

ProtoCOL 3

User Manual



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Version 19.1

Published by Synoptics Limited

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Getting started

Introduction

ProtoCOL 3 is a combined hardware and software system for automatically counting colonies and measuring antibiotic susceptibility or inhibition zones.

Note ProtoCOL 3 administrators can control which features of the program are available for different groups of users (see *Managing ProtoCOL 3 users*, page 181), 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 system has been set up, all you have to do in order to count the colonies or measure the zones on a series of plates is to put each plate in the plate holder and press a button. The counting / measurement of each plate takes no more than a few seconds and the results are shown instantly in a table.

ProtoCOL 3 allows you to enter plate identifiers and dilutions manually, to use auto-incrementing plate identifiers or to enter lists of plate identifiers and dilution series within ProtoCOL 3 from a CSV file or a LIMS. If you use auto-incrementing plate identifiers or a plate list, ProtoCOL 3 will prompt you for which plate to insert next. The plate identifiers and dilutions are then automatically assigned to the results and recorded. You can also read plate identifiers and dilutions using a barcode reader.

When you are counting colonies, you can use ProtoCOL 3:

- to distinguish between different types of colony according to their color, size and/or shape, and to distinguish between colonies, debris or other artefacts;
- to apply sophisticated algorithms to distinguish touching colonies;
- to add or remove colonies manually after an automatic count – you can also perform a completely manual count if required;
- to define 'exclude regions' on the plate if there are any problem areas on an individual plate where colonies cannot be distinguished for some reason – this is then taken into account when the software calculates the total count for the plate.

When you are measuring zones, ProtoCOL 3 can apply sophisticated algorithms to measure the zones automatically, making appropriate allowance for any discs or wells. However, you can also measure the zones manually after an automatic measurement.

All counting and measurement results are displayed and saved automatically, and any manual changes you make 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 can be reloaded at a later time for auditing. You can also view a full audit history for any changes you have made to a result.

Once you have completed the counts/measurements, you can compile a report showing the results in Open Office, Excel or PDF format.

This introduction has only mentioned a few of the features that make ProtoCOL 3 such a powerful but easy tool to use. The rest of the Manual gives full instructions for using all of its many functions.

Structure of the Manual

Note The cross-references in this manual assume that it is printed or viewed with odd numbered pages on the right-hand side. If you are using Adobe Acrobat to read this manual on-screen, you can display two pages side-by-side by choosing **Two Page View** from the **View** **Page Display** submenu, and you can then choose **Show Cover Page in Two Page View** from the same submenu to display the odd numbered pages on the right-hand side.

The rest of this Manual is structured as follows:

- The next section, *Using ProtoCOL 3 – overview* in this *Getting Started* chapter provides a brief overview of the way you use ProtoCOL 3 to count colonies and measure zones. This involves two main phases:
 - using *the Batch Designer* to set up the system for either detecting and counting colonies or measuring zones on a series of plates;
 - using *Measurement mode* to actually count the colonies or measure the zones on the plates and record the results.

The next section also introduces you to the idea of a 'batch', which is used to hold the settings made in *the Batch Designer* and then to store the results produced in *Measurement mode*.

The final two sections of this *Getting Started* chapter give instructions for starting ProtoCOL 3 (see *Starting ProtoCOL 3*, page 4) and then for logging out and closing ProtoCOL 3 (see *Logging out and closing ProtoCOL 3*, page 5).

The remaining chapters are:

- *Capturing images*, page 7, which shows you how to load plates into ProtoCOL 3 and capture an image of the plate.

These procedures are used in the Batch Designer and Measurement mode.

- *Creating a new batch*, page 19, which shows you how to use the Batch Designer to create a new batch and specify how colonies should be counted or inhibition zones measured.

Some of these procedures can also be applied in Measurement mode.

- *Using Measurement Mode to count colonies and measure zones*, page 139, which shows you how to count the colonies or measure the inhibition zones on a series of plates.
- *Working with results*, page 157, which shows you how to view and edit the results of your measurements and how to create reports using the results.
- *Configuring ProtoCOL 3*, page 177, which shows ProtoCOL 3 administrators how to configure ProtoCOL 3 in a variety of ways, including how to set up and manage user accounts to protect access to the program and to choose which features are available to different groups of users.

Using ProtoCOL 3 – overview

ProtoCOL 3 uses 'batches' to define the settings used to detect colonies or measure zones on plates and to store the results of the measurements you make using those settings. This means that before you can use ProtoCOL 3 to take any measurements, you must have an open batch. You can do this by opening an existing batch or by creating a new batch. You can either create a completely new batch or base a new batch on an existing one in order to use the same settings,

or as a starting point for defining new settings. ProtoCOL 3 automatically saves your results in the current batch, so once you have created and accepted a batch design, you do not need to take any action to save it again.

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 (Pour Plate, Spiral Plate, Dilution Series, Chromogenic Media, Multi-Sector Plate, Multiwell, Ames, OPKA, SBA, Antibiotic Susceptibility or Inhibition Zone).

Creating a colony counting batch

For a colony counting batch, if you are just interested in a total count of all the colonies on the plate, all you need to do in most cases is just specify whether the colonies are lighter or darker than the background.

However, for difficult cases, or if you want to distinguish between different types of colony, you can create a colony 'classification' according to the color of the colonies – you can also add size and/or shape classifications if required. The color classification is the primary classification and is used to distinguish between colonies and the plate background (medium) and any debris or other artifacts, and between different types of colony if you want to distinguish between them. For most batches, the classification can be performed completely automatically using wizards – all you need to do is choose how many classifications you want and specify how the results should be interpreted: for example, whether a particular color represents a type of colony, background or debris. However, you can also customize the detection settings to handle problem cases.

Creating a zone measuring batch

For a zone measuring batch, all you usually need to do is indicate where the zones are located on the image by placing 'frames' over them, and then click on areas in the image to indicate which colors in the image correspond to the background and which correspond to the zones (and wells/discs if present). In many cases, this is all you need to do, but for difficult cases you can fine tune the detection parameters manually to optimize the zone detection.

Accepting a batch

While you are working in the Batch Designer, you can perform test measurements and experiment with and change the settings used to detect and classify the colonies or zones. Once you are satisfied with the design, the next step is to 'accept' the batch and switch ProtoCOL 3 to *Measurement* mode, which you then use to count the colonies or measure the zones on each plate, and record the results.

Once you have accepted the batch, most of the batch settings, including the definitions of any color, size and shape or zone classifications, are fixed and cannot be changed. However, there are some settings that can be changed in *Measurement* mode, but, if required, they can also be locked by the ProtoCOL 3 administrator to prevent them being changed.

Taking measurements

Once the batch has been accepted for *Measurement* mode, the process of counting the colonies is very straightforward: put a plate in the plate holder, press a button and move on to the next plate.

In most cases this is all you will need to do, but if there are any problems with difficult plates, you can make manual adjustments before carrying out the measurement. For example, for

Getting started

colony counting batches, you can add 'exclude regions', carry out a 'test measurement' and optimize the colony detection settings, or perform a 'manual colony count'.

After you have carried out a measurement, the results are recorded in a results table, and you can edit the individual results in a number of ways. For example, for a colony counting batch you can add exclude regions and adjust the colony detection settings before carrying out an automatic colony recount, or you can add and remove colonies manually; and for a zone measuring batch, you can adjust the individual measurements manually.

Any changes you make to a result are recorded in an audit history for that result, and the ProtoCOL 3 administrator can set the program to require users to add a comment explaining any changes they make.

Finally, once you have taken all the measurements, all you need to do to compile a report on the results is press a few buttons.

Starting ProtoCOL 3

Note You can control ProtoCOL 3 using a mouse or keyboard shortcuts.

See the ProtoCOL 3 *Quick Guide* for how to set up the hardware ready for use.

To turn on the ProtoCOL 3 system:

1. Make sure the system is connected to a suitable mains power supply.
2. Switch on the system using the toggle switch on the rear of the unit.
3. If using a ProtoCOL 3 Plus, follow the quick guide for set up help.
4. If using a separate PC, connect with the USB lead provided and switch on.

To start the ProtoCOL 3 colony counting program:

1. Double-click on the ProtoCOL 3 icon on the Windows Desktop:



If the ProtoCOL 3 administrator has disabled user authentication (see *Managing ProtoCOL 3 users*, page 184), the program will open immediately and you will not need to follow the remaining steps in this procedure. If not installed and using separate PC, please refer to the software installation quick guide.

Otherwise, the **Please log on to ProtoCOL 3** dialog box will be displayed:



Note If user authentication is enabled, this dialog box is also displayed when you log out from ProtoCOL 3 or if you are logged out automatically by ProtoCOL 3 because you have not used it for a set period of time (5 minutes by default) – see *Logging out and closing ProtoCOL 3*, below, for more details.

2. Enter your Windows **Username** – the name you use to log on to Windows on this PC.
 3. Enter your Windows **Password** – the password you use to log on to Windows on this PC.
-

Notes ProtoCOL 3 checks that the user name/password is a valid combination to log on to Windows on this PC, but does not check that it is the currently logged on user. This means that you can log on to ProtoCOL 3 while someone else is logged on to Windows on this PC.

If using a ProtoCOL3 Plus, the default **Username** is P3Admin and the default **Password** is the serial number of your instrument, for example, P3PC/xxxx.

If using a separate PC provided by Synbiosis the **Password** would be P3/xxxx.

Press **OK**.

The ProtoCOL 3 Application window will open. You can either create a new 'batch' (see *Creating a completely new batch*, page 20) or open an existing batch if there is one (see *Opening and selecting batches*, page 139) and start counting colonies or measuring zones immediately.

Logging out and closing ProtoCOL 3

To log out from ProtoCOL 3:

Press the **Log out** button near the top right-hand corner of the ProtoCOL 3 application window:



If user authentication is enabled, the **Please log on to ProtoCOL 3** dialog box will be displayed.

Note You may also be logged out automatically and this dialog box displayed if you do not use ProtoCOL 3 for more than a set period of time (5 minutes by default) – see *System settings*, page 178, for how to set the timeout period.

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You (or someone else) can then:

- Log on again – see *Starting ProtoCOL 3*, page 4.
- Press **Close** to exit ProtoCOL 3.

Alternatively, to exit ProtoCOL 3:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Press



Capturing images

This chapter shows you how to capture and work with images. You will find out about:

- *The Image tab*, below
- *Choosing an external camera or scanner*, page 9
- *Loading plates into the instrument*, page 9
- *Capturing an image*, page 10
- *Setting the exposure*, page 10
- *Zooming the image*, page 11
- *Exporting the image to a file*, page 12
- *Importing an image from a file*, page 13
- *Calibrating the image*, page 13
- *Viewing batch images*, page 15.

The 'View Batch Images' procedure is used to view images captured during the measurement process, so is only available in Measurement mode. All of the other procedures described in this chapter are used in both the Batch Designer and Measurement mode.

The Image tab

To display the image controls:

Press the **Image** tab on the right hand side of the screen:



Capturing images

You use the controls on the **Image** tab to capture and work with images:



The View Batch Images button is enabled when you are working in Measurement mode (see *Using ProtoCOL 3 – overview*, page 2, for more information about the Batch Designer and Measurement mode).

For instructions on how to use the image controls, see the following sections:

- *Choosing an external camera or scanner*, on the facing page
- *Capturing an image*, page 10
- *Setting the exposure*, page 10
- *Exporting the image to a file*, page 12
- *Importing an image from a file*, page 13
- *Viewing batch images*, page 15.

Choosing an external camera or scanner

To choose whether to use the internal camera in the ProtoCOL 3 system, an external camera or a scanner:

1. If you want to use:
 - A scanner:
 - a. Make sure a suitable TWAIN scanner has been installed on the PC and is currently connected to the PC and switched on.
 - b. If there is more than one TWAIN device attached to the PC, select the required scanner – see *Selecting a scanner*, page 182.
 - An external camera:
 - a. Make sure ProtoCOL 3 has been configured to use the camera (see *Configuring an external camera*, page 183).
 - b. Make sure the camera is currently connected to the PC and switched on.
2. Press the camera button at the top of the Image tab (see *The Image tab*, page 7) to display the source menu:



Note The **TWAIN Device** command only appears on this menu if a suitable TWAIN Source has been installed and is currently connected to the PC and switched on.

3. Choose whether to use the **Internal Camera**, an **External Camera** or a **Scanner (TWAIN Device)**.

Note If you are using an external camera with ProtoCOL 3, you will need to check the **Live Image** box on the **Image** tab to display a live image from the external camera – see the separate documentation on using ProtoCOL 3 with an external camera.

Full instructions for using ProtoCOL 3 with the internal camera are provided in this Manual; see the separate documentation for using ProtoCOL 3 with an external camera and the documentation supplied with your scanner software for how to control the scanner and use it to scan an image into ProtoCOL 3.

Loading plates into the instrument

To load a sample plate into the instrument:

1. Open the doors at the front of the instrument by sliding them into the instrument.
2. If required, change the plate holder fitted in the instrument:

Three-pillared plate holders should be fitted with the plate stop pillars to the left and rear

Capturing images

Note ProtoCOL 3 is supplied with three sample holders: transparent, translucent white and opaque black holders for circular plates; translucent white and opaque black holders for rectangular plates are offered as a purchasable option. Apart from the shape of plate, the choice of which type to use depends on the properties of the samples you are using and is best determined by experiment.

3. Place the sample plate on the holder and push it up against the plate stop pillars to fix its position.

For rectangular plates, use a three-pillared plate holder and push the plate against the two side pillars first, then slide it back along the side pillars until it touches the rear pillar.

Note ProtoCOL 3 automatically displays a live image when you insert a new plate. However, if required, you can choose to display a live image manually by checking the **Live Image** box on the *Image tab*, page 7. If you have purchased the option to use an external camera with ProtoCOL 3, you will need to check the **Live Image** box to display a live image from the external camera – see the separate documentation on using ProtoCOL 3 with an external camera.

4. Close the instrument doors.

Capturing an image

To capture an image in ProtoCOL 3:

1. Press the **Image** tab



to display the image controls.

2. Press



The image will be captured and displayed in the ProtoCOL 3 window.

Note In fact, three images will be captured, with red, green and blue illumination respectively, and the results combine to form a color image – this procedure produces more accurate results than can be obtained using a color camera.

If the image is too light or too dark, you can adjust the exposure (see the next section, *Setting the exposure*) and capture the image again.

Setting the exposure

Note You can adjust the exposure in both the Batch Designer and Measurement Mode, provided in the latter case that the batch has not been restricted (see *Setting batch restrictions*, page 133).

To set the exposure for capturing an image:

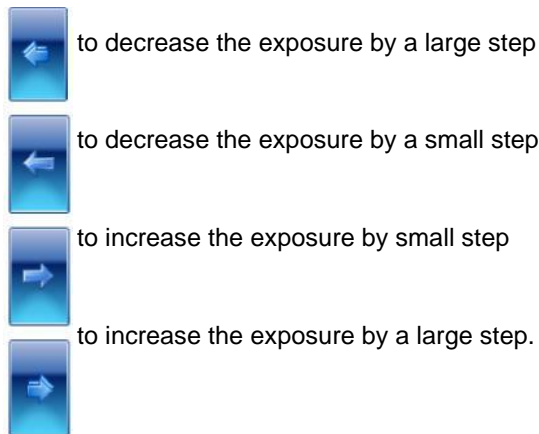
1. Press the **Image** tab



to display the image controls, including the exposure controls:



2. The current exposure time is shown between the arrow buttons. To change the exposure time, press:



Or:

Drag the handle in the exposure slider bar:



Zooming the image

By default, the image is scaled to fit the image pane in the ProtoCOL 3 window. To zoom the image:

Press



When it is zoomed, the button shows the scaling factor:



Capturing images

To zoom out:

Press



To return to fitting the image to the image pane:

Press



Note The zoom controls are also displayed with the image in dialog boxes – you use them in exactly the same way as in the main ProtoCOL 3 window.

Exporting the image to a file

To export the current captured image in the ProtoCOL 3 window to a file:

1. Press the **Image** tab



to display the image controls.

2. Press



to display the **Export Image** dialog box – this is a standard Windows 'Save As' dialog box.

3. Use the **Export Image** dialog box to:
 - select an image format from the **Save as type** drop-down list
 - select a folder to hold the image
 - enter a file name for the image.
4. Press **Save** to save the file.

See also

Viewing batch images, page 15, for how to export previous images from a batch.

