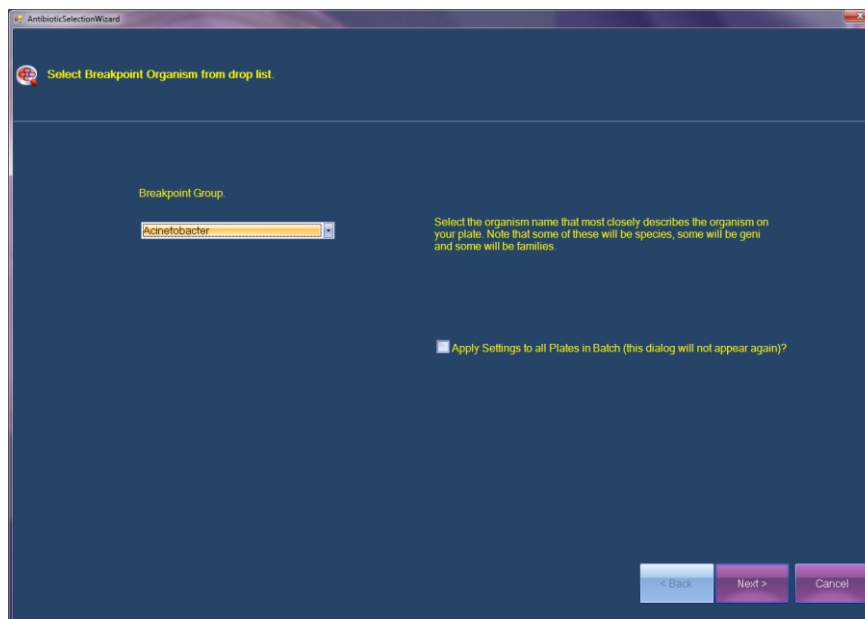
The batch measurement screen will appear.



AST BATCH MEASURE SCREEN

24. Click **Measure Plate**

The Antibiotic Selection Wizard screen opens.



ANTIBIOTIC SELECTION WIZARD SCREEN

25. Select the **Breakpoint Group** from the drop down menu

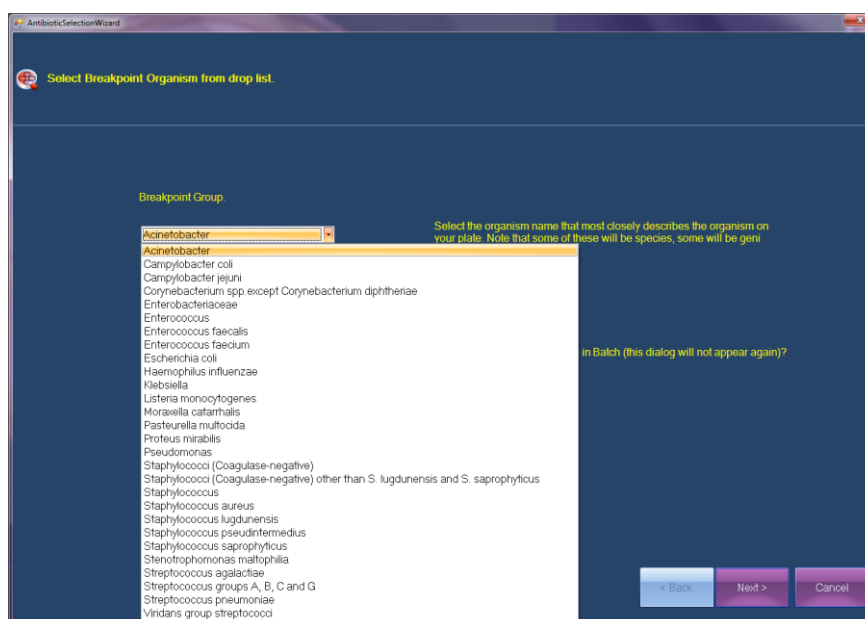
Note: The drop down menu will contain bacteria genus's and individual bacteria names.

26. Select if settings are to be applied to all plates

☐ Apply Settings to all Plates in Batch (this dialog will not appear again)?

APPLY SETTINGS TO ALL PLATES IN BATCH OPTION

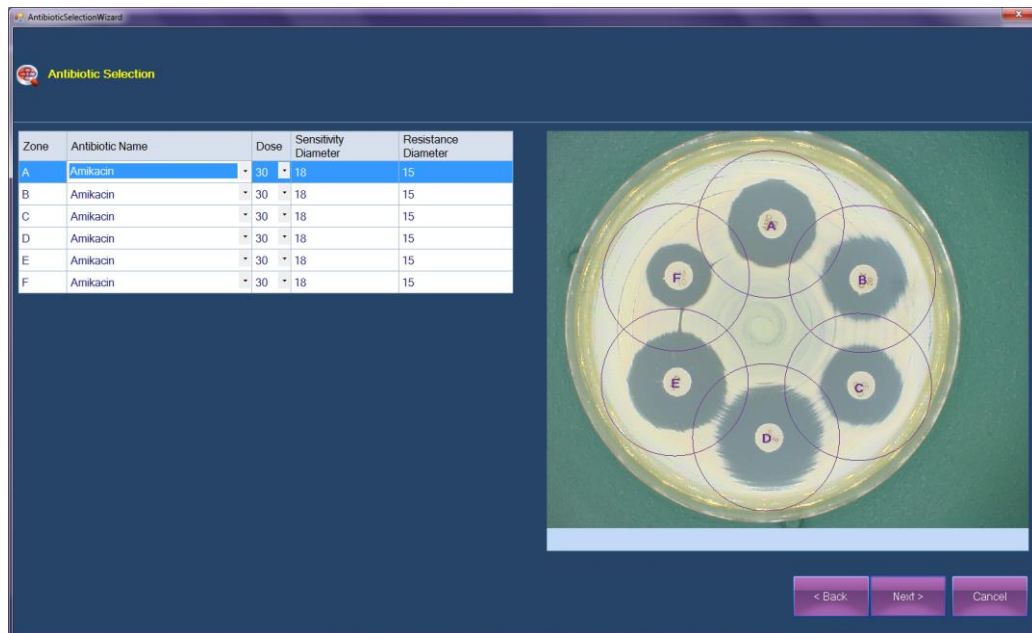
Note: Selecting Apply Settings to All Plates in Batch will **not appear again if selected**. All further plates in the batch will be for the same breakpoint organism and applied antibiotics.