Importing an image from a file

To import an image from a file into the ProtoCOL 3 window:

1. Press the **Image** tab



to display the image controls.

2. Press



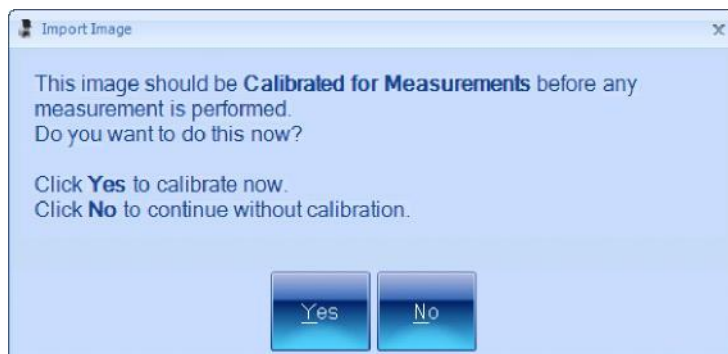
to display the **Import Image** dialog box – this is a standard Windows ‘Open’ dialog box.

3. Use the **Import Image** dialog box to locate the folder holding the required image and select the image.
4. Press **Open** to open the file and load it into the ProtoCOL 3 window.

A warning dialog box will be displayed asking you to calibrate the image – see the next section, *Calibrating the image*, for details.

Calibrating the image

When you import an image from a file (see *Importing an image from a file*, above) or capture an image from an external camera, or try to perform a measurement from an uncalibrated imported image, a warning dialog box will be displayed asking if you want to calibrate the image, for example:



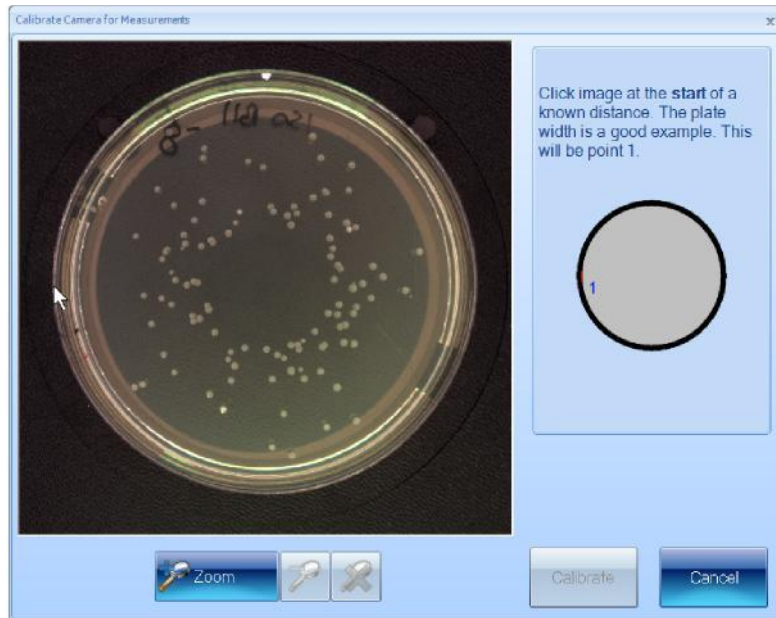
Note In order to create an accurate calibration you need to know the actual size of some feature in the image, for example a petri dish.

To calibrate the image:

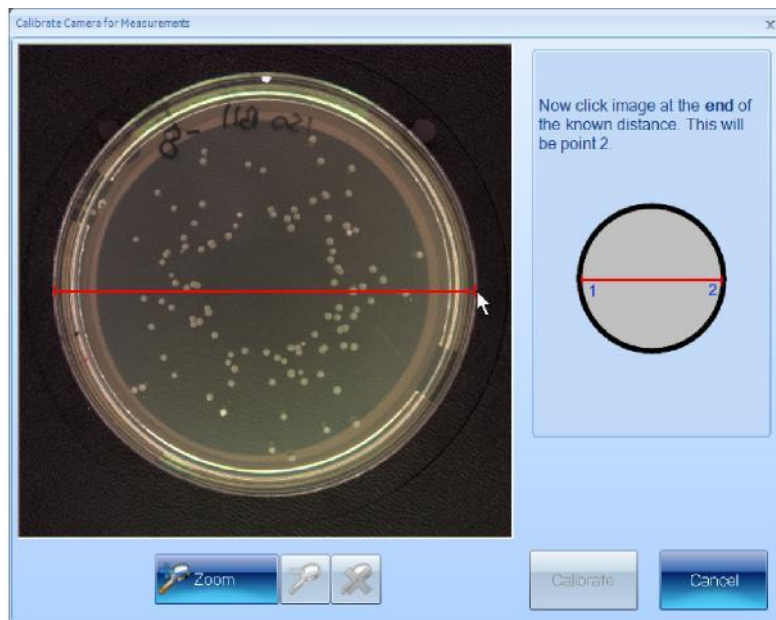
1. Press **Yes** in the warning dialog box to display the **Calibrate Camera for Measurements** dialog box.

Capturing images

2. Choose some horizontal or vertical feature of known length in the image, such as the plate itself, and click on one end of it, for example:

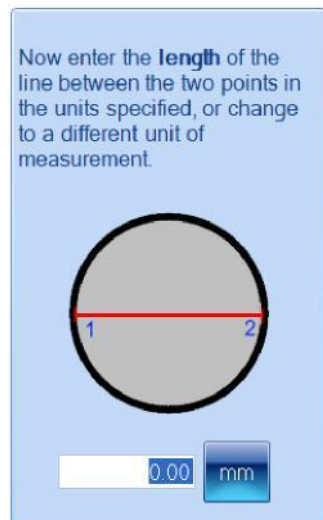


3. Move the pointer to the other end of the feature – as you do this, a line will be drawn back to the starting point, for example:



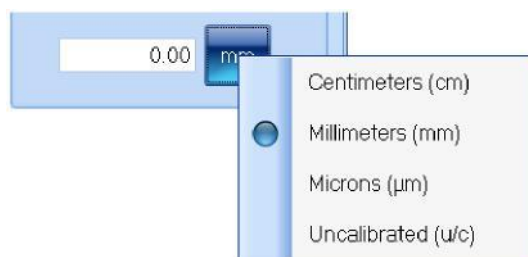
4. Click to mark the other end of the feature.

The calibration edit box and units button will be displayed:



5. If required, to change the units:

a. Press the units button to display the units menu:



b. Select the required units from the menu.

6. Type the length of the known feature into the calibration edit box:



7. Press



to set the calibration and return to the main ProtoCOL 3 window.

Viewing batch images

By default (see *System settings*, page 178), when you create a new batch, the image used to set it up (see *Setting up batches*, page 27) is saved with the batch, and then, after you have accepted the batch design, each time you take a measurement in Measurement mode, the image is also saved with the result in the batch.

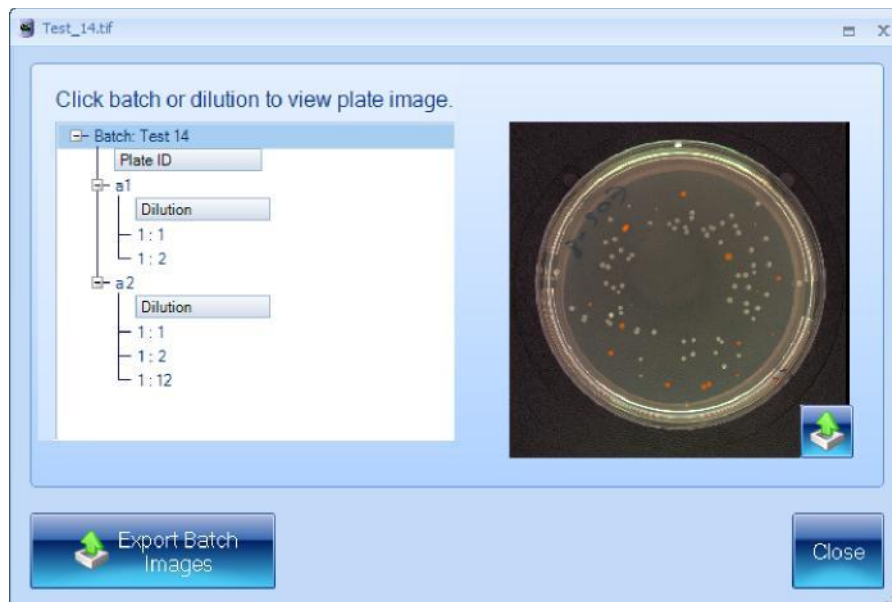
Capturing images

To view the batch setup image and images used for measurements:

1. Press



to display a dialog box:



The box on the left-hand side lists the batch images, beginning with the batch setup image, which is followed by the measurement images listed in order of their plate identifiers and, in the case of dilution series, dilutions.

2. To view a measurement image, click on the plate identifier or, in the case of dilution series, the dilution line within the plate identifier entry.

To view the batch setup image, click on the batch identifier (the top line).

3. To export all the batch images:

- a. Press




to display the **Browse for Folder** dialog box.

- b. Select the folder you want to export the images to – if required, press **Make New Folder** to add a new folder to the selected folder.
- c. Press **OK** to save the batch images to the selected folder.

The batch setup image will be saved using the name of the batch; the measurement images will be saved using the image identifier – if the same identifier has been used for more than one measurement, for example, with a dilution series batch, '_2', '_3' etc, will be added to the identifier for the second, third, etc images.

An xml file containing information about the images will also be saved in the folder.

4. To export a single batch image:
 - a. Select the required image – see Step 2.
 - b. Press  to display a Standard Windows **Save As** dialog box.
 - c. Use the **Save As** dialog box to select a folder and enter a filename for saving the image.
 - d. Press **Save** to save the selected image.
5. Press **Close** to close the dialog box and return to the main ProtoCOL 3 window.

Creating a new batch

This chapter shows you how to create and set up a new batch for counting colonies or measuring inhibition zones. It also describes a number of batch management procedures for use once you have created the batch.

The process of creating a new batch is divided into two main stages, which are covered in the following main sections:

- *Creating the batch* – see the next section

This section shows you how you can create:

- A completely new batch from scratch – see *Creating a completely new batch*, on the next page.
- A new batch based on an existing batch or an exported batch file – see *Creating a new batch based on an existing batch or an exported batch file*, page 24.
- *Setting up batches*, page 27.

This section describes:

- *Giving the batch a name*, page 28
- *The Classification tab*, page 28, for specifying how colonies or zones are detected – this section is divided into two major subsections:
 - *The Classification tab – colony counting batches*, page 29
 - *The Classification tab – zone measurement batches*, page 76.
- *The Configuration tab*, page 107, for setting up how plate identifiers will be assigned to plates when they are measured.
- *The Measure tab*, page 123, including:
 - *Plate ID*, page 124
 - *Dilution*, page 124
 - *Setting Count Restrictions*, page 128
 - *Test Measure Plate*, page 129.
- *The Results tab*, page 131, for choosing what to display in the Results table.

Once you have completed all the settings, you will need to accept the batch so that you can use it for measuring plates – see *Accepting the batch design*, page 132.

Note The sections described above show you how to use the Batch Designer, but many of the operations covered may also be carried out when you are working in Measurement mode.

The final sections of this chapter describe a number of batch management operations – for details, see:

- *Exporting batch details*, page 133
- *Setting batch restrictions*, page 133
- *Deleting batches*, page 135.

Creating a new batch

Creating the batch

Before you can start counting colonies or measuring antibiotic susceptibility or inhibition zones on plates you need to set up and create a 'batch' to define how the counts/measurements should be carried out, and to hold the results.

You can create:

- A completely new batch from scratch – see *Creating a completely new batch*, below.
- A new batch based on an existing batch or an exported batch file – see *Creating a new batch based on an existing batch or an exported batch file*, page 24.

Note Once you have created the new batch, you will still need to set it up and test it before you can start using it to count colonies or measure antibiotic susceptibility or inhibition zones – see *Setting up batches*, page 27.

For more in depth information regarding the Ames, AST, Chromogenic, OPKA or SBA modules, please review the appendices at the end of this document.

Creating a completely new batch

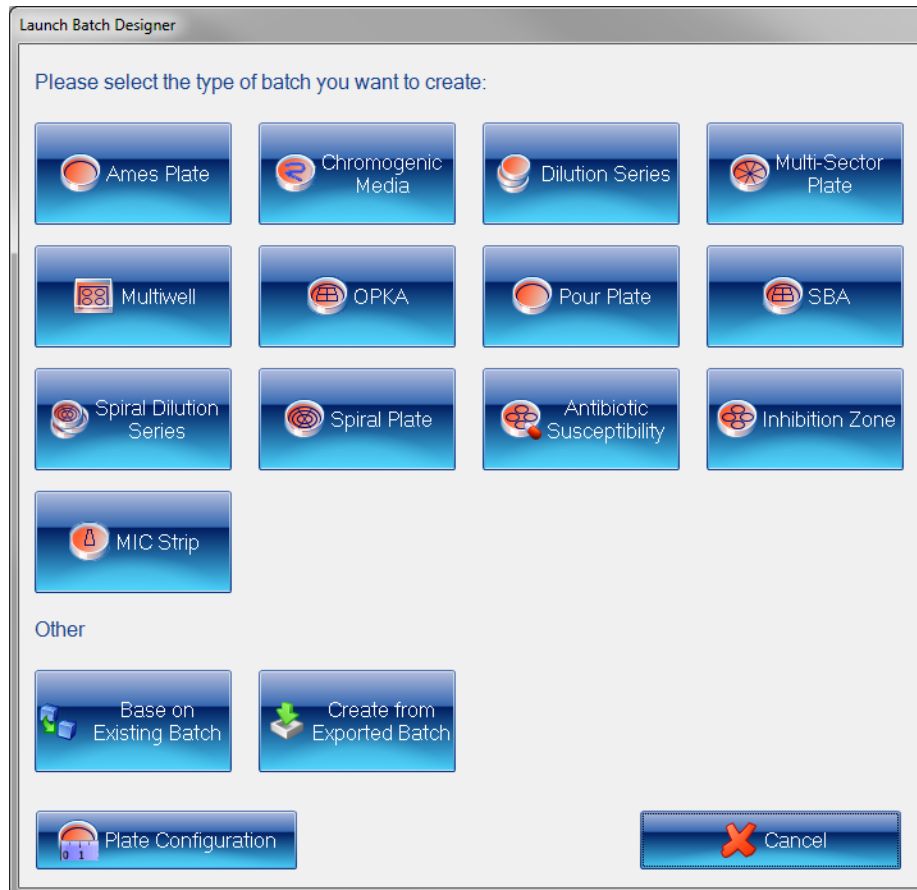
Note See *Creating a new batch based on an existing batch or an exported batch file*, page 24, for how to create a batch with the same settings as an existing or exported batch.

To create a completely new batch:

1. Press



to display the **Launch Batch Designer** dialog box:



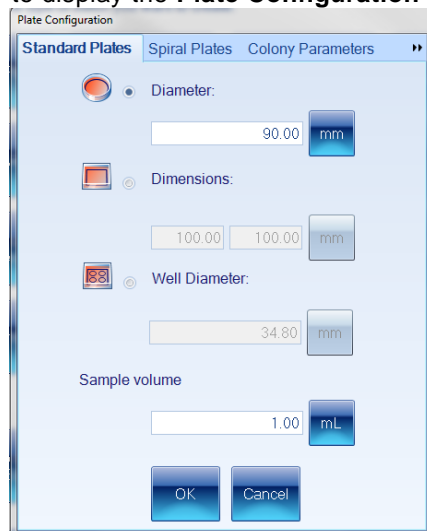
Note The buttons displayed depend on which module options you have purchased with ProtoCOL 3.

2. To set the plate configuration for a colony counting batch:

a. Press



to display the **Plate Configuration** dialog box:



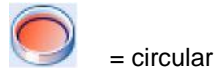
Creating a new batch

If you are going to use the batch for spiral plates, go to Step c; otherwise, go to Step b.

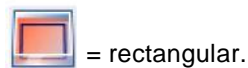
Note The settings in the **Plate Configuration** dialog box have no effect on zone measuring batches.

b. For non-spiral plates:

- i. Click on the **Standard Plates** tab if it is not already selected – see picture in Step a.
- ii. Press the appropriate radio button to specify whether the plates you are using are:



or

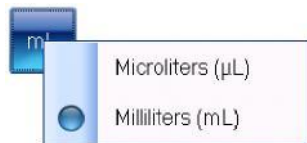


- iii. For a circular plate, enter the **Diameter** of the plate; for a rectangular plate, enter the **Dimensions**.
- iv. To choose the units for the **Diameter** or **Dimensions**, press the corresponding units button to display the units menu



and select the required unit.

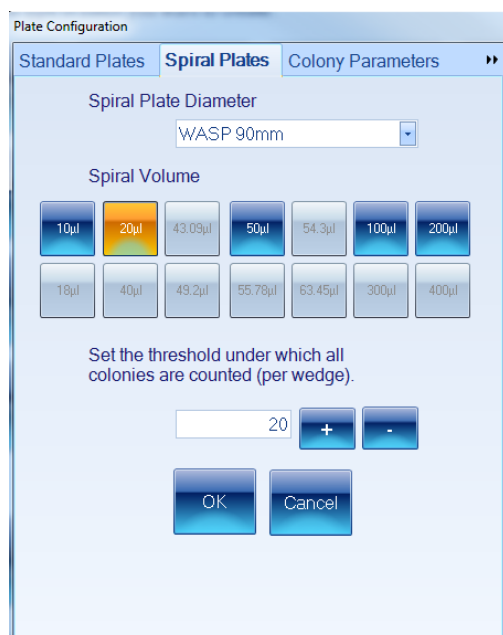
- v. Enter the **Sample volume** you will be using.
- vi. To choose the units for the **Sample volume**, press the units button to display the units menu



and select the required unit.

- vii. Press **OK** to confirm the settings, close the dialog box and return to the **Launch Batch Designer** dialog box – go to Step 3.

- c. For spiral plates:
- i. Click on the **Spiral Plates** tab to display the **Spiral Plates** page:



- ii. Select the **Spiral Plate Diameter** you will be using from the drop down menu.
- iii. Click on the button corresponding to the **Spiral Volume** you will be using.
- iv. If you are using a two-sector counting frame and the number of colonies counted within the first sector is less than a set threshold, the whole frame will be used instead.
 To **Set the threshold under which all colonies will be counted**, type in a value directly or press the **+** or **-** button to increase or decrease the current value.
- v. Press **OK** to confirm the settings, close the dialog box and return to the **Launch Batch Designer** dialog box.

Note If you have the appropriate permissions (see *User permissions*, page 189), you will be able to change the **Spiral Volume** and **threshold** settings for a spiral batch after the batch has been created (see *Spiral plate properties*, page 150). However, you will not be able to change the **Spiral Plate Diameter** or *any* of the settings for the other plate types after you have created the batch in the next step.

In this window you can also adjust the colony parameters and MIC strip properties.

3. To create the new batch, press the button for the type of batch required. Depending on which options you have purchased, you can create batches for the following plate types:
 - Ames Plate
 - Chromogenic Media
 - Dilution Series
 - Multi-Sector Plate
 - Multiwell
 - OPKA
 - Pour Plate

Creating a new batch

- SBA
- Spiral Dilution series
- Spiral Plate
- Antibiotic Susceptibility
- Inhibition Zone
- MIC strip

The Batch Designer will be launched for the selected plate type so that you can complete the process of setting up the new batch – see *Setting up batches*, page 27, for details.

For more detailed information and help setting up individual batches, please refer to the quick guides.

Creating a new batch based on an existing batch or an exported batch file

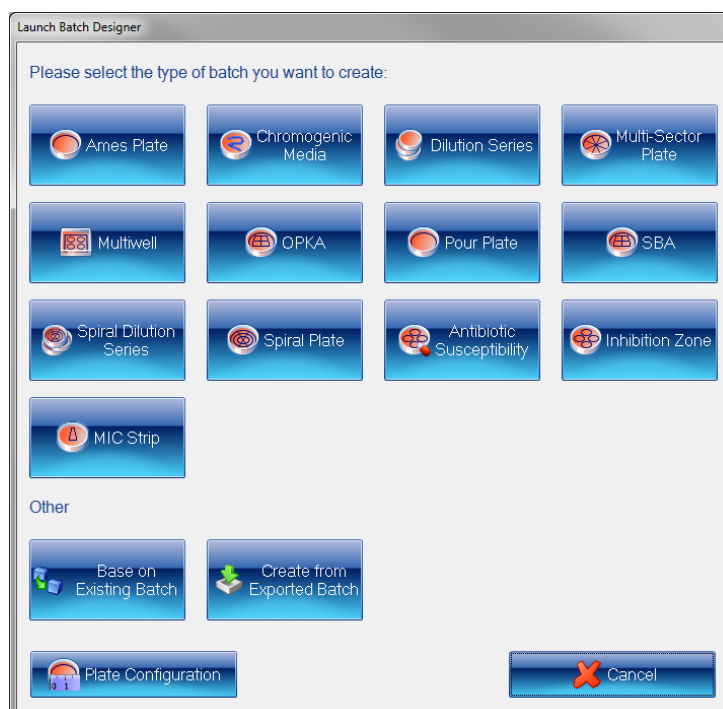
Note See *Creating a completely new batch*, page 20, for how to create a new batch from scratch.

To create a new batch based on an existing batch or an exported batch file: 1.

Press



to display the **Launch Batch Designer** dialog box:

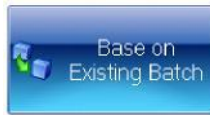


Go to Step 2 if you want to create a new batch from an existing batch; go to Step 3 if you want to create a new batch from an exported batch file.

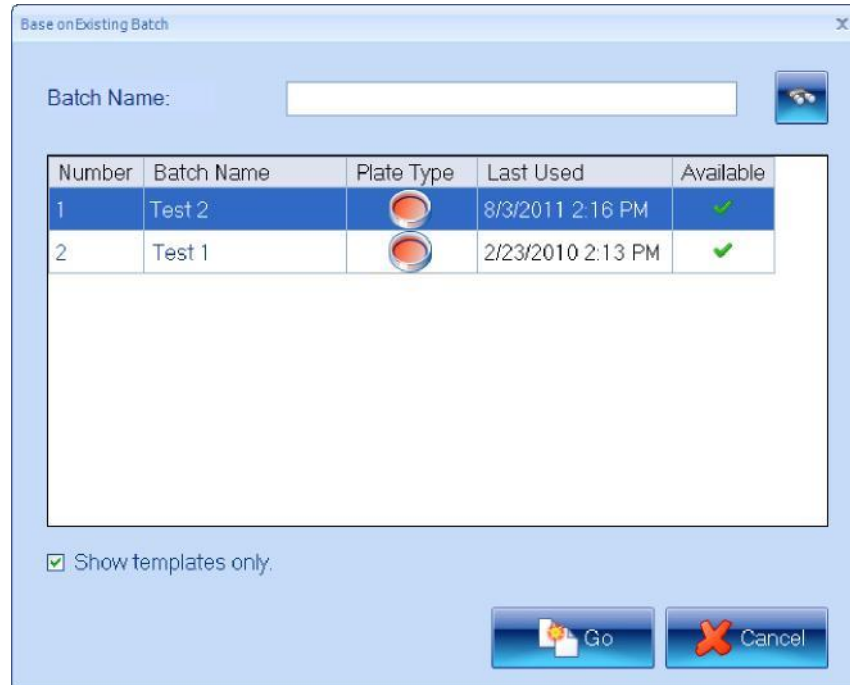
Note The plate configuration will be inherited from the existing batch and any settings you make using the **Plate Configuration** button will be ignored.

2. If you want the new batch to have the same settings as an existing batch: a.

Press



to display the **Base on Existing Batch** dialog box:



Note Any batches that are currently open will be marked with a tick in the **Available** column. You will not be prevented from choosing to base the new batch on one of these, but you will be warned when you confirm the selection – see Step f.

- b. Select **Show templates only** to hide any batches in the list that were based on an existing batch or an exported batch file.
- c. If there are a large number of existing batches, the batch you want to use as a basis for the new batch may not be shown in the list. To search for an existing batch:
 - i. Type the name, or a part of the name, of the required batch into the **Batch Name** box.
 - ii. Press

Only batches with names that contain the text you entered will be listed.
- d. Select the required batch in the list.
- e. Press

Creating a new batch

- f. If you chose an existing batch that is currently in use, you will be warned:



Press **Yes** to create the new batch anyway; press **No** to close the warning dialog box and return to Step **C** to choose another batch.

The new batch will be created; see the paragraph following Step 3 for what to do next.

3. If you want the new batch to have the same settings as an exported batch (see *Exporting batch details*, page 133, for how to export batches):

- a. Press



to display the **Create from Exported Batch** dialog box:



- b. Press



to display the **Please select a batch file to import** dialog box – this is a standard Windows **Open** dialog box.

- c. Use the dialog box to locate and select the required exported batch file.
d. Press **Open** to select the file and return to the **Create from Exported Batch** dialog box.
e. Press



to create the new batch.

The new batch will be created and loaded into the Batch Designer with the same settings as the selected existing batch or exported batch file. You will be able to edit some of these settings in the Batch Designer – see *Setting up batches*, on the facing page, for how to customize the batch settings in the Batch Designer.

Note With the exception of some spiral plate settings (see *Spiral plate properties*, page 150), you will not be able to edit the settings made in the **Plate Configuration** dialog box when the original batch was created – see *Creating a completely new batch*, page 20, for details of the settings made in the **Plate Configuration** dialog box.

Setting up batches

Once you have created a new batch (see *Creating the batch*, page 20), it will be loaded into the Batch Designer for final set up and testing before you use it to carry out and record measurements.

While the Batch Designer is active:

- The message area at the bottom of the ProtoCOL 3 window contains an edit box allowing you to enter a name for the batch (see *Giving the batch a name*, on the next page):



- The **Discard New Batch** button appears near the top left corner of the ProtoCOL 3 window:



Press **Discard New Batch** to abort the new batch – you will be asked to confirm that you want to do this.

- The **Accept New Batch** button appears near the top left corner of the ProtoCOL 3 window. Initially, the button will be disabled:



When you have completed the steps *required* to set up the batch and 'Test Measure', the button will become enabled:



See *Accepting the batch design*, page 132, for further details.

The steps you will need to follow before you can start carrying out measurements depend on the type of plate you are using and on whether you based the new batch on an existing batch. However, in both cases you will need to give the new batch a name – see the next section *Giving the batch a name*.

Creating a new batch

The other settings you will need to make when you are creating a completely new batch, or the settings you can edit if the new batch is based on an existing one, are grouped on a series of tabs – see the following sections for details:

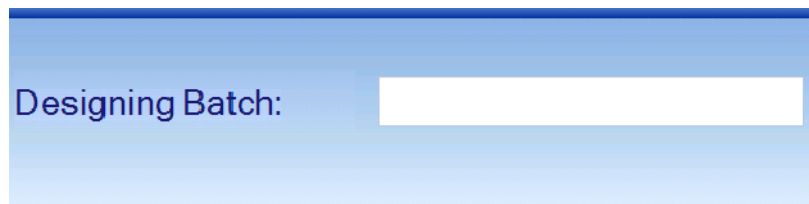
- *The Classification tab*, below – in particular, see:
 - *The Classification tab – colony counting batches*, on the facing page
 - *The Classification tab – zone measurement batches*, page 76.
- *The Measure tab*, page 123
- *The Configuration tab*, page 107
- *The Results tab*, page 131.

Giving the batch a name

After you have created a new batch (see *Creating the batch*, page 20), you must give it a name before you can accept the design (see *Accepting the batch design*, page 132).

To give a name to a batch:

Type the name into the **Designing Batch** box at the bottom of the **Batch Designer** screen:

A screenshot of a software interface. It features a light blue background with a darker blue header bar. Below the header, the text 'Designing Batch:' is displayed in a dark blue font. To the right of this text is a white rectangular input field with a thin blue border, intended for entering a batch name.

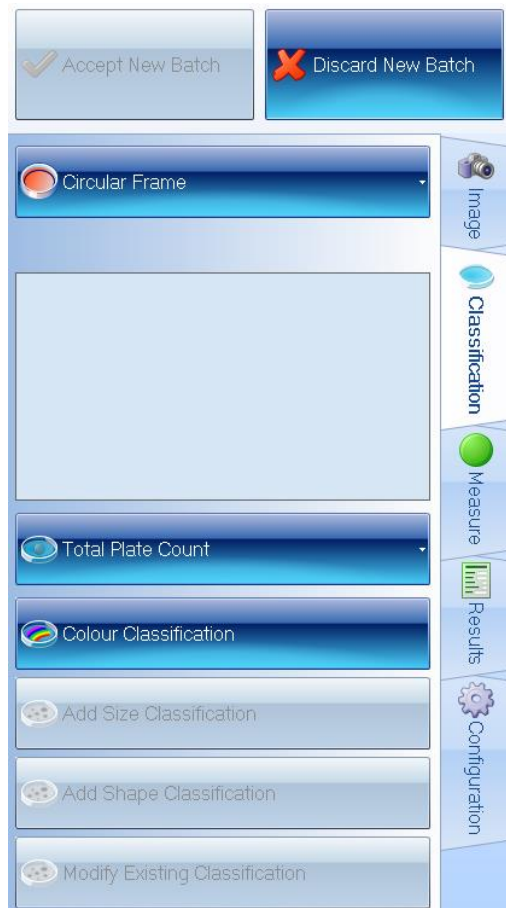
See *Setting up batches*, on the previous page, for a list of other steps in setting up a new batch.

The Classification tab

You use the **Classification** tab to specify how colonies or zones are automatically detected by ProtoCOL 3. The content of the tab and the procedures required depend on the type of batch – for details see:

- *The Classification tab – colony counting batches*, on the facing page
- *The Classification tab – zone measurement batches*, page 76.

The Classification tab – colony counting batches



To display the classification controls in the Batch Designer:

Press the **Classification** tab:



In order to use the **Classification** tab, you will need a sample image to work with:

1. Insert a typical plate from the batch into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
2. Capture the image – see *Capturing an image*, page 10.

The **Classification** tab in the Batch Designer for counting colonies allows you to:

- set the frame – see *Frame*, on the next page
 - configure a simple total plate count – see *Total Plate Count*, page 38
- view details of the current classification and set the properties of the markers used to identify detected colonies on the image – see *The Classification panel*, page 41

Creating a new batch

- create a color classification – see *Color classification*, page 43
- create a size sub-classification – see *Size classification*, page 63
- create a shape sub-classification – see *Shape classification*, page 67
- modify an existing classification – see *Changing an existing classification*, page 70.

Note By default, if you base a new batch on an existing batch, it will use the same total plate count or classification settings as the existing batch (though you can change them if required). If you are creating a completely new batch, you will not be able to accept it for taking measurements until you have defined a total plate count or color classified it.

Frame

The following sections tell you about:

- *Choosing the type of frame to use*
- *Adjusting the position and size of frames*, page 33.

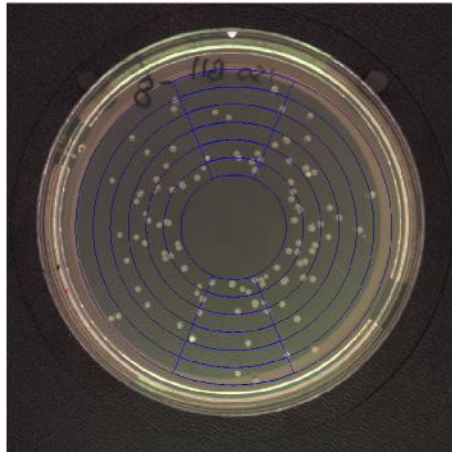
Choosing the type of frame to use

The 'frame' defines the area within which colonies will be counted. The choice of frames depends on the type of batch:

Different frames / multiple frames will give you a separate recorded count for each plate

- **Pour Plate, Dilution Series, Chromogenic and Ames:** the frame can be circular, rectangular or a single sector of a circle occupying 1/10, 1/8, 1/4 or 1/2 of the total area.
- **Multi-Sector Plate:**
 - Multi-Sector Frame: the frame is a circle divided into the selected number of sectors
 - Air Plate Frame: the frame consists of two concentric circles (an annulus), with the area between them divided into the selected number of sectors.
- **OPKA and SBA:** the frame is a rectangle divided into a grid of rectangular sector sub-frames.
- **Multiwell:** the frame is a rectangular grid of circular sector sub-frames.
- **Spiral Plate:**
 - Whole Frame – Whole Frame: the frame is circular
 - Whole Frame – Annulus Frame: the frame consists of two concentric circles

- Two Sector: a series of concentric circles, with two opposite highlighted sectors. For example:



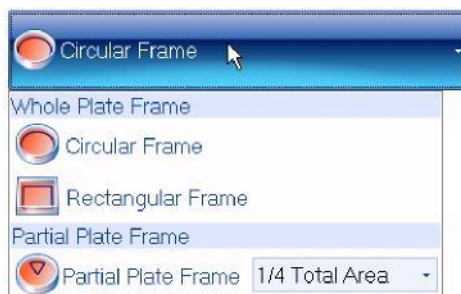
To change the type of frame used to define the region for counting colonies:

1. Press the frame button to display the frame menu.
2. Choose the required option – the contents of the menu depend on the type of plate and are described in the following sections:
 - *Pour Plate, Dilution Series and Ames batches*, below
 - *Spiral Plate batches*, on the next page
 - *Multi-Sector Plate batches*, on the next page
 - *OPKA and SBA batches*, on the next page
 - *Multi well batches*, page 33.