SELECT BREAKPOINT ORGANISM DROP DOWN

27. Once selections have been made, click **Next >**

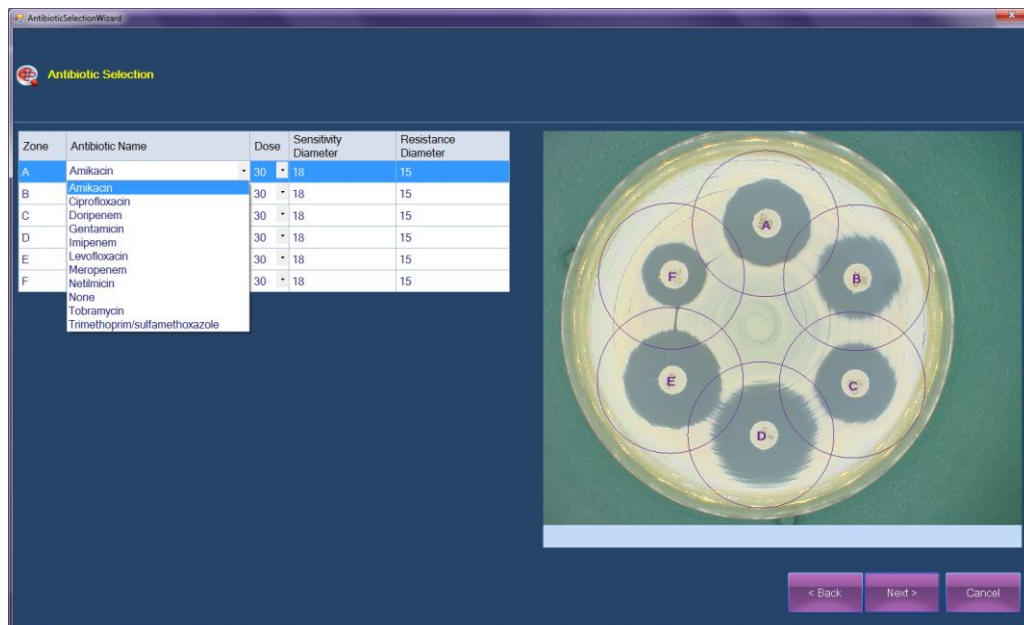
The Antibiotics Selection screen will appear.



ANTIBIOTIC SELECTION SCREEN

28. Select which antibiotics are present in each zone by using the drop down menu

Note: Antibiotic selection is specific to the breakpoint organism selected. Eg only antibiotics known to have an effect on the selected breakpoint organism will appear in the drop down menu.



ANTIBIOTIC SELECTION DROP DOWN

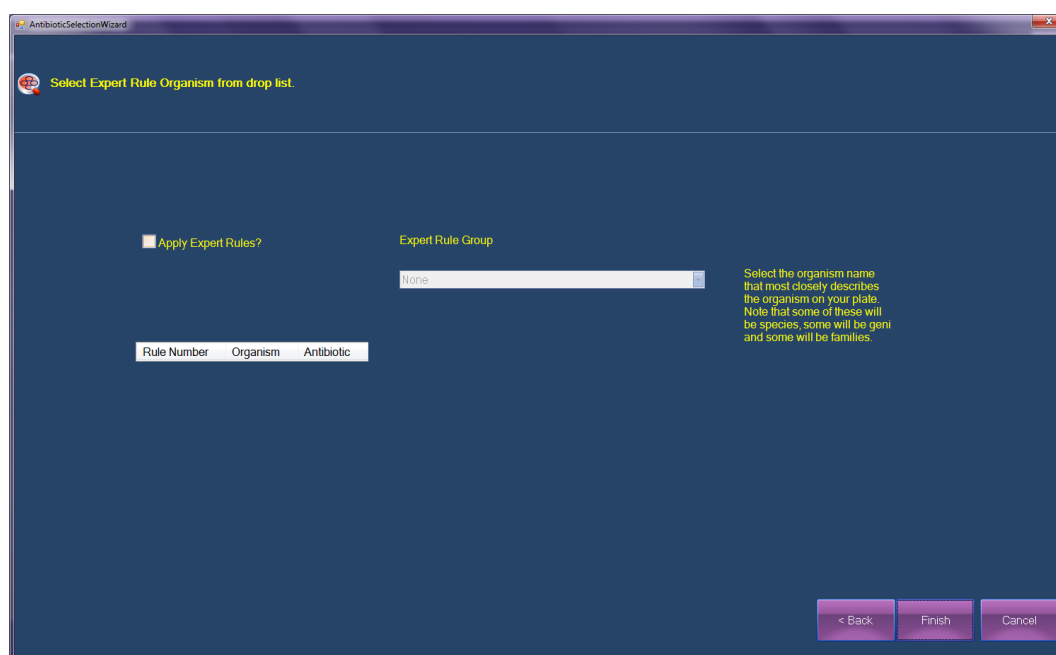
29. When an antibiotic is selected, pre-programmed doses, sensitivity and resistance diameters will be applied according to EUCAST guidelines. These appear in the table next to the selected antibiotic



ANTIBIOTIC SELECTION SHOWING SPECIFIC VALUES FOR DOSE AND SENSITIVITY & RESISTANCE DIAMETERS PER ANTIBIOTIC

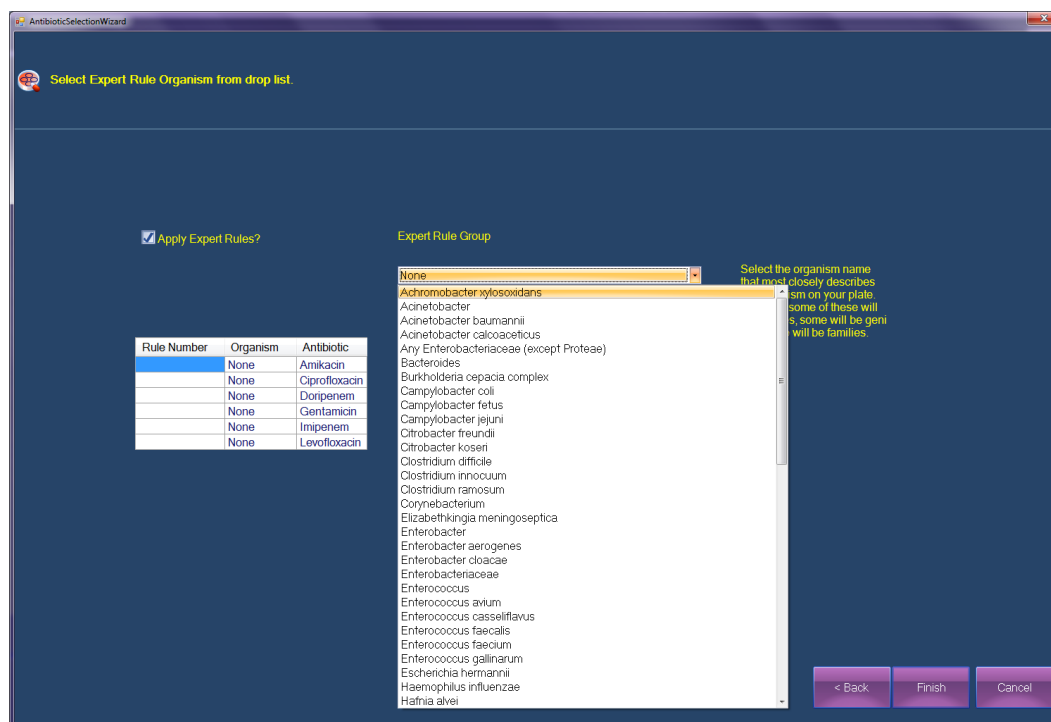
30. Once antibiotic selection is complete, click **Next >**

The **Select Expert Rule Organism from Drop List** screen will appear.