Note You can change the type of frame in the Batch Designer or Measurement mode.

Pour Plate, Dilution Series, Chromogenic and Ames batches

You can choose **Circular Frame** or **Rectangular Frame**, or, to use a frame covering a single sector only, choose **1/2**, **1/4**, **1/8** or **1/10** from the **Partial Plate Frame** submenu:



Partial plate frames can be useful when you have plates that are heavily loaded with bacteria, or if there is some problem with a plate and you just want to read results from an unaffected part. This means that you are more likely to want to choose a partial plate frame for an individual plate while you are working in Measurement mode rather than for all the plates in a batch when you are creating it in the Batch Designer.

Creating a new batch

Note You can also avoid counting in problematic areas of individual plates by placing Exclude regions over them – see *Exclude regions*, page 149.

Spiral Plate batches

You can choose **Whole Frame**, **Annulus Frame** or **2 Sector**:



Notes As a general rule, if there has only been a small amount of colony growth, you should use one of the **Whole Frame** options (use **Annulus Frame** if the spiral plater does not reach the center of the plate; use **Whole Frame** if it does reach the center), otherwise, use **2 Sector**.

If you choose **2 Sector** and the number of colonies counted in the first sector falls below the **threshold under which all colonies are counted** (see *Creating a completely new batch*, page 20, or *Spiral plate properties*, page 150), ProtoCOL 3 will count the whole frame.

Multi-Sector Plate batches

You can use the **Multi-Sector Frame** submenu to choose between a **Multi-Sector Frame** and an **Air Plate Frame**, and the number of sectors required in each case. For an **Air Plate Frame**, you can also set the size of the hole by dragging the **Hole size** slider, or increase or decrease it by one step by clicking on the **+** or **-** buttons:



OPKA and SBA batches

You can choose the number of columns and rows in the grid of OPKA or SBA frame sectors from the **OPKA Frame/SBA Frame** submenu:



Multiwell batches

You can use the **Multiwell Frame** submenu to choose the number of columns and rows in the grid of sector sub-frames. You can also set the size of the sub-frames by dragging the slider, or increase or decrease it by one step by clicking on the + or – buttons (this changes the size of all the sub-frames together):



Adjusting the position and size of frames

The following sections describe how to adjust the position and size of frames:

Moving frames, below

Resizing frames, page 35

Rotating frames, page 36.

Note You should load a plate from the batch into the instrument and capture an image before adjusting the frame – see *Loading plates into the instrument*, page 9, and *Capturing an image*, page 10.

Moving frames

To move a frame:

1. For a Multiwell, OPKA or SBA frame, to choose whether to move the frame as a whole or the individual sector sub-frames:
 - a. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



- b. Press:



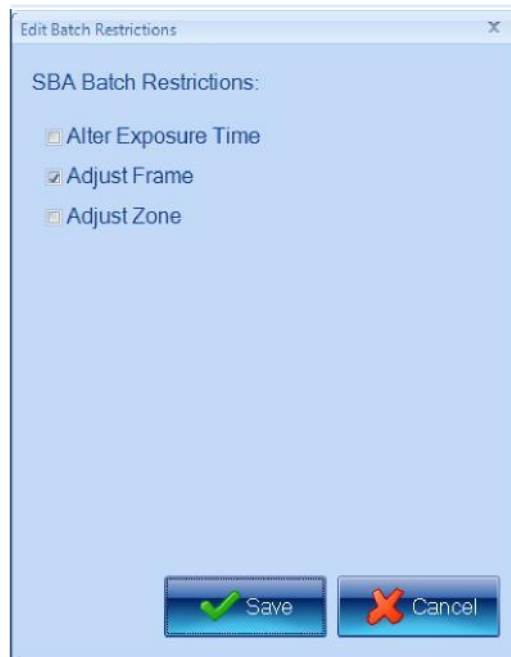
to display the **Batch Management** submenu.

- c. Press:



Creating a new batch

to display the **Edit Batch Restrictions** dialog box:



- d. Check **Adjust Frame** if you want to move the frame as a whole; check **Adjust Zone** if you want to move the individual sector sub-frames.
 - e. Press **Save** to close the **Edit Batch Restrictions**.
2. For a Multiwell, OPKA or SBA frame, if you chose **Adjust Zone** in the previous step (so that you move the individual sector sub-frames rather than the frame as a whole), either:
 - Press the **Select Multiple Zones** button below the preview pane:



to move a selection of sub-frames together (the button label changes to the **Select Individual Zones** after it is pressed).

Or

- Press the **Select Individual Zones** button below the preview pane:



to move individual sub-frames one at a time (the button label changes to the **Select Multiple Zones** after it is pressed).

3. For a Multiwell, OPKA or SBA frame, if you chose **Select Multiple Zones** in the previous step:
 - To choose multiple sub-frames:

Click in them – the selected sub-frames will be highlighted in yellow.
 - To deselect a selected sub-frame:

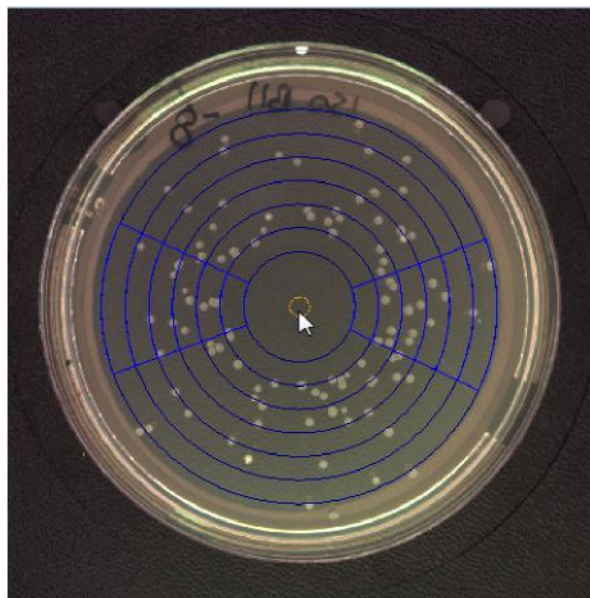
Click in it – the yellow highlighting will be removed.

- To deselect all selected sub-frames:

Press the **Deselect Zones** button below the preview pane:



4. Move the pointer near to the center of the frame (see following notes if you want to move individual Multiwell, OPKA or SBA sector sub-frames) – an orange circular drag handle will appear in the center of the frame. For example:



Notes If the orange circle does not appear, make sure **Adjust Frame** is not restricted – see *Setting batch restrictions*, page 133.

If you have selected **Adjust Zone** to move individual Multiwell, OPKA or SBA sector sub-frames, the individual sub-frame(s) will be highlighted instead of a circular drag handle appearing.

5. Drag the frame or sub-frame(s) and drop it/them in the required position.

Resizing frames

Generally, frames should be made as large as possible within the area of the plate but avoiding the very edge (as this may produce spurious results) and allowing some margin for variation between plates. You can adjust the size for individual plates in Measurement mode, but it will save time if you can use a single setting for all the plates in a batch. For counting outside the frame, please refer to the quick guide

Note See *Multiwell batches*, page 33, for how to resize the sector sub-frames within a Multiwell frame, and *Multi-Sector Plate batches*, page 32, for how to resize the hole at the center of an air plate frame. You cannot resize the sector sub-frames for SBA and OPKA frames independently of the complete frame.

To resize a frame:

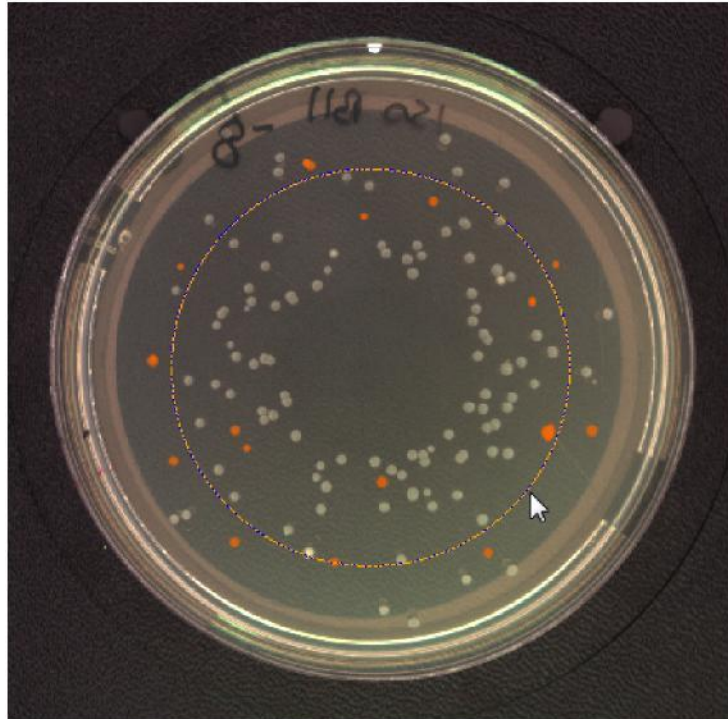
1. Make sure **Adjust Frame** is selected in the **Edit Batch Restrictions** dialog box (this is the default) – see *Setting batch restrictions*, page 133.

Note If **Adjust Zone** is selected for a Multiwell, SBA or OPKA batch, the following procedure will move the sector sub-frame, not resize it.

Creating a new batch

2. Move the pointer near to the frame boundary (for Partial Plate frames, the frame boundary is the circle of which the frame forms a sector).

The frame boundary will turn orange – showing that you can adjust its size. For example:



3. Drag the frame boundary to resize/reshape the frame.

For rectangular frames, drag the top or bottom side to adjust the height; drag the left or right side to adjust the width; drag a corner if you want the frame to maintain the same shape (aspect ratio) when you adjust the size.

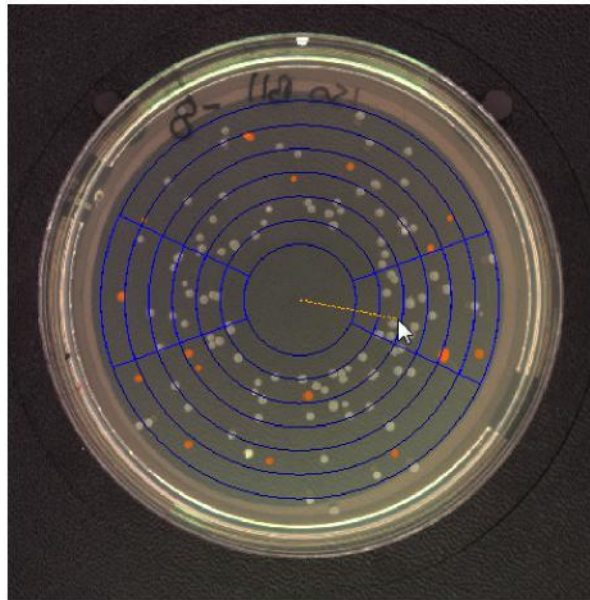
Rotating frames

You can rotate 2-Sector Spiral Plate frames, Partial Plate frames (any type) and Multi-Sector frames.

To rotate a frame:

1. Move the pointer to a point about half way between the center and edge of the frame.

An orange line will appear joining the pointer to the center. For example:



Note If the orange line does not appear, make sure **Adjust Frame** is selected in the **Edit Batch Restrictions** dialog box (this is the default) – see *Setting batch restrictions*, page 133.

2. Drag the line around the center to rotate the frame.

Changing the grid axis for an OPKA frame

By default, the sub-frames in an OPKA frame are numbered in column order, for example:

1	4	7
2	5	8
3	6	9

If required, you can change to numbering in row order, for example:

1	2	3
4	5	6
7	8	9

Creating a new batch

To change the number ordering for an OPKA frame:

Press the **Change Grid Axis** button below the preview pane:



Total Plate Count

You can use the **Total Plate Count** button to set simple criteria for detecting and counting the total number of colonies on a plate according to their color – see *Color classification*, page 43, for how to set more sophisticated color criteria, including the ability to distinguish between different colony colors; see also, *Adding sub-classifications*, page 62, for how to further distinguish between colonies according to their size and/or shape.

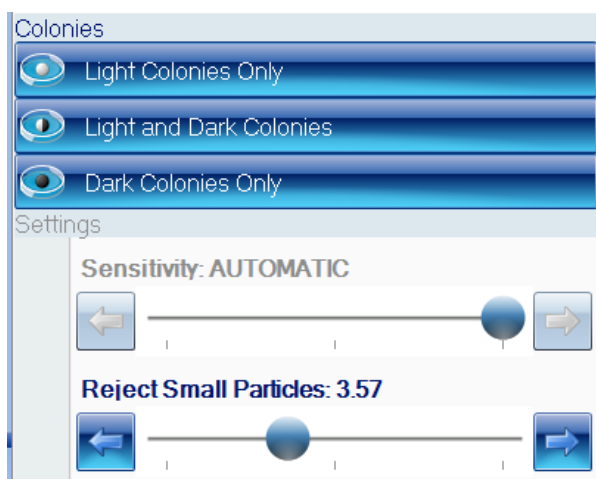
Note If you press the **Total Plate Count** button after adding a color classification, that classification, and any sub-classifications you have added, will be removed.

To set simple **Total Plate Count** criteria:

1. Press the **Classification** tab in the Batch Designer – see *The Classification tab*, page 28.
2. To specify whether the colonies to be counted are lighter than or darker than the background:
 - a. Press:



to display the **Total Plate Count** controls:



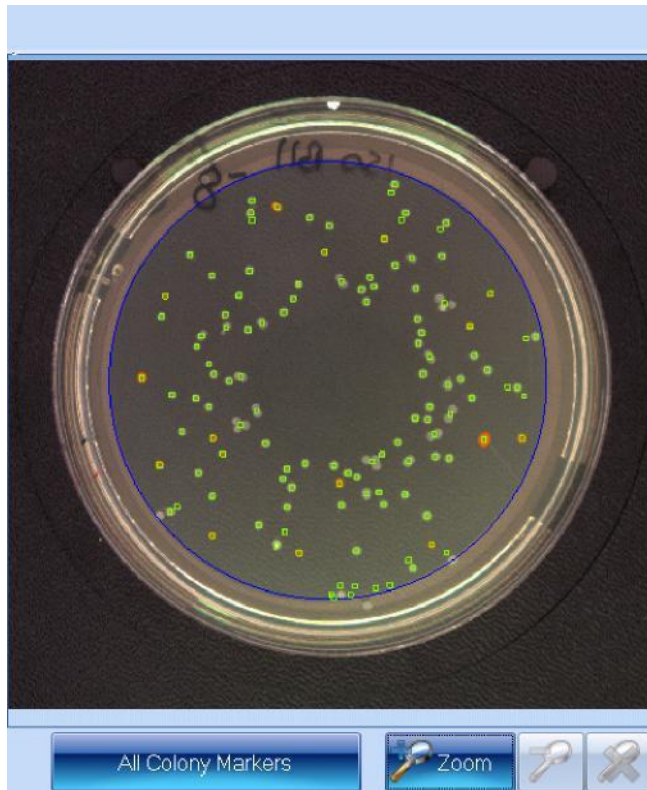
Note If there is no current image, an image will be automatically captured when you press the **Total Plate Count** button. If the image shown in the viewer is too light or too dark, press the **Image** tab and adjust the exposure as required (see *Setting the exposure*, page 10), then go back to Step 1. The light and dark control is for the plates which you feel have mixed colonies.

- b. Press the appropriate button to choose whether to count:
 - Light Colonies Only** – the number of colonies lighter than the background
 - Dark Colonies Only** – the number of colonies darker than the background.