EXPERT RULES SCREEN

31. Select **Apply Expert Rules** and select the tested organism from the drop down menu



EXPERT RULES ORGANISM DROP DOWN

Note: Once the organism has been selected then the expert rules for each antibiotic will be applied and displayed.

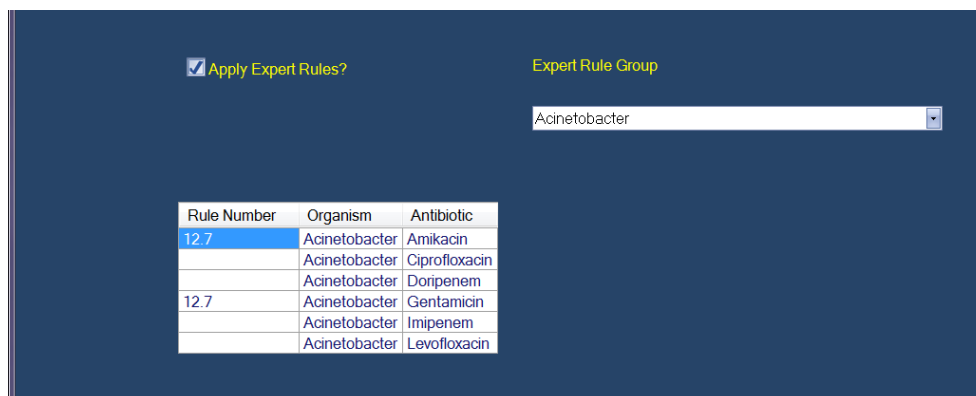


TABLE DISPLAYING EXPERT RULES FOR SELECTED ANITBIOTICS BASED ON ORGANISM SELECTED

32. Click **Finish**

The plate will be analysed by the software and results displayed in the side bar and below the plate image in the results window.



BATCH HOME SCREEN SHOWING PLATE RESULTS

The results window will display the Antibiotic Susceptibility calculated from the sensitivity and resistance diameter.

33. To read the next plate within the batch, remove the current plate and replace with the next within the ChromaZona imaging platform, then click **Measure Plate**

Note: If **Apply Settings to All Plates in a Batch** was selected when creating the measurement settings, this menu will not appear again when measuring further plates. All subsequent measurements of new plates will have the same settings applied e.g. the same organism and antibiotics as well as the location of the antibiotics. To reactivate the option to change the settings for each plate select **Disable Apply AST to All Plates** (see pg. 20 for instructions).

34. To access a plate report spreadsheet, click on the required plate within the results window, this will make the **Edit/ Review** tab appear.



SELECTED PLATE RESULTS WITHIN MEASURE SCREEN

35. Select the **Edit/ Review** tab

The **Edit/ Review** screen will appear



EDIT/ REVIEW SCREEN

36. Select **Print Plate Report**

Selecting this option will automatically open an Excel spreadsheet containing the results for that specific plate

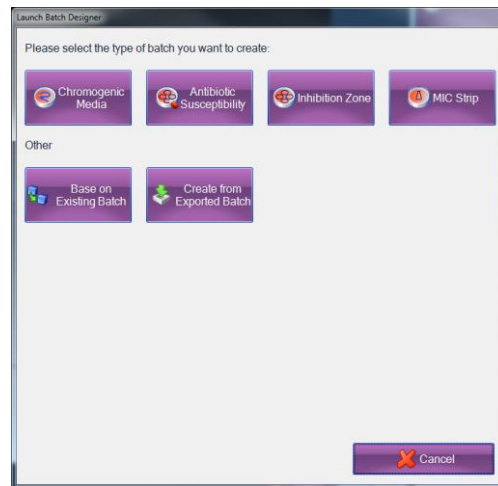
Sector	Zone Name	Zone Diameter (mm)	Antibiotic Susceptibility	Flags
1	A	20.2000076	Intermediate	
2	B	18.5099977	Intermediate	
3	C	18.46999931	Intermediate	
4	D	23.79999924	Sensitive	
5	E	22.46999931	Sensitive	
6	F	14.64999962	Resistant	

AST BATCH PLATE REPORT WITHIN EXCEL

Creating an Inhibition Zone Batch

1. On the **Home Screen** select the **New Batch** button.

The **Launch Batch Designer** pop-up appears.



LAUNCH BATCH DESIGNER POP-UP

2. Select the **Plate Configuration** button and adjust the parameters to the required plate types
3. On the **Launch Batch Designer** pop-up select the **Inhibition Zone** button

The **Inhibition Zone** home screen will appear



INHIBITION ZONE HOME SCREEN

4. In the **Designing Batch:** field enter a name for the new Batch

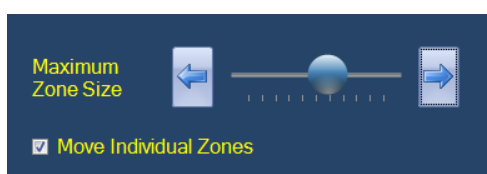
5. Within the Measure tab, In the **Plate ID** field, enter a Plate ID for the first Plate in the new Batch. If using a barcode reader, select the **Configuration** tab and check the barcode checkbox. If using a LIMS or CVS file system select configure and then select which system you are using (see pg 66, USING AN EXTERNAL PLATE NAMING SOURCE)
6. Select the **Zone Classification** button

The **Configure Zone Frame** window opens



7. Select the layout and number of zones required for the plate being measured

Note: Once the number of zones has been selected for the batch, that will be the number measured in future measurements. For plates with more or less zones than the created batch, a new batch would need to be created.



ZONE EDITING FUNCTIONS

8. Adjust the placement of the zones so the letter for each zone lies directly above the disc/well. Move Individual Zones by clicking on the Move Individual Zones button
9. Increase or decrease the Zone size using the **Maximum Zone Size** adjustable slider. Ensure the zone size is larger than the zone present so the software can correctly measure the zone size.
10. Once all zones have been placed, click **Next >**

The **Configure Type of Analysis** screen will appear.



CONFIGURE TYPE OF ANALYSIS WINDOW

11. Select if the plate contains Disc/ Wells in each zone or if there are no Disc/ wells present
12. If there are Disc/ Wells present, the size needs to be set so the software can read the zones present. Disc/ Well size can be entered manually or by using the up/ down arrows. Ensure the Disc/ Well diameter is larger than the Disc/ Well on the image. The software will automatically position a disc diameter over one of the Disc/ Wells present on the plate, this can be moved to be more centred by using the mouse and clicking on the centre of the circle
13. Once the Disc/ Well size is set, click **Next >**

The **Identify Zone Colours** screen will appear.



IDENTIFY ZONE COLOURS WINDOW

14. Select the Disc, Zone/Edge and background colour

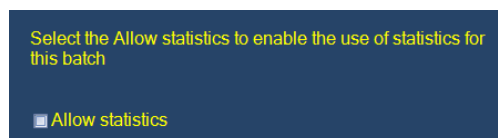
- Select the Disc Colour by selecting the clear circle and then selecting the centre of one of the discs present
- Select the Zone/Edge colour by selecting the clear circle and then selecting anywhere within one of the zones present on the plate being measured
- Select the background colour by selecting the clear circle and then selecting anywhere on the background colony growth
- Multiple colour samples can be taken to help aid the software in distinguishing different colony colours; this is particularly useful for plates which may have a slight lighting difference from one side of the plate to the other



IDENTIFY ZONE COLOURS WINDOW - 2

15. Once the colours for each section have been selected, click **Next >**

The **Configure Statistics** screen appears.



ALLOW STATISTICS CHECKBOX

16. If using a statistics package to analyse results, select the **Allow Statistics** checkbox and then click **Next >**

The software will analyse the image and open the **Review Zone Measurement** window.



REVIEW ZONE MEASUREMENTS WINDOW

17. Ensure the measured zones are aligned correctly and then click **Finish**

The batch measure screen will appear.



INHIBITION ZONE BATCH MEASURE SCREEN

18. Click on **Test Measure Plate**

Note: A test measure of the plate must be performed before the software will allow the new batch to be accepted.

19. Once a test measure has been performed, click **Accept New Batch**

The batch will be saved and measurements can begin.



MEASUREMENT TEST RESULTS SCREEN

20. Click **Measure Plate**

The plate will be analysed by the software and results displayed in the side bar and below the plate image in the results window.



INHIBITION ZONE BATCH MEASURE SCREEN WITH RESULTS WINDOW

21. To read the next plate within the batch, remove the current plate and replace with the next within the ChromaZona imaging platform, then click **Measure Plate**

22. To access a plate report spreadsheet, click on the required plate within the results window, this will make the **Edit/ Review** tab appear

23. Select the **Edit/ Review** tab

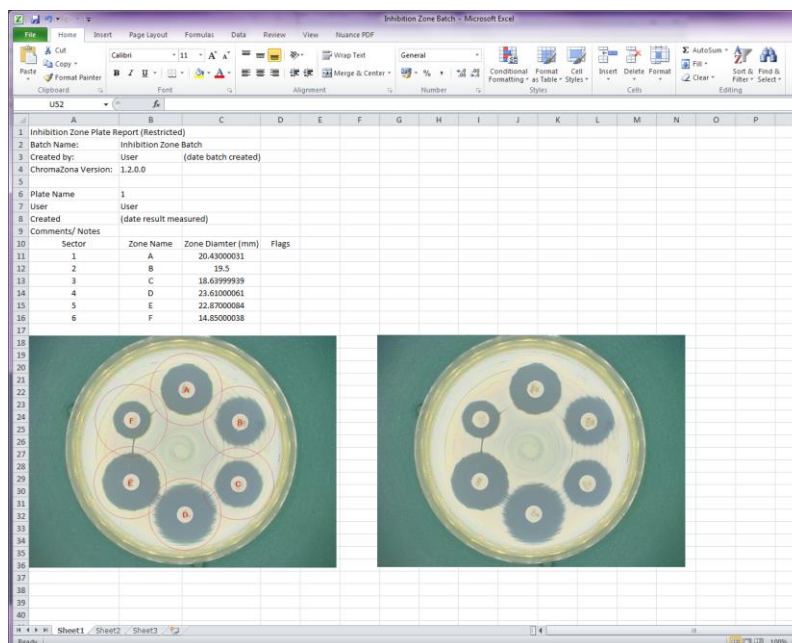
The **Edit/ Review** screen will appear



EDIT/ REVIEW SCREEN

24. Select **Print Plate Report**

Selecting this option will automatically open an Excel spreadsheet containing the results for that specific plate

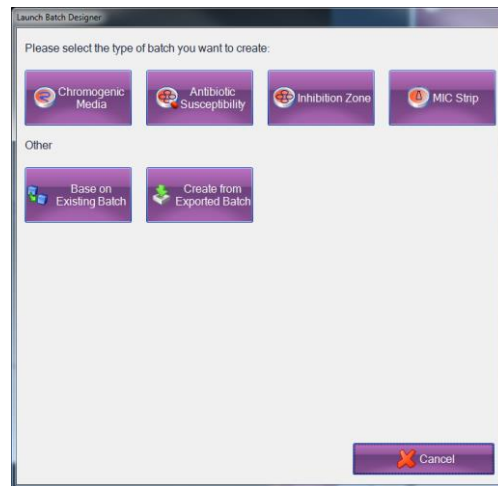


INHIBITION ZONE BATCH PLATE REPORT WITHIN EXCEL

Creating and using a MIC Strip Batch

1. On the **Home Screen** select the **New Batch** button.

The **Launch Batch Designer** pop-up appears.



LAUNCH BATCH DESIGNER POP-UP

2. Select the **Plate Configuration** button and adjust the parameters to the required plate types
3. On the **Launch Batch Designer** pop-up select the **MIC Strip** button

The **MIS Strip** home screen will appear



MIC STRIP CALIBRATION SCREEN

4. In the **Designing Batch:** field enter a name for the new Batch
5. If using a barcode reader, select the **Configuration** tab and check the barcode checkbox. If using a LIMS or CVS file system select configure and then select which system you are using (see pg 66, USING AN EXTERNAL PLATE NAMING SOURCE)

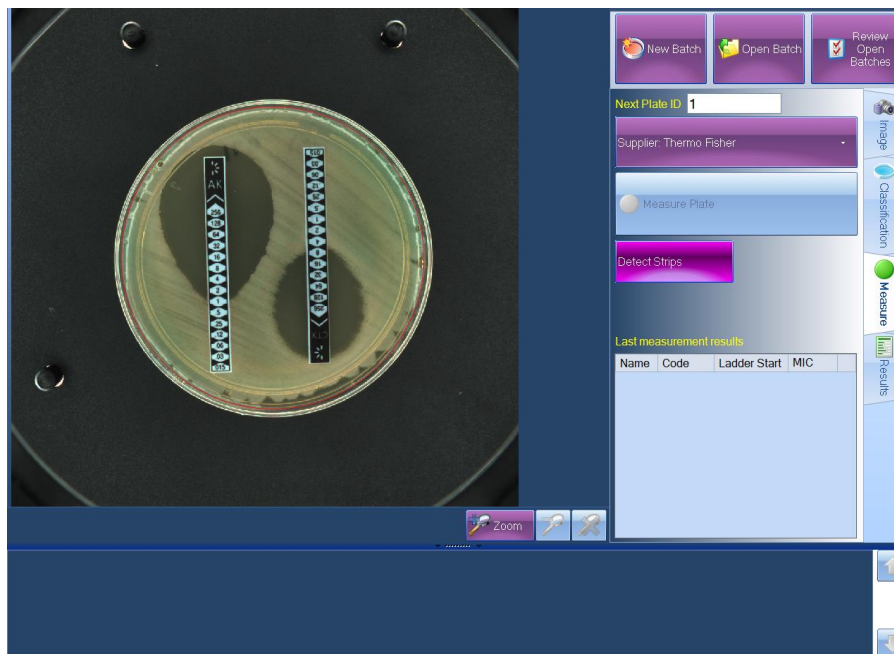
6. Within the Measure tab, in the **Plate ID** field, enter a Plate ID for the first Plate in the new Batch