The **Total Plate Count** controls will be hidden and the icon on the **Total Plate Count** will show the selected option:



- c. If the button below the image is labeled **No Colony Markers**, press the button and select **All Colony Markers** to display the detected colonies on the image:



For more detailed information about the precise areas identified as colonies, you can also select **All Colony Outlines** or **All Colony Regions** instead.

If required, you can zoom into the image to check the colony detection – see *Zooming the Image*, page 11.

If required, you can change the symbol and color used to mark the colonies – for details, see the next section, *The Classification panel*.

3. To adjust the settings used when counting colonies using a Total Plate Count (if the button below the image is labeled **All Colony Markers**, **All Colony Outlines** or **All Colony Regions**, the detected colonies will be shown on the image when you make the adjustments):

- a. Press:



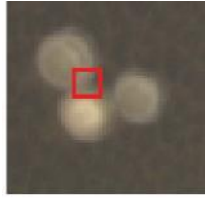
to display the **Total Plate Count** controls again – see picture in Step 1.

- b. Click on the **Split Touching Colonies** check button to specify whether to use the colony splitter for colonies or not.

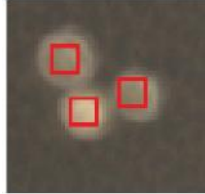
Creating a new batch

For example:

- Split Touching Colonies** unchecked:



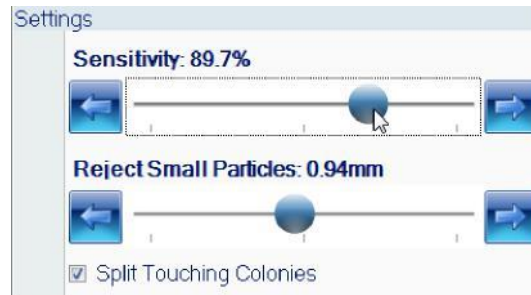
- Split Touching Colonies** checked:



- c. In most cases, the automatic sensitivity setting will give excellent results, but if the colonies are not being detected correctly, you can experiment by adjusting the sensitivity manually until you get an optimal result.

To adjust the detection sensitivity:

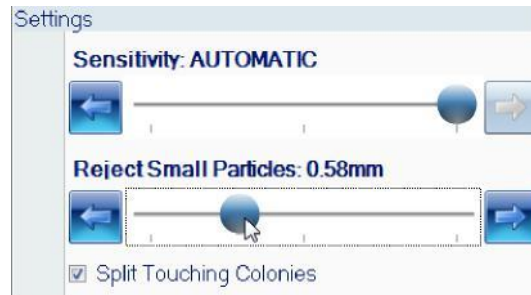
Drag the **Sensitivity** slider; click the arrow buttons at the ends of the slider to adjust the slider by a single step; or press anywhere on the slider bar to set the slider to that position – as you adjust the setting, the control shows the sensitivity set. As a rule of thumb, a sensitivity of 95% works well for most plates:



To set the **Sensitivity** back to **AUTOMATIC**, drag the slider back to the right-hand end – in fact, this corresponds to a manual sensitivity setting at the middle of the scale.

- d. To set a lower limit to the size colonies must be in order to be counted (you can use this, for example, to prevent small particles of debris being counted as colonies):

Drag the **Reject Small Particles** slider; click the arrow buttons at the ends of the slider to adjust the slider by a single step; or press anywhere on the slider bar to set the slider to that position – as you adjust the setting, the control shows the minimum colony size set:



To switch small particle filtering off, drag the slider to the left-hand side.

Note The **Sensitivity**, **Reject Small Particles** and **Split Touching Colonies** settings you make here will be used to perform the initial counts for plates when you are working in Measurement mode. However, you will be able to vary these settings for individual plates by carrying out a test count in Measurement mode (see *Test measurement and adjusting settings*, page 151), or by editing the result after you have carried out a count (see *Rejecting small particles in a result*, page 166, *Changing the Sensitivity setting for a result*, page 166, and *Splitting colonies in a result*, page 167).

4. Press



to hide the **Total Plate Count** controls again.

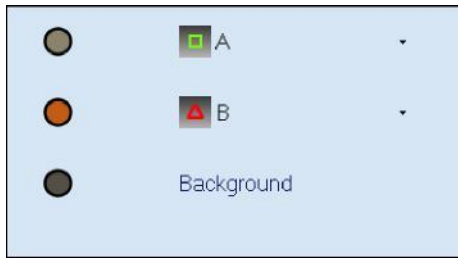
The Classification panel

Once you have created your settings and accepted the batch, the Classification panel appears below the frame button in the classification tab. If you have selected **Total Plate Count** (see *Total Plate Count*, page 38), it shows a key for the markers used to identify the detected colonies on the image:



Creating a new batch

If you have set up a color classification (see *Color classification*, on the facing page), it will show the classified colors and a key for the markers used to identify the detected colonies on the image:



In this example, the only classification is a color classification with two colony types **A** and **B** along with the **Background** (classification items can also be classified as debris – see *Classification Details*, page 60).

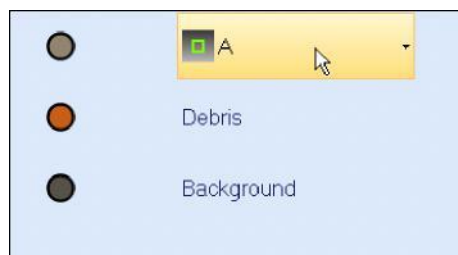
If you add sub-classifications (see *Adding sub-classifications*, page 62), the classification will appear as a tree – for example:



As well as showing you the current classification, the Classification panel allows you to change the colony marker and color (but not the name, or whether colors correspond to colonies, debris or background).

To change the colony marker or color:

1. Move the pointer over the colony item to display the colony button:



2. Press the colony button to display the marker controls:

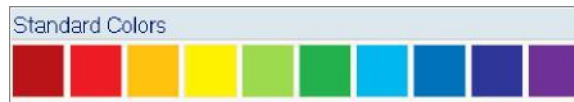


3. Either:

Select the shape of colony marker you want to use from the marker controls

Or:

- a. Press **Color** to display the **Standard Colors** control:



- b. Click on the required color in the **Standard Colors** control.

Color classification

In many cases you can use the **Total Plate Count** (see *Total Plate Count*, page 38) to make accurate and reliable counts of the colonies on the plates in the batch. However, you can use the more powerful features offered by the color classification tool for difficult cases, or if you want to make separate counts of differently colored colonies, or to use size and shape to distinguish colonies (see *Adding sub-classifications*, page 62).

Note You can only perform size or shape classifications *after* you have performed a color classification.

To carry out a color classification procedure:

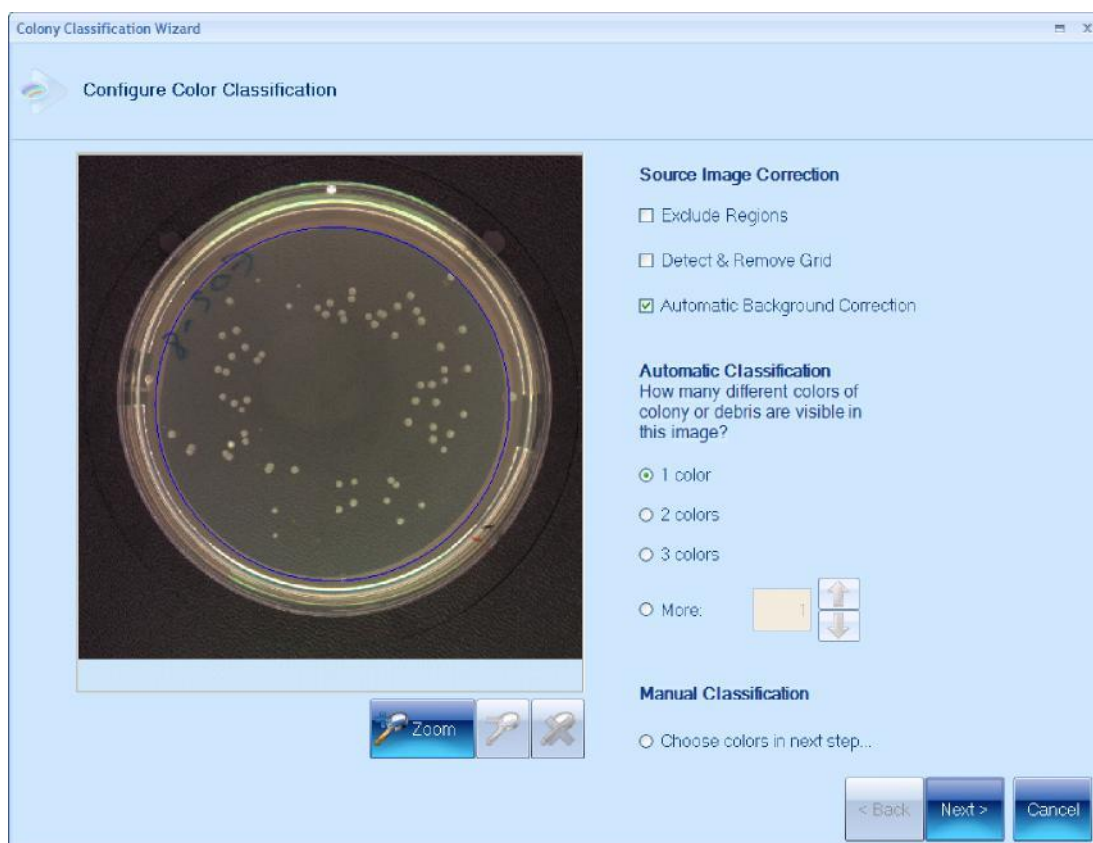
1. Capture the image of a typical plate from the batch – see *Loading plates into the instrument*, page 9, and *Capturing an image*, page 10.
2. Press



to start the **Colony Classification Wizard** for color classification.

Creating a new batch

The **Configure Color Classification** page will be displayed in the wizard:



Note If there is no current image, an image will be automatically captured when you press the **Color Classification** button. If the image shown in the wizard is too light or too dark, press **Cancel** to close the wizard and adjust the exposure accordingly (see *Setting the exposure*, page 10), then press the **Color Classification** button again to restart the wizard.

3. If required, you can move and/or resize the frame shown on the image using the same techniques as in *Adjusting the position and size of frames*, page 33. This may be useful in restricting the area used for color classification – see Step 5 for another way of limiting the area of the plate used for color classification.

Note The frame in the **Colony Classification Wizard** defines the area used for color classification – adjusting the color classification frame has no effect on the size or position of the main colony counting frame.

4. If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.
5. If one or more parts of the plate you are using for the color classification has some problem, such as a label or other artifacts, you can create one or more exclude regions so that these areas are not included in the color classification.

If you want to create one or more exclude regions on the plate image before the color classification is carried out, check **Exclude Regions**.

The **Exclude Regions** page of the wizard will be displayed when you click **Next** in the **Configure Color Classification** page – see *Excluding regions during color classification*, page 47, for details.

6. When you are using plates that have a grid pattern, the grid lines may interfere with the detection of colonies that have a similar color to the grid. To remove the effects of the grid on the count, check **Detect & Remove Grid**.

The **Detect & Remove Grid** page of the wizard will be displayed before the color classification is carried out – see *Detecting and removing a grid*, page 51, for details.

7. By default, ProtoCOL 3 automatically compensates for any irregularities in the illumination of the plate – this is called *background correction*. In most cases, automatic background correction produces excellent results, but you may find that if the plates in a batch have some unusual properties, you can get better results by manually adjusting the background correction parameters, or by disabling background correction completely.

Note You should only use manual background correction after you have tried using automatic background correction and found that it does not produce satisfactory results.

To set the background correction manually, or to disable background correction:

Press the **Automatic background correction** check box so that it becomes unchecked (see *Manual background correction*, page 52, for how to carry out the manual background correction procedure).

To allow ProtoCOL 3 to carry out the background correction automatically:

Leave **Automatic background correction** checked.

8. In this step you choose whether to make ProtoCOL 3 carry out an automatic color classification of the colonies or to carry out a manual color classification yourself.

To make ProtoCOL 3 carry out an automatic color classification of the colonies:

- a. Decide on the number of colors to be classified on the plate (excluding the background); this is the number of distinct colony types plus the number of distinct colors of debris.

Note As an example, suppose the plates have colonies with two different colors but no debris. If you want the results to show separate totals for the two different colors of colony, you should set the number of colony types to **2**, but if you just want the total number of colonies, irrespective of color, the number of colony types should be set to **1**. However, the automatic color classification may have problems if there are more than two colors and you want to distinguish between some of them, but not others: for example, if there are two colony colors that you want to count together, but also some debris that you want to distinguish from the colonies. In this case, you could:

- Set the number of distinct colony types plus the number of distinct colors of debris to **2**, and check whether the automatic color classification successfully distinguishes the colonies from the debris (see *Review Color Classification*, page 57) – if it does not, you can press **Back** to return to the **Configure Color Classification** page to try one of the following alternatives.
- Set the number of colors for automatic color classification to be three and combine the results for the different colony colors later (outside ProtoCOL 3).
- Use manual color classification.
 - b. Select the **Automatic Classification** radio button corresponding to the number of different colors to be classified. If you want to enter a number greater than 3, select **More** and press the arrow buttons to set the required number.

Creating a new batch

- To set the color classification manually:

Select the **Choose colors in next step** radio button (the button is labeled **Choose colors in later step** if you have not selected the default options for other settings).

9. Press **Next**.

What happens next depends on the selections you have made:

- If you have selected **Exclude Regions**, the wizard will display the **Exclude Regions** page – see *Excluding regions during color classification*, on the facing page, for instructions.
- Otherwise, if you have selected **Detect & Remove Grid**, the wizard will display the **Detect & Remove Grid** page – see *Detecting and removing a grid*, page 51, for instructions.
- Otherwise, if you have not selected **Automatic background correction**, the wizard will display the **Background Correction** page – see *Manual background correction*, page 52, for instructions.
- Otherwise (ie you have not selected **Exclude Regions** or **Detect & Remove Grid** and you have selected **Automatic background correction**):
 - If you have selected manual color classification, the wizard will display the **Manual Color Classification** page – see *Manual color classification*, page 54, for instructions.
 - Otherwise (ie you have selected automatic color classification), the automatic colony color classification will be carried out immediately and the wizard will then display the **Review Color Classification** page showing the results – see *Review Color Classification*, page 57).

Excluding regions during color classification

The **Exclude Regions** page in the **Colony Classification Wizard** is displayed if **Exclude Regions** is selected when you press **Next** in the **Configure Color Classification** page (see *Color classification*, page 43):



On occasions, you may want to avoid counting colonies on part of the plate used for color classification because of some problem with it. You can do this by creating one or more exclude regions, which will be ignored when ProtoCOL 3 classifies the colonies.