MIC STRIP BATCH – ACCEPT NEW BATCH

7. Select **Accept New Batch**

The MIC Strip **Measure** screen will appear



MIC STRIP BATCH MEASURE SCREEN

8. Select Strip manufacturer from drop down list
9. Click **Detect Strip**

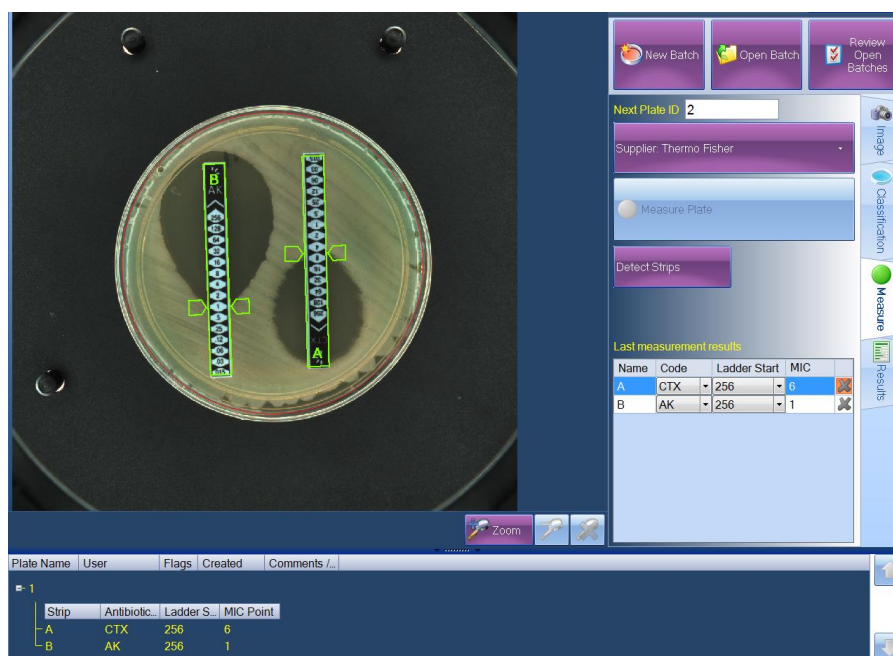
The software will detect the MIC strips present on the plate and will calculate the type of MIC strips present, the number of strips present and the ladder measurement.



MIC STRIP DETECTION

10. Click **Measure Plate**

The software will analyse the bacterial growth around the MIC strip and determine the MIC point. Results are shown within the measurement window below the measured image.



MIC STRIP BATCH MEASUREMENT RESULTS

11. To read the next plate within the batch, remove the current plate and replace with the next within the ChromaZona imaging platform. Click **Detect Strips**. Click **Measure Plate**.

12. To access a plate report spreadsheet, click on the required plate within the results window, this will make the **Edit/ Review** tab appear.

13. Select the **Edit/ Review** tab

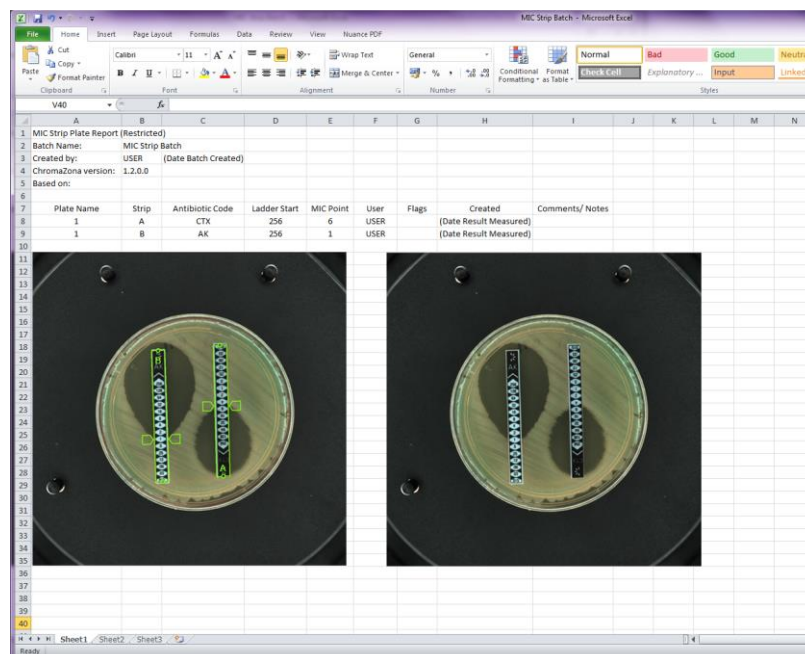
The **Edit/ Review** screen will appear



MIC STRIP EDIT/ REVIEW SCREEN

14. Select **Print Plate Report**

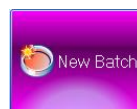
Selecting this option will automatically open an Excel spreadsheet containing the results for that specific plate



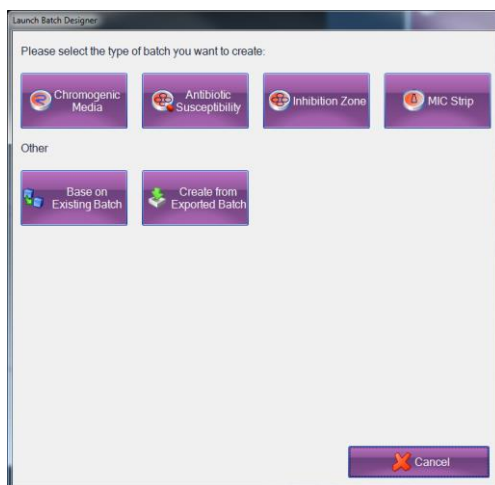
MIC STRIP BATCH PLATE REPORT WITHIN EXCEL

Create and Save a Batch from an Existing Batch

1. From the Home screen, select the **New Batch** button



The **Launch Batch Designer** dialogue box appears.