To define an exclude region on the classification image:

1. Press



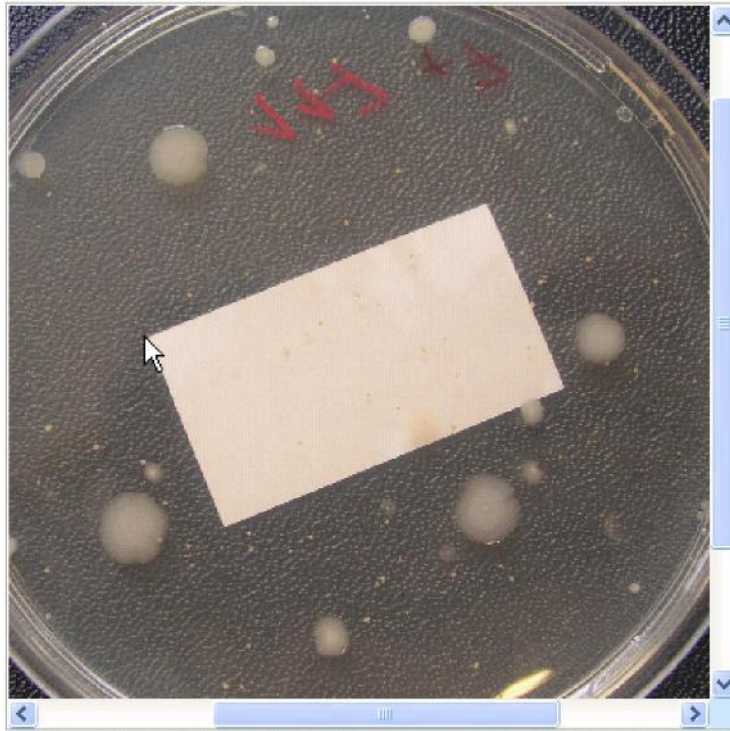
to select it:



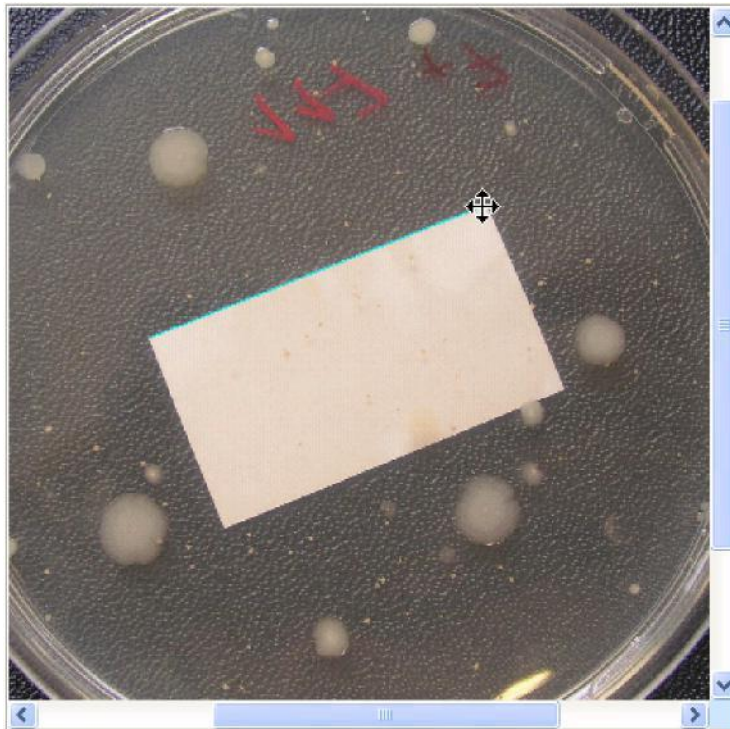
Note You may find it helpful to zoom the image before placing the exclude region on the image – see *Zooming the image*, page 11.

Creating a new batch

2. Move the pointer to the position you want to place the first vertex of the exclude region:

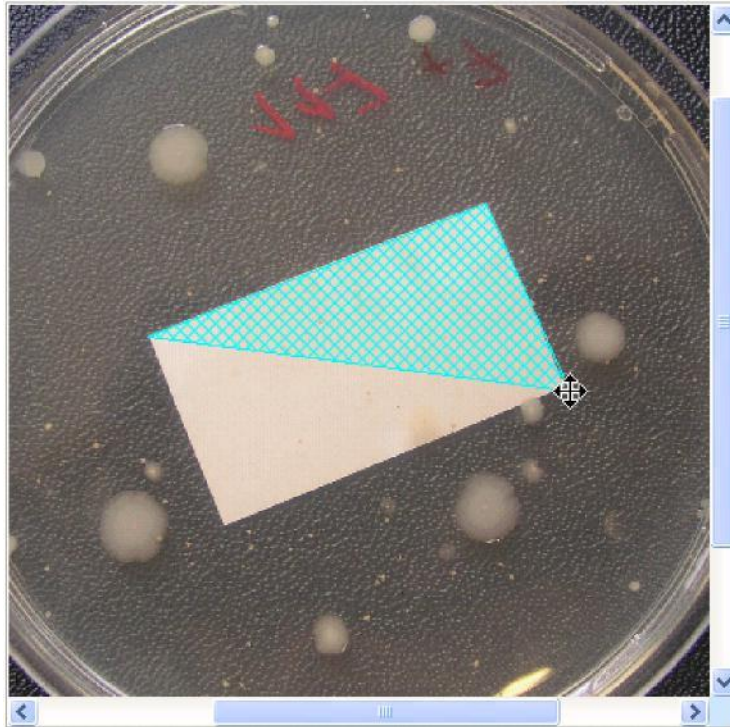


3. Click to place the first corner of the exclude region.
4. Move the pointer to the position you want to place the next vertex of the exclude region – a line will be dragged out from the first vertex:

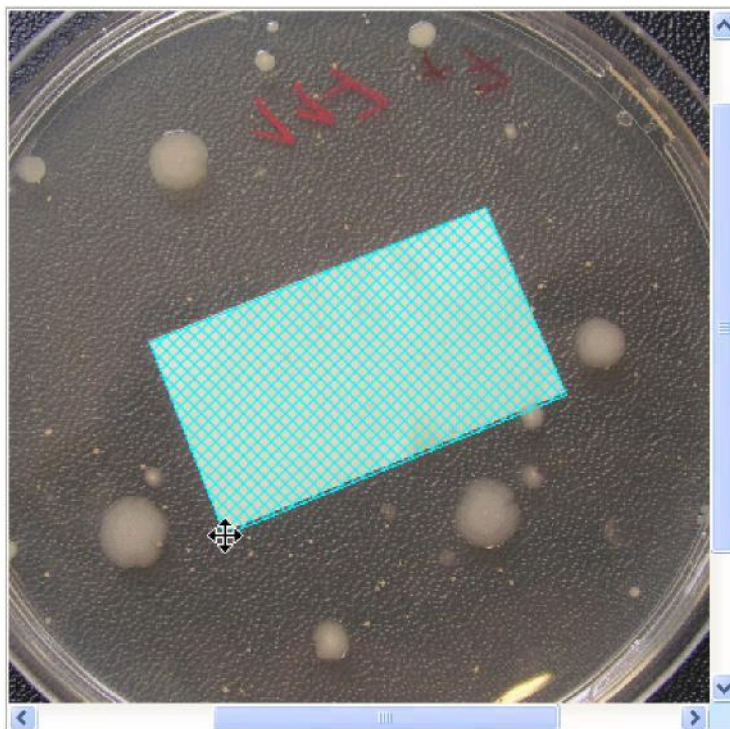


5. Click to add the new vertex for the exclude region.

- Repeat Steps 4 and 5, as required, to place any further vertices until you have placed the penultimate one (the included area is shown as you drag the mouse):



- Move the pointer to the final vertex of the exclude region.
- Double-click to add the vertex and complete the region.



Creating a new batch

To remove the last exclude region you added to the image:

Press



You can repeat these procedures to add and remove as many exclude regions as required.

Note The regions defined here will only be excluded from the color classification process itself – see *Exclude regions*, page 149, for how to define exclude regions for individual plates during the measurement process.

To proceed to the next step in the color classification procedure:

Press **Next**.

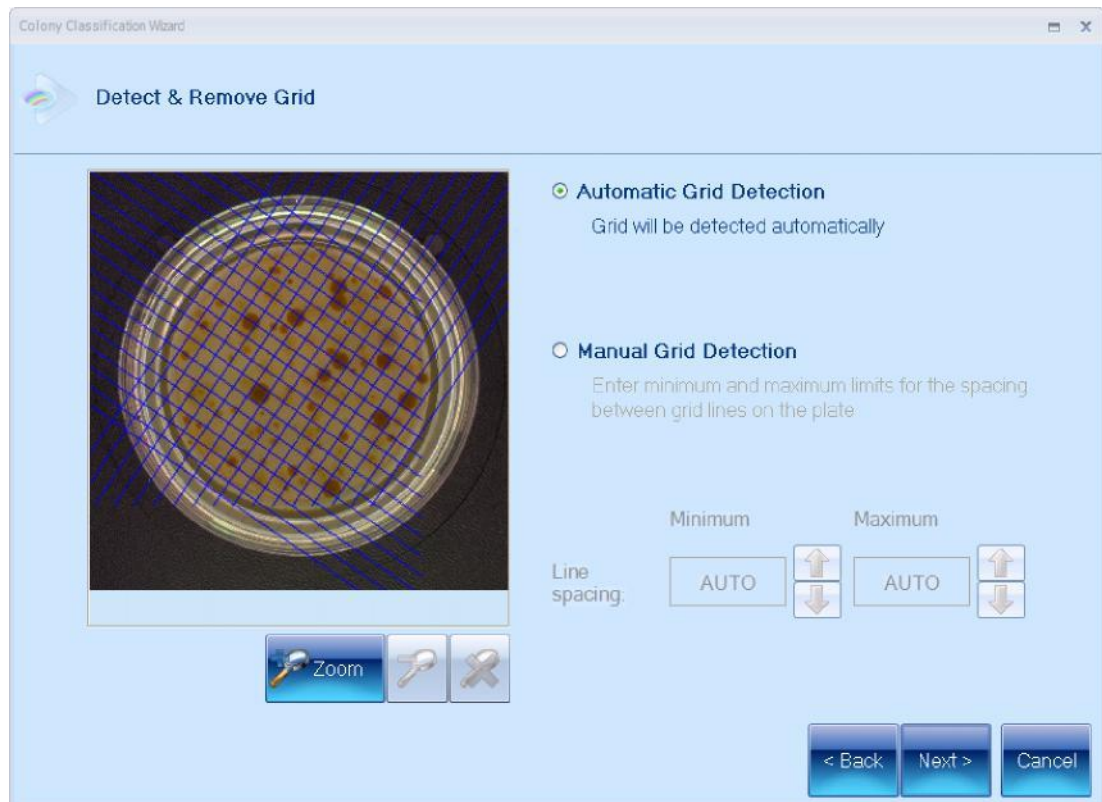
The next step in the color classification procedure depends on selections you made in the **Configure Color Classification** page (see *Color classification*, page 43):

- If you selected **Detect & Remove Grid**, the wizard will display the **Detect & Remove Grid** page – see *Detecting and removing a grid*, on the facing page, for instructions.
- Otherwise, if you did not select **Automatic background correction**, the wizard will display the **Background Correction** page – see *Manual background correction*, page 52, for instructions.
- Otherwise (ie you did not select **Detect & Remove Grid** and selected **Automatic background correction**):
 - If you selected manual color classification, the wizard will display the **Manual Color Classification** page – see *Manual color classification*, page 54, for instructions.
 - Otherwise (ie you selected automatic color classification), the automatic colony color classification will be carried out immediately and the wizard will then display the **Review Color Classification** page showing the results – see *Review Color Classification*, page 57).

Detecting and removing a grid

When you are using plates that have a grid pattern, the grid lines may interfere with the detection of colonies that have a similar color to the grid. The **Detect & Remove Grid** page allows you to remove the effects of the grid. The **Detect & Remove Grid** page in the **Colony Classification Wizard** is displayed during color classification if **Detect & Remove Grid** is selected in the **Configure Color Classification** page (see *Color classification*, page 43).

When the **Detect & Remove Grid** page is displayed, it performs automatic grid detection and marks the detected grid on the image:



If the automatic grid detection has succeeded in locating the grid on the plate, press **Next** to proceed to the next step in the color classification procedure – see the end of this section for details.

Note It may be easier to see the results if you zoom in on areas of the image: you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.

To narrow the grid search if the grid has not been correctly detected:

1. Select **Manual Grid Detection** – the **Line spacing** controls will become enabled:



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2. Set **Minimum** and **Maximum** limits for the grid **Line Spacing** in the search for the grid by typing directly into the edit boxes or by clicking on the up or down arrow buttons attached to the right-hand side of the edit boxes.

As you adjust the figures, the **Colony Classification Wizard** will carry out a new search for the grid.

3. Repeat Step 2 using narrower search criteria until the grid has been detected successfully.
4. Press **Next** to proceed to the next step in the color classification procedure.

The next step in the color classification procedure depends on selections you made in the **Configure Color Classification** page (see *Color classification*, page 43):

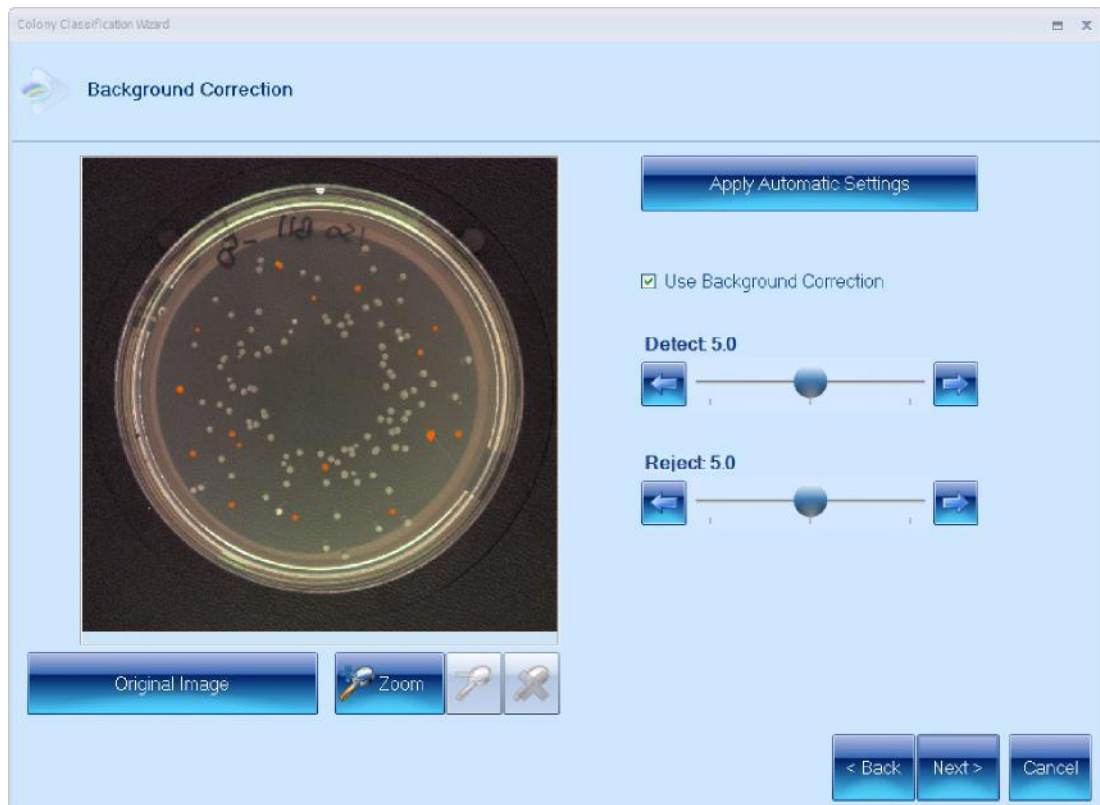
- If you did not select **Automatic background correction**, the wizard will display the **Background Correction** page – see *Manual background correction*, below, for instructions.
- Otherwise (i.e. you selected **Automatic background correction**):
 - If you selected manual color classification, the wizard will display the **Manual Color Classification** page – see *Manual color classification*, page 54, for instructions.
 - Otherwise (i.e. you selected automatic color classification), the automatic colony color classification will be carried out immediately and the wizard will then display the **Review Color Classification** page showing the results – see *Review Color Classification*, page 57).

Manual background correction

In most cases the ProtoCOL 3 automatic background correction produces very good results and should always be used in the first instance. In exceptional cases, however, you may find you can get better results by using manual background correction or no background correction at all.

Note You should only use manual background correction after you have tried using automatic background correction and found that it does not produce satisfactory results.

If you chose not to carry out automatic background correction (see Step 8 in *Color classification*, page 43), the **Background Correction** page is displayed in the **Colony Classification Wizard**:



To carry out a manual background correction:

1. If you do *not* want to apply any background correction, press the **Use Background Correction** check box to uncheck it and go to Step 5.

Otherwise, leave **Use Background Correction** checked and go on to the next step.
2. If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.
3. Adjust the **Detect** and **Reject** sliders to optimize the background correction so that the areas shown as background/non-background features in the colormap image correspond as accurately as possible to the actual background/non-background in the image (see following note).

You can drag the sliders, press the arrow buttons to adjust the sliders by a single step or click anywhere on a slider bar to set the slider to that position.

Note You can view the effect of the background correction by pressing the display button (labeled **Original Image** in the example picture). You can view:

- Colormap Image**: this uses contrasting colors to show which areas of the image have been detected as the background and which as foreground features
- Original image**: the uncorrected image
- Corrected image**: the image after the background correction has been applied to the image.