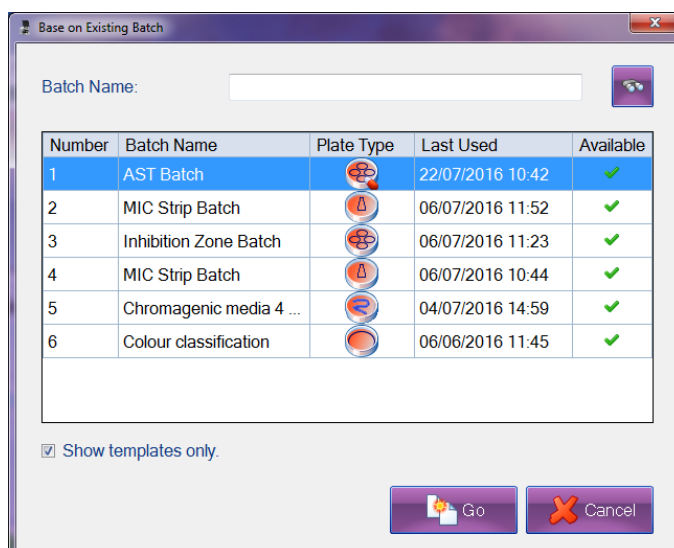


LAUNCH BATCH DESIGNER DIALOGUE BOX

2. Select the **Base on Existing Batch** button




The **Base on Existing Batch** dialogue box appears. This displays a list of available Batches



BASE ON EXISTING BATCH DIALOGUE BOX

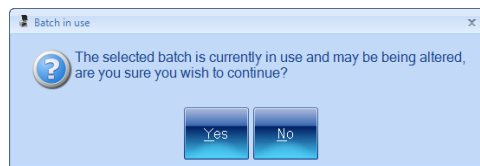
- Currently open Batches are marked with a tick ✓
- The new Batch can be based on an open Batch, but a warning will be displayed at the point when the selection has to be confirmed

- Select the **Show templates only** checkbox to hide any Batches that were based on an Existing Batch or an Exported Batch file
- If there is a large number of existing Batches and the desired Batch is not shown in the list, type the name, or a part of the name, into the **Batch Name** box and press the search button . Only Batches from the list that contain the entered search text will be displayed.

3. Select the required Batch in the list

4. Select the **Go** button 

- If the selected Batch is a currently open Batch, the following **Batch in use** warning is displayed:



BATCH CURRENTLY IN USE WARNING

- Select the **Yes** button to create the new Batch from the selected currently open Batch
 - Select the **No** button to close the **Batch in use** warning and return to step 3. to select a different Batch from the list
5. The new Batch will be created

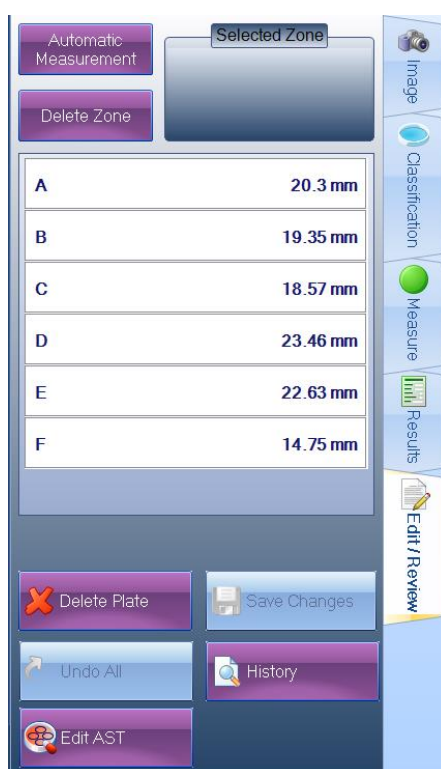
MANUAL EDITING OF PLATE MEASUREMENTS

Selecting individual plates within any batch results window will make the Edit/ Review tab appear. Within this tab changes can be made to measured plates depending on the batch applied.

Antibiotic Susceptibility Batch Editing

Within the Antibiotic Susceptibility batch manual adjustments can be carried out by the user to:

- Edit the measured zone size
- Edit AST
- Deleting a zone measurement
- Deleting the entire plate from the batch



AST EDIT/ REVIEW TAB LAYOUT

Zone Editing

Zone editing enables the user to adjust the software measured zone size to a manual measured size.

1. Click on the zone that requires a manual change within the Edit/ Review tab

The Selected zone will be highlighted in magenta within the results list and on the plate itself.



SELECTED ZONE FOR MANUAL EDITING

- Using the mouse to adjust the zone size by clicking on the zone to be edited and dragging it to the required size (larger or smaller)

The edited zone boundary will appear yellow on the plate image.



MANUALLY ADJUSTED ZONE SIZE

- If the user wishes to undo any changes made to zone measurements, Click **Undo All**
- Once the zone has been edited to the required size, click **Save**.
- A pop-up window will appear asking for a reason for the changes made to the plate. Enter the reason and click **OK**

The recorded zone measurement will have been adjusted to the new manual value and a flag (M) will be present in the plate results signifying a manual edit has occurred.

Edit AST

The Edit AST function enables the user to adjust the entered EUCAST or CLSI Breakpoint and Expert Rules settings applied to the specific plate. Click on the **Edit AST** button to make adjustments (see pg. 89 to 89 for instructions).

Deleting a Zone Measurement

Deleting a zone measurement removes the software measurement and replaces the value with zero.

1. Select the zone measurement to be deleted within the Edit/ Review tab

The selected zone will be highlighted in magenta within the results list and on the plate itself.

2. Now select **Delete Zone**

The selected zone measurement will be deleted and replaced with zero.



DELETE ZONE

3. If the user wishes to undo deleting any zone measurements, Click **Undo All**
4. Once the selected zone measurement(s) have been deleted, click **Save**
5. A pop-up window will appear asking for a reason for the changes made to the plate. Enter the reason and click **OK**

The recorded zone measurement will have been adjusted to the new manual value and a flag (M) will be present in the plate results signifying a manual edit has occurred.

Deleting an Entire Plate

Selecting **Delete Plate** will delete the recorded plate from the batch.

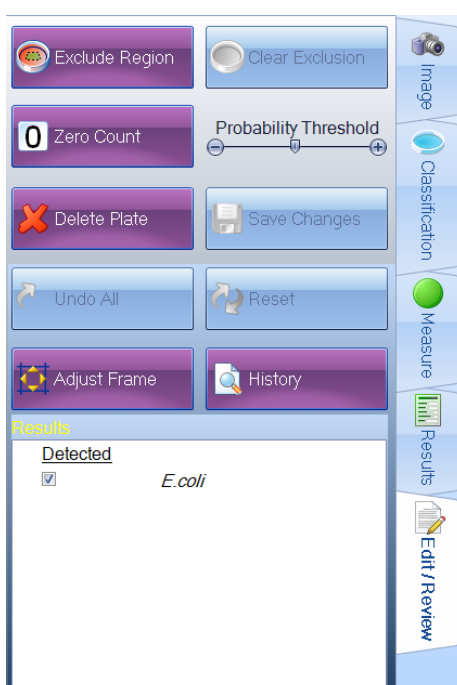


1. Select **Delete Plate**
2. Now click **Save**
3. Enter audit reason

The plate measurements will be removed from the batch. Although the result is deleted from the results table and can no longer be viewed within ChromaZona, it is not removed from the ChromaZona database and can be viewed there is required for audit purposes.

Chromogenic Media Batch Editing

Within the Chromogenic Media batch, manual adjustments can be carried out by the user.



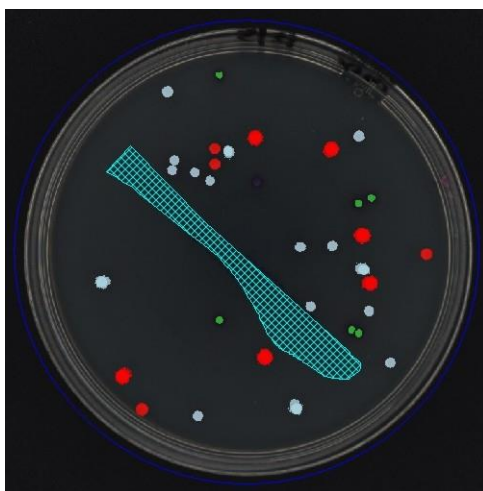
CHROMOGENIC MEDIA EDIT/ REVIEW TAB LAYOUT

Exclude Regions

If one or more areas of the plate have a problem affecting plate measurement, e.g. a label or other artefact, one or more exclude regions can be created. Exclude regions are defined using the **Exclude Regions** function.

1. Select the **Exclude Regions** button to use this function.
2. A hatched area can be overlaid on the image of the Plate in the Image Pane
3. Select a point on the image to start defining an exclude region
4. Hold down the left mouse button to drag the selector

5. Single left click to draw a line
6. Single right click to add a node
7. Double left click to end drawing the current hatched area (exclude region)
8. To draw another exclude region, select the **Exclude Region** button again



EXAMPLE EXCLUDE REGION

9. To remove the last exclusion region drawn, select the **Clear Exclusion** button.

Zero Count



Zero count is for plates which have zero growth present. Zero count can be applied after plate measurement has been carried out.

Adjust Frame



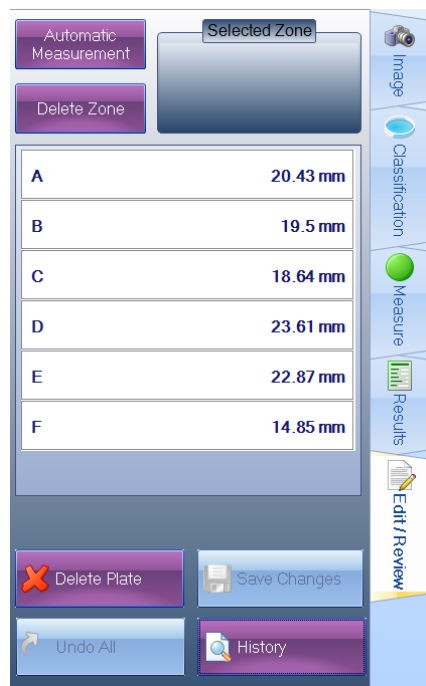
The Adjust Zone function enables the plate frame to be adjusted.

Inhibition Zone Batch Editing

Within the Inhibition Zone batch, manual adjustments can be carried out by the user to:

- Edit the measured zone size
- Deleting a zone measurement
- Deleting the entire plate from the batch

These adjustments are the same as those explained within the Antibiotic Susceptibility batch editing.

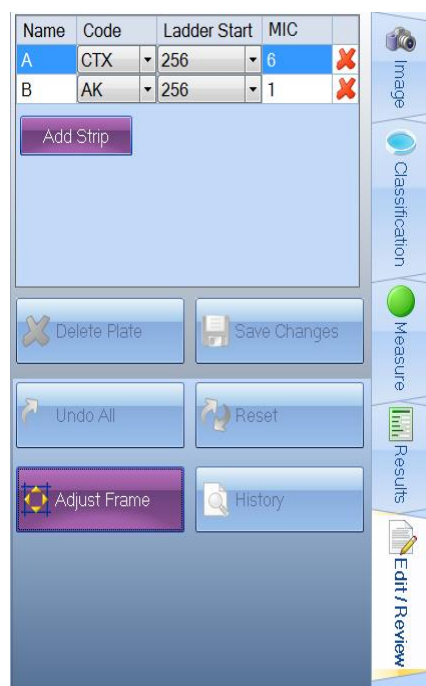


INHIBITION ZONE EDIT/ REVIEW TAB LAYOUT

MIC Strip Batch Editing

Within the MIC Strip batch, manual adjustments can be carried out by the user to:

- Add a strip
- Adjust the MIC Strip measuring zone
- Deleting the entire plate from the batch



MIC STRIP EDIT/ REVIEW TAB LAYOUT

Add Strip

Clicking Add Strip will add a MIC strip frame to the plate for the user to place over a MIC strip.



Adjust Frame

Adjust frame can be used to change the automatically placed MIC Strip detection frame. The frame can be lengthed or shortened and the position moved.



Delete Plate

Deleting a plate from a batch is explained within the Antibiotic Susceptibility batch editing section.

GENERATING REPORTS & AUDIT TRAILS

ChromaZona documents and records all measurements, settings and any manual changes made by a user to a result enabling a full audit history.