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- If you wish to revert to the automatic background correction settings (**Detect** and **Reject** both set to **5.0**), press



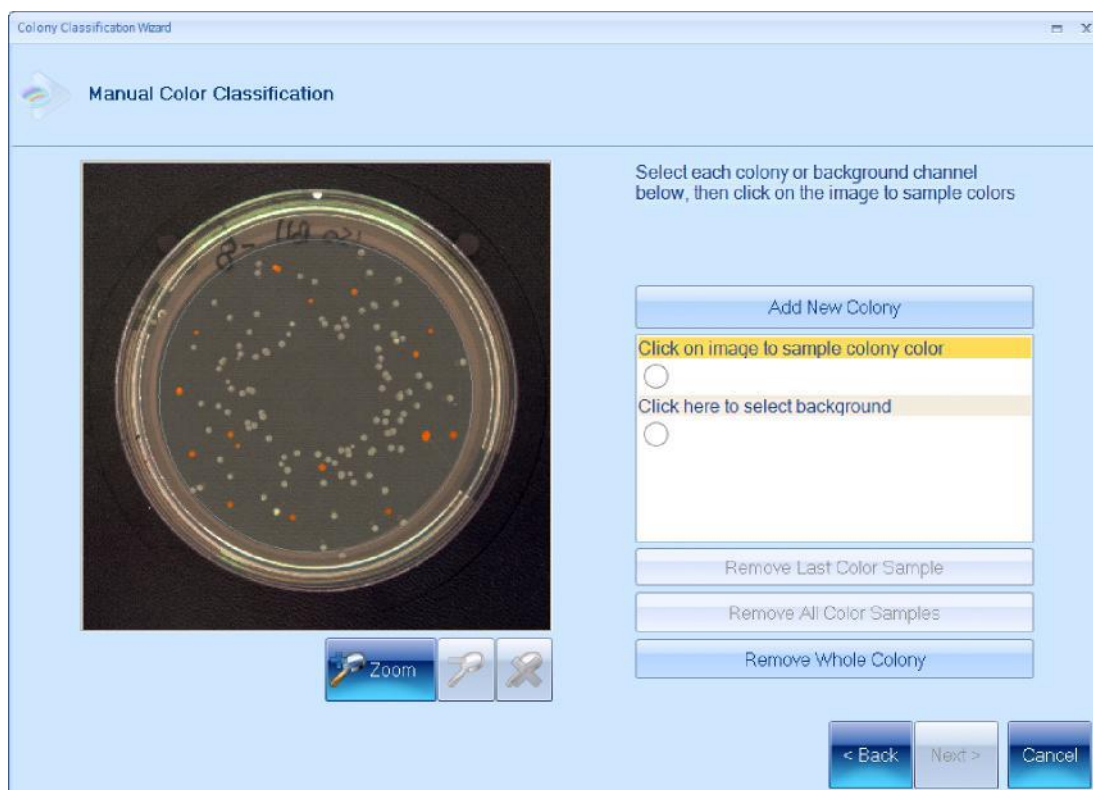
- When you are satisfied with the background correction, press **Next**.

What happens next depends on the selections you made earlier (see *Color classification*, page 43):

- If you selected manual color classification, the wizard will display the **Manual Color Classification** page – see the next section, *Manual color classification*, for instructions.
- Otherwise (ie you selected automatic color classification) the automatic color classification will be carried out immediately and the wizard will then display the **Review Color Classification** page showing the results – see *Review Color Classification*, page 57).

Manual color classification

If you selected **Choose colors in next step** or **Choose colors in later step** in the first page of the wizard (see step 9 in *Color classification*, page 43), the **Manual Color Classification** page is displayed in the **Colony Classification Wizard**:



You use the **Manual Color Classification** page to enable ProtoCOL 3 to distinguish the colonies you want to count from the background and any debris you want ignored. You do this by identifying the colors of the distinct colony types, the debris and the background appearing in the image and creating a 'color channel' for each of them. You then define the color of each color channel by taking samples of the corresponding color from the image.

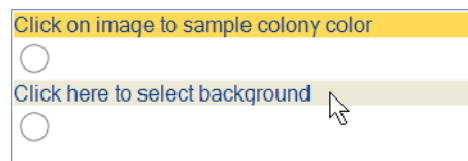
At this stage, one of the color channels is identified as a **background** color and the other channels as **colony colors**. However, the **Classification Details** page later in the wizard (see

Classification Details, page 60) will allow you to redefine each of the colony colors independently as a colony type, debris or background color. For example, if the background has some patches of different colors, you can assign one color to the **background** color channel and the other colors in the background to separate **colony colors**, and then redefine these later as background colors in the **Classification Details** page.

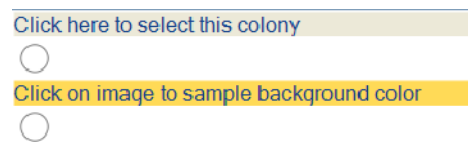
You can add as many colony channels as required. However, you should bear in mind that when you perform a count, the results will show a separate count for each colony channel you define. This means that if the plates have colonies with different colors, but you do not want to distinguish between them in the results (you just want the total number of colonies, irrespective of color), you should define a single color channel for these colonies, and sample all of the different colony colors to define that channel.

To carry out a manual color classification:

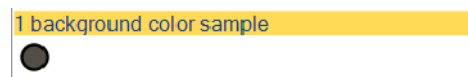
1. Click on the **Background color sample** item at the bottom of the channel list to select it:



The selected item will be highlighted in orange and the wording changed:

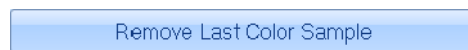


2. In the image, click on the background to sample its color. When you click, a circle showing a sample of the color you clicked on will appear in the color channel:



3. If you click at the wrong point and want to remove the last color sample added:

Press



If you want to remove all the color samples added:

Press



Note This removes the color sample(s) from the *currently selected* color channel – if the channel you want to remove the sample from is not currently selected, click on it first to select it.

4. Repeat Steps 2–3 as required to select a representative sample covering the full range of the background color as it appears in the image.
5. Identify the next color in the image for which you want to define a color channel: it may be a colony color, a debris color or another background color – you will specify

Creating a new batch

which it is in the **Classification Details** page later in the wizard (see *Classification Details*, page 60).

6. Either, if an undefined color channel already exists:

Click on the **Click here to select this colony** item for the channel to select it.

Or, if there are currently no undefined color channels:

Press



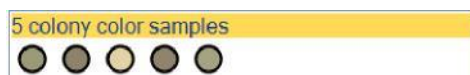
to create a new channel – the new channel will be selected.

The selected channel will be highlighted in orange and labeled **Click on image to sample colony color** (see pictures in Step 1).

7. If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11. Zooming the image may help you click accurately on colonies or debris when you are selecting color samples in Step 8.
8. In the image, click on areas of the image (colony, debris or background) that have the required color – you should select a representative sample covering the full range of the color as it appears for these objects in the image.

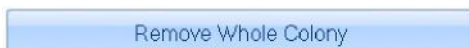
Note Remember that when you perform a count, the results will show a separate count for each *colony* channel you define – if you do not want the results to distinguish between different colored colonies, you should sample all of the colony colors for a single colony channel.

When you click, a circle showing a sample of the color you clicked on will appear in the color channel:



See Step 3 if you want to remove the last sample added.

9. When you have added a representative number of samples to the channel:
- If you want to add another color channel, go back to Step 5.
 - If you do not want to add another color channel, go to Step 10.
10. If after adding samples for all the colors appearing in the image there are any unused channels (colony definitions), or if you want to remove a channel you have added:
- a. Click on the **Click here to select this colony** item for the first channel you want to remove to select it (you cannot remove the default background item).
 - b. Press



c. Repeat Steps **a** and **b** for any other channels you want to remove.

11. Press **Next** to display the **Review Color Classification** page (see the next section, *Review Color Classification*).

(**Next** is disabled if there are any unused channels – see previous step.)

Review Color Classification

The **Review Color Classification** page is displayed in the **Colony Classification Wizard** after you have carried out an automatic or manual color classification (see *Color classification*, page 43, or *Manual color classification*, page 54, respectively):



To review the color classification:

1. Examine the detected colonies and image carefully to check that:
 - the colonies in the image are being detected successfully
 - different colors of colony are being distinguished successfully
 - the colonies are distinguished from debris with a different color.

Note It may help to change the way the image is displayed and/or the way the colonies are marked – see *Image and colony marker display* following these instructions. Also, it may be easier to see the results if you zoom in on areas of the image: you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.

2. If you are happy that the color classification is as good as possible, press **Next** to display the **Classification Details** page (see *Classification Details*, page 60).

Otherwise, proceed to the next step.

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- If colors are being wrongly classified (for example, if colonies are not being detected because they are being treated as background, or if colonies and debris with different colors are not being distinguished accurately):
 - If you performed an automatic color classification, try pressing **Back** until you get to the **Configure Color Classification** page and try increasing or decreasing the number of colors you selected, or try using a manual color classification instead – see *Manual color classification*, page 54.
 - If you performed an automatic background correction, try pressing **Back** until you get to the **Configure Color Classification** page and use a manual background correction instead – see *Manual background correction*, page 52.
- ProtoCOL 3 applies a size filter to remove the effect of small colored regions of the image that may be wrongly interpreted as colonies. You can use the **Reject Small Particles** slider bar to set the level of size filtering used.

To adjust the **Reject Small Particles** slider:

Drag the slider; click the arrow buttons at the ends of the slider to adjust the slider by a single step; or press anywhere on the slider bar to set the slider to that position.

- 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.
- If the colonies have indistinct fringes around them, they may be interpreted as separate colonies.

To remove the fringes from colonies if they are causing problems:

Check **Remove Fringes Around Colonies**.

- To choose whether to include or reject colonies that the frame passes through:

Check **Complete Colonies Across Frame** to include the colonies; uncheck it to reject them.
- If there is a problem with overlapping colonies being counted as a single colony:

Click on the **Split** check button next to the colony type:

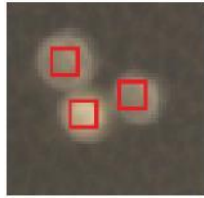


For example:

- Split** unchecked:



- **Split** checked:



8. When you are happy that the color classification is as good as possible, press **Next** to display the **Classification Details** page (see the next section, *Classification Details*).

Note The settings for the **Reject Small Particles** slider and colony splitter you make in the **Review Color Classification** page will be used to perform the initial counts for plates when you are working in Measurement mode. However, you will be able to vary these settings for individual plates by editing the measurement after you have performed the count – see *Rejecting small particles in a result*, page 166, and *Splitting colonies in a result*, page 167, for details.

Image and colony marker display

You can press the display button (labeled **Original Image All Colony Markers** in the example picture) and choose to display the image as the:

- **Colormap Image:** this uses contrasting colors to show which areas of the image have been detected as the background and which as foreground features
- **Original Image:** the original uncorrected image
- **Corrected Image:** the image after the background correction has been applied to the image.

You can also press the display button to choose how to mark the detected 'colonies' on the image.

Note Here 'colonies' means non-background parts of the image that have one of the classified colors, which may include different types of colony, different colors of the same type of colony, or debris. You will be able to specify whether each of these 'colony' types should be counted as a colony in the **Classification Details** page later (see *Classification Details*, on the next page).

You can choose to display:

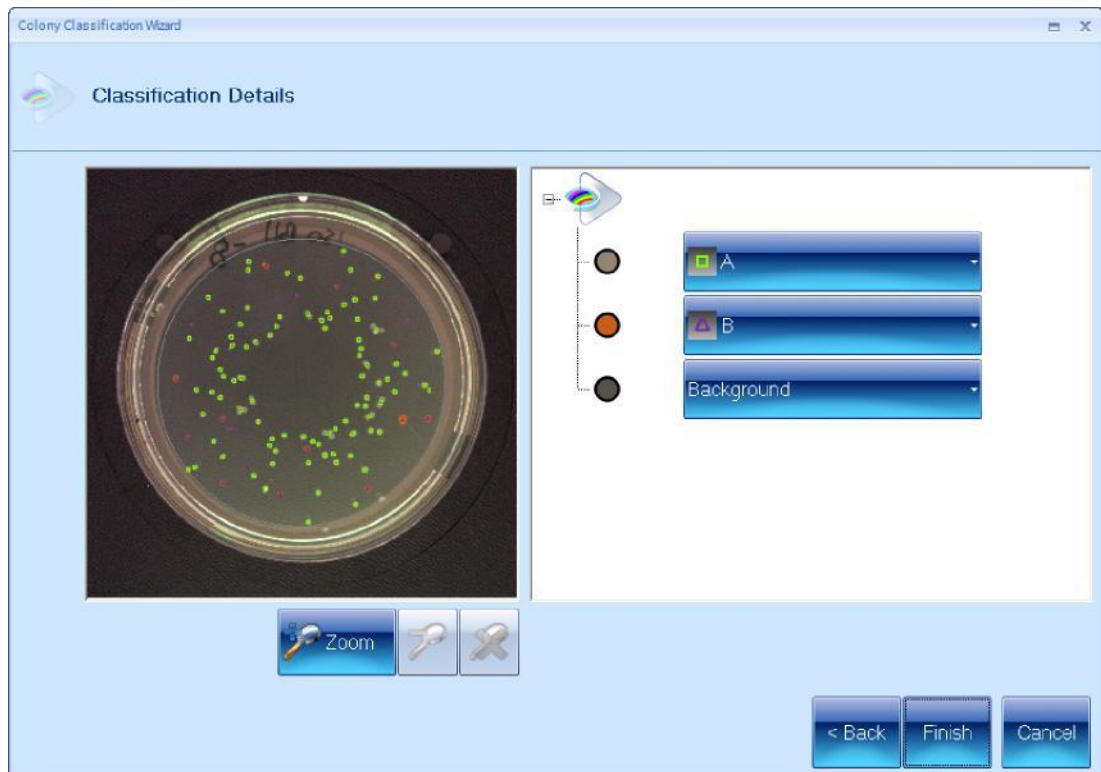
- **No Colony Markers**
- **All Colony Markers:** each detected colony is identified with a symbol showing the type of colony (color classification) – a key to the colony symbols is shown to the right of the image
- **All Colony Outlines:** the boundary of each detected colony is marked in the color of the symbol representing the type of colony
- **All Colony Regions:** the area of each detected colony is marked in the color of the symbol representing the type of colony.

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Classification Details

You use the **Classification Details** page to specify whether the detected colors (and sizes or shapes for a size or shape sub-classification) correspond to colonies, debris or background, and, if there is more than one type of colony color (size or shape), what organisms they correspond to.

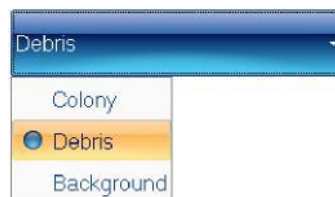
Note The following instructions show you how to change the classification details for a colony type produced by a color classification. However, this page is also displayed for the other classifications (size or shape – see *Adding sub-classifications*, page 62), and the same procedure, with the obvious wording changes, is also used for them.



The left-hand pane of the **Classification Details** page shows the image with the colony markers superimposed. The right-hand pane lists the classified colors in the image and shows whether they represent colonies, debris or the background, with a button allowing you to change the classification: the button for a colony type is labeled with the colony name and marker, otherwise it is labeled **Debris** or **Background**.

To change the classification for a color:

1. Press the classification button to display the classification menu:



Note When the current classification is **Colony**, the classification details panel is shown alongside the classification menu – see example in next instructions.

The Classification tab

2. Select **Colony**, **Debris** or **Background**, as required.

If necessary, you can assign more than one color to **Debris** or **Background**.


The button will show the selected classification type: **Debris**, **Background** or the name of the colony type.

Note The following instructions show you how to change the classification details for a colony type produced by the color classification. However, if you intend to use one of the other classifications (size or shape – see *Adding sub-classifications*, on the next page) as a sub-classification for this colony type, you can omit this step, as any settings you make here will be overridden by the sub-classification settings.

To change the classification details for a colony type:

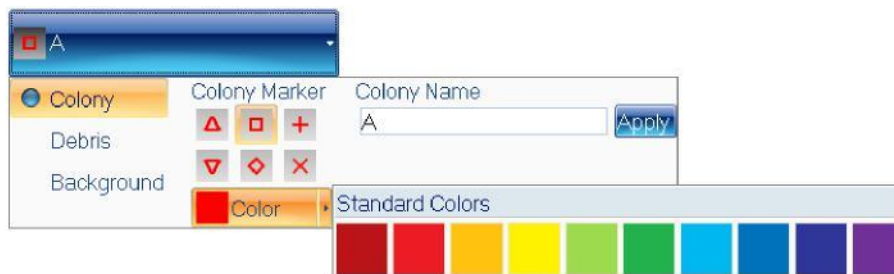
1. Press the colony classification button to display the classification details panel next to the classification menu:



2. Edit the **Colony Name** as required – for example, to show the name of the organism forming the colonies.
 3. If you do not want to change the colony marker or color, press the Return key or press .
 4. Select the shape of colony marker you want to use from the **Colony Marker** panel.
-

Note The classification menu and panel will close after you have selected the colony marker, so you will need to go back to Step 1 if you want to carry out any more changes.