Generating Reports

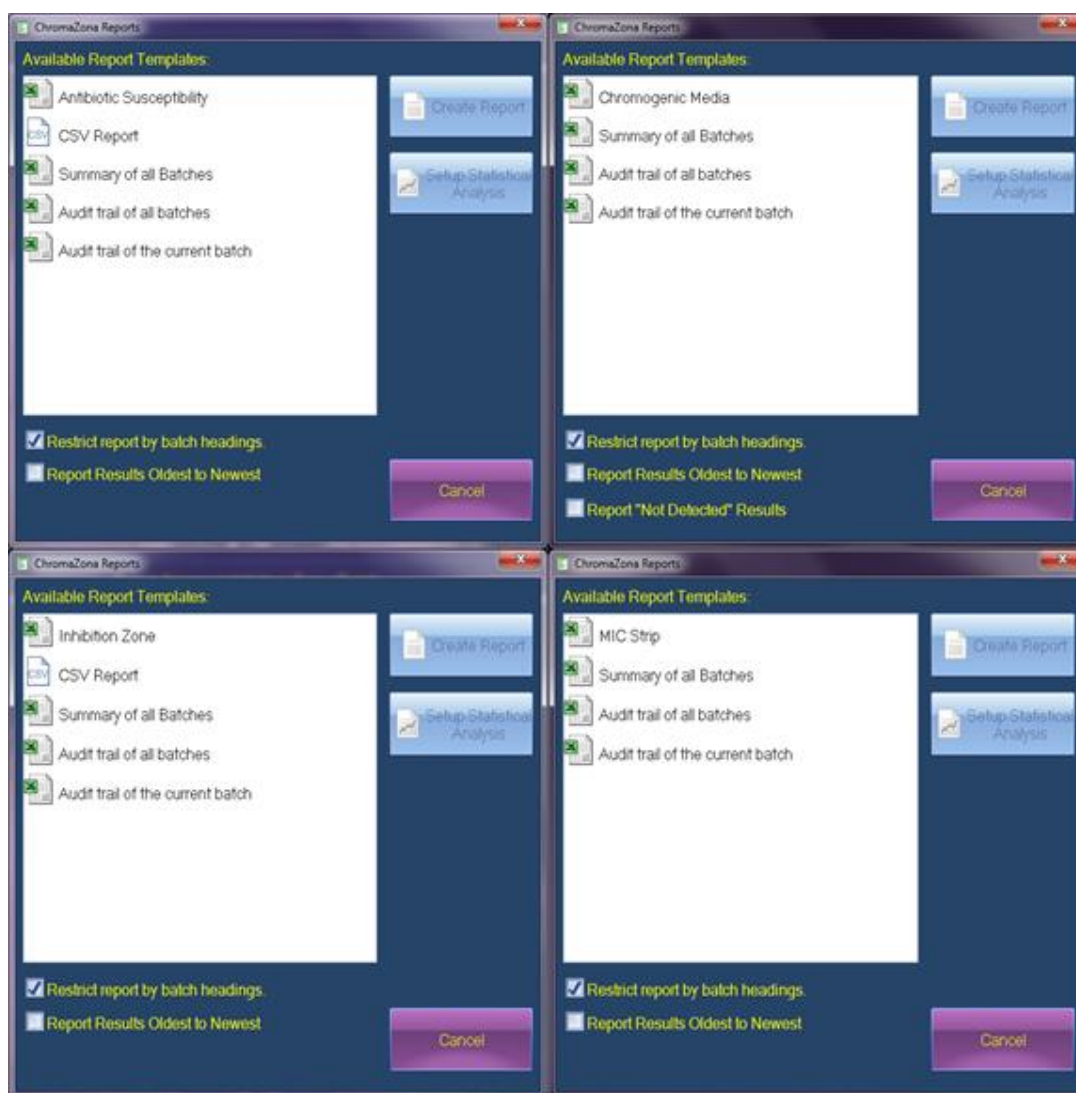
To compile a report:

- Select the **Results** tab within a batch
- Then select **Create Report**.



CREATE REPORTS BUTTON

The ChromaZona Reports window for the current batch will open



CHROMAZONA REPORTS WINDOW FOR EACH BATCH TYPE

- Select which type of report is required within the batch
- Click create report
- An Excel or openoffice spreadsheet will automatically open displaying recorded information

Below the available results templates there are checkboxes present, these enable minor changes to be made to the information which will appear in the reports generated.

- Restrict Report by Batch Headings
 - Information in the batch report will be limited to the selected headings checked within the results tab. This setting has no effect on summary or audit trail reports.
- Report Results Oldest to Newest
 - Information in the report will be listed showing the oldest results first
- Report “Not Detected” Results (only appears within a chromogenic media batch)
 - This selection will enable non-detected bacteria that could be isolated from the selective agar to be shown

Batch Report

Creates a detailed report of all the results and changes made within the current batch

Summary of All Batches Report

Creates a summary report of all the batches created within the ChromaZona database

Audit Trail of All Batches

Creates an audit report of all the batches created within the ChromaZona database

Audit Trail of the Current Batch

Creates an audit report for the current batch open

Plate History



Plate history enables a user to view any saved changes made to a specific plate within the software. It is located by selecting an individual plate within any batch results window and selecting the Edit/Review tab. Once selected a pop-up box will appear showing the following:

- | | |
|-------------------|----------------|
| • Date | • New Value |
| • Action | • User |
| • Element Changed | • Audit Reason |
| • Old Value | |

OPERATOR SUPPORT

Contacting Synbiosis

If it becomes necessary to contact Synbiosis the following information will be required:

- Instrument Type
- Unit Serial Number
- ChromaZona Software Version
- Database Version

Instrument Type is dependent on the range/model of instrument being operated.

The Unit Serial Number can be found on a sticker on the back of the instrument.

The Software and Database Version information can be found from the Home screen, as shown below:

1. Select the Main Menu button  to display the Main Menu.



CHROMAZONA MAIN MENU

2. Select the **About ChromaZona...** button to display the **About ChromaZona** pop-up
(Continued on next page)



ABOUT CHROMAZONA POP-UP

3. Select the **Save Details...** button to display a standard Windows Save As dialogue box, the details can then be saved as a text file.
4. Select the **Close** button to close the pop-up.

Further information concerning the instruments that use the ChromaZona Software can be found on the Synbiosis website; www.synbiosis.com. Here you can access; Application Notes, Technical Articles, FAQs and Quick Guides.

Technical support can be accessed by telephone or email:

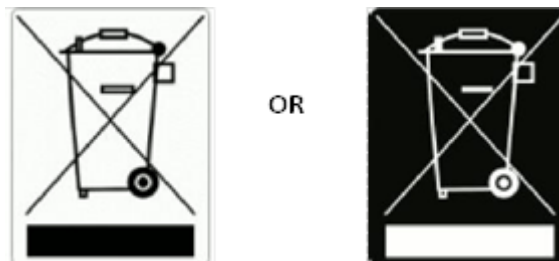
Tel: +44 (0)1223 727123

Email: support@synbiosis.com

DISPOSAL

Disposing of a ChromaZona

The Waste Electrical and Electronic Equipment (WEEE) Directive



A label with a crossed-out wheeled bin symbol and a rectangular bar indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and must not be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately, and in accordance with the regulatory guidelines in a local area.

The objectives of the WEEE Directive are to preserve, protect and improve the quality of the environment, protect human health, and utilise natural resources prudently and rationally. Specific treatment of WEEE is indispensable in order to avoid the dispersion of pollutants into the recycled material or waste stream. Such treatment is the most effective means of protecting the environment.

WEEE Instructions for ChromaZona Instrument

The requirements for waste collection re-use, recycling, and recovery programs are set by the local regulatory authority. Contact your local Responsible Person (such as the Laboratory Manager) or authorised representative for information regarding applicable disposal regulations. For information specific to the ChromaZona Instrument, contact Synbiosis at:

Website: www.Synbiosis.com

Email: sales@synbiosis.com

Mail and telephone:

Synbiosis UK office

Beacon House

Nuffield Road

Cambridge

CB4 1TF

United Kingdom

Tel: +44 (0)1223 727125

Synbiosis USA office

5103 Pegasus Court, Suite L

Frederick

MD 21704

USA

Tel: 800 686 4451/301 662 2863

Note: Products from other manufacturers may form a part of your ChromaZona Instrument. These other manufacturers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these manufacturers directly before disposing of any of their products.