5. To change the color used for the colony marker, press the **Color** button to display the **Standard Colors** control:



6. Click on the required color in the **Standard Colors** control.

When you have completed the classification details, press **Finish** to complete the color classification and return to the main ProtoCOL 3 window – the color classification will be shown in the Classification panel – see *The Classification panel*, page 41.

Creating a new batch

Adding sub-classifications

Once you have added a color classification to the batch, the other classification buttons become enabled – the first step in adding a sub-classification is to choose where to add the sub-classification – see the next section, *Choosing where to add the sub-classification*.

Notes You cannot add a color sub-classification – if you press the **Color Classification** button to create a new color classification, you will be warned that if you continue, the current color classification and all of its sub-classifications will be replaced by the new color classification.

You can add a *Size classification*, see on the facing page, or *Shape classification*, see page 67, as a sub-classification to any existing colony type.

Choosing where to add the sub-classification

When you press one of the sub-classification buttons, the appropriate wizard opens. However, if there is already more than one colony type, the first step in all of these wizards is the same, for example:



The left-hand panel shows the image with colony markers; the right-hand panel shows the current colony classification as a list. The first step in adding a new classification is to specify which of the existing colony types you want to divide with the new classification.

Note The wizards will skip this step if there is currently only one colony type, since in that case there is no choice to be made.

To choose where to add the new classification:

Click in the classification list on the existing colony that you want to divide.

Size classification

The **Add Size Classification** button becomes enabled once you have performed a color classification (see *Color classification*, page 43).

You can use a size classification 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.

Note You can use the **Reject Small Particles** slider in the **Review color classification** page of the **Color classification** wizard to set a minimum size for colonies of *all* colony types (see *Review Color Classification*, page 57).

To add a size classification to the batch so that you can distinguish between colonies or between colonies and debris according to their sizes:

1. If the current image does not have the full range of sizes appearing on plates in the batch, you should replace it by loading a plate having the full distribution (see *Loading plates into the instrument*, page 9) and capturing a new image (see *Capturing an image*, page 10).
2. Press



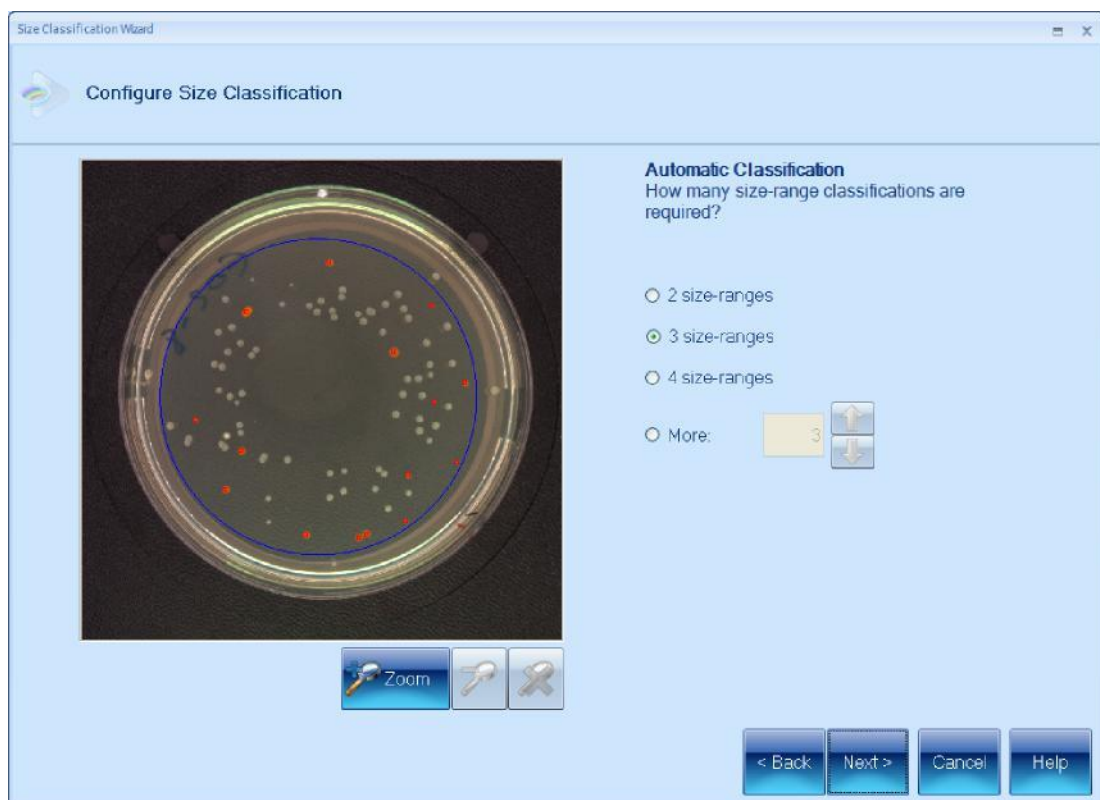
to start up the **Size Classification Wizard**.

If there is already more than one colony type in the colony classification, the **Add New Size Sub-Classification** page will be displayed – go to Step 3; otherwise (if there is currently only one type of colony in the colony classification), the wizard will skip straight to the **Configure Size Classification** page – go to Step 5.

3. In the **Add New Size Sub-Classification** page, click on the existing colony type that you want to subdivide with the new size classification (see *Adding sub-classifications*, on the previous page, for more details).

Creating a new batch

4. Press **Next** to display the **Configure Size Classification** page, for example:



5. In the **Configure Size Classification** page (see picture in previous step), click on the radio button for the number of size sub-classifications you want to add or, if you want to add more than four, click on **More** and use the arrow buttons to set the required number.

- Press **Next** to carry out an automatic size classification and display the results in the **Review Size Classification** page, for example:



The **Review Size Classification** page allows you to review and adjust the automatic size classification results.

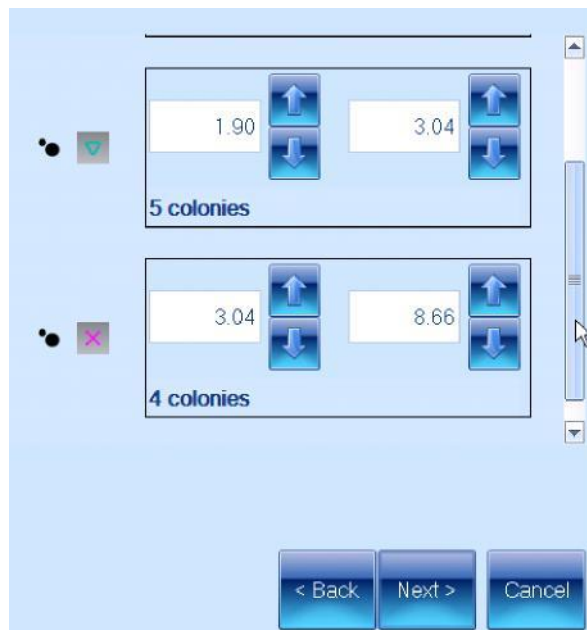
- Examine the marked colonies on the image to check whether the size classification is as required.

It may be easier to see the results if you zoom in on areas of the image (as in the example above): you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.

It may also help to change the way the colonies are marked by pressing the display button (labeled **All Colony Markers** in the picture).

Creating a new batch

8. If required, adjust the boundaries between the different size classifications:
 - a. If the number of size ranges is larger than the space available, a scroll bar will appear so that you can display the required controls, for example:

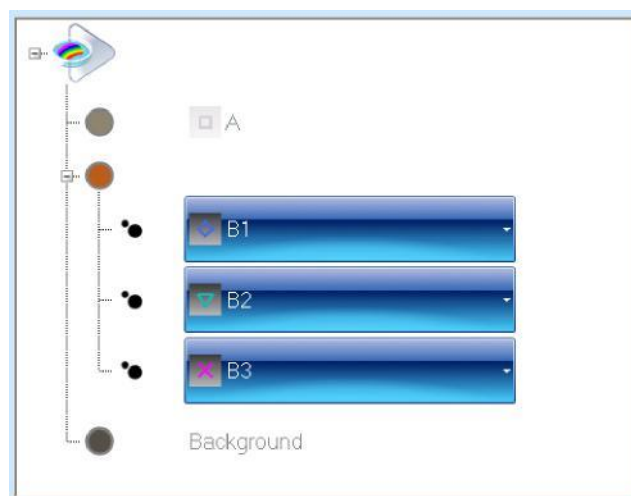


If necessary, scroll to the classification you want to adjust.

- b. Adjust the upper or lower size boundary for the classification by typing in the edit box or clicking on the up or down arrow buttons attached to the right-hand side of the edit box.

The lower/upper boundary of the adjacent classification will be adjusted accordingly and the results of the new classification shown.

9. When you are satisfied with the classification, press **Next** to display the **Classification Details** page showing the new classification tree:



10. Use the classification tree controls in the **Classification Details** page in the same way as for color classification (see *Classification Details*, page 60) to specify for each sub-classification:
 - whether it corresponds to colonies or debris (you cannot specify that it is part of the background)and for a colony sub-classification (if you are not going to sub-classify it any further):
 - the name to be used to identify it
 - the marker used to identify it on the image
 - the color used for the marker.
11. When you have completed the classification details, press **Finish** to return to the main ProtoCOL 3 window, which will show the updated classification tree.

Shape classification

The **Add Shape Classification** button becomes enabled once you have performed a color classification (see *Color classification*, page 43).

You can use a shape classification to distinguish differently shaped colonies as different types or classes of colony, or to distinguish between colonies and unevenly shaped debris.

To add a shape classification to the batch so that you can distinguish between colonies and/or debris according to their shape (how close they are to being circular):

1. If the current image does not have the full range of shapes appearing on plates in the batch, you should replace it by loading a plate having the full distribution (see *Loading plates into the instrument*, page 9) and capturing a new image (see *Capturing an image*, page 10).
2. Press



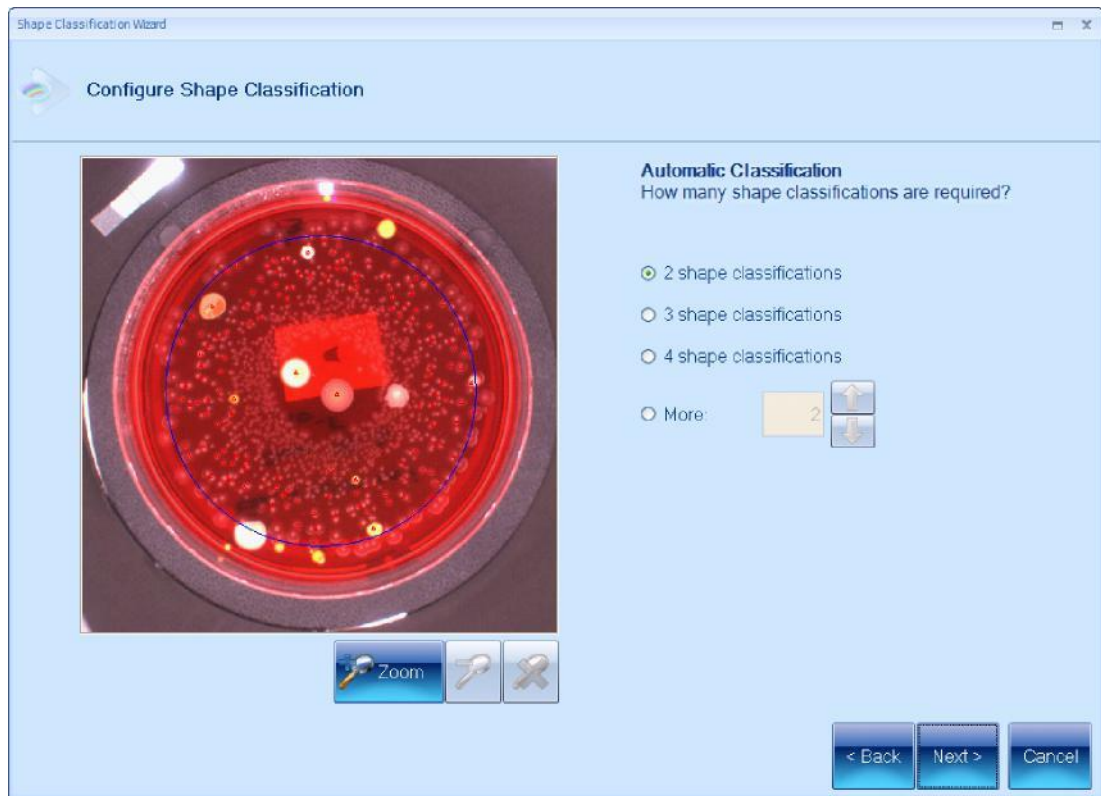
to start up the **Shape Classification Wizard**.

If there is already more than one colony type in the colony classification, the **Add New Shape Sub-Classification** page will be displayed – go to Step 3; otherwise (if there is currently only one type of colony in the colony classification), the wizard will skip straight to the **Configure Shape Classification** page – go to Step 5.

3. In the **Add New Shape Sub-Classification** page, click on the existing colony type that you want to subdivide with the new shape classification (see *Adding sub-classifications*, page 62, for more details).

Creating a new batch

4. Press **Next** to display the **Configure Shape Classification** page, for example:



5. In the **Configure Shape Classification** page (see picture in previous step), click on the radio button for the number of shape sub-classifications you want to add or, if you want to add more than four, click on **More** and use the arrow buttons to set the required number.

6. Press **Next** to carry out an automatic shape classification and display the results in the **Review Shape Classification** page, for example:



The **Review Shape Classification** page allows you to review the automatic shape classification results.

7. Examine the marked colonies on the image to check whether the shape classification is as required.

It may be easier to see the results if you zoom in on areas of the image (as in the example above): you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.

It may also help to change the way the colonies are marked by pressing the display button (labeled **All Colony Markers** in the picture).

8. If the automatic shape classification has not produced the required results, press **Back** to return to the **Configure Shape Classification** page and try selecting a different number of sub-classifications – see Step 5.

Creating a new batch

9. When you are satisfied with the new classification, press **Next** to display the **Classification Details** page showing the new classification tree:



10. Use the classification tree controls in the **Classification Details** page in the same way as for color classification (see *Classification Details*, page 60) to specify for each sub-classification:

whether it corresponds to colonies, debris or background

and for a colony sub-classification (if you are not going to sub-classify it any further):

the name to be used to identify it

the marker used to identify it on the image

the color used for the marker.

11. When you have completed the classification details, press **Finish** to return to the main ProtoCOL 3 window, which will show the updated classification tree.

Changing an existing classification

Once you have accepted a batch for use in Measurement mode (see *Accepting the batch design*, page 132), you cannot make significant changes to the way colonies are detected and classified.

Notes If you are using a **Total Plate Count**, you will be able to carry out a test measurement and change the settings for the **Reject Small Particles** and **Sensitivity** sliders and the colony splitter before recording the result (see *Test measurement and adjusting settings*, page 151).

You can also edit a result by adjusting these settings after you have recorded it – see *Rejecting small particles in a result*, page 166, *Changing the Sensitivity setting for a result*, page 166, and *Splitting colonies in a result*, page 167, for details. You can also change the colony markers and colors in Measurement mode (see *The Classification panel*, page 41).

However, you can change the way colonies are detected and classified while you are still working on the batch in the Batch Designer. You cannot do this by editing the definition of an existing classification (for example, the color samples defining the background, the boundary between two size classes or the number of shape classes), but you can remove an existing (sub-)classification and replace it with a new one with the required properties.

Note This section and the following ones are concerned with changing an existing classification, but you can also replace a classification (or Total Plate Count) with a new Total Plate Count – see *Total Plate Count*, page 38.

The next section, *Modifying an existing classification*, describes how to change classifications using the **Modify Existing Classification** button. However, some of these operations can be carried out in other ways. In particular, you can:

- Add a new *Size classification*, see page 63, or *Shape classification*, see page 67, using the procedures described in previous sections.
- Create a new color classification replacing the existing one and any sub-classifications it may have by pressing the **Color Classification** button (see *Removing a (sub-) classification*, page 74, for how to remove sub-classifications *individually*).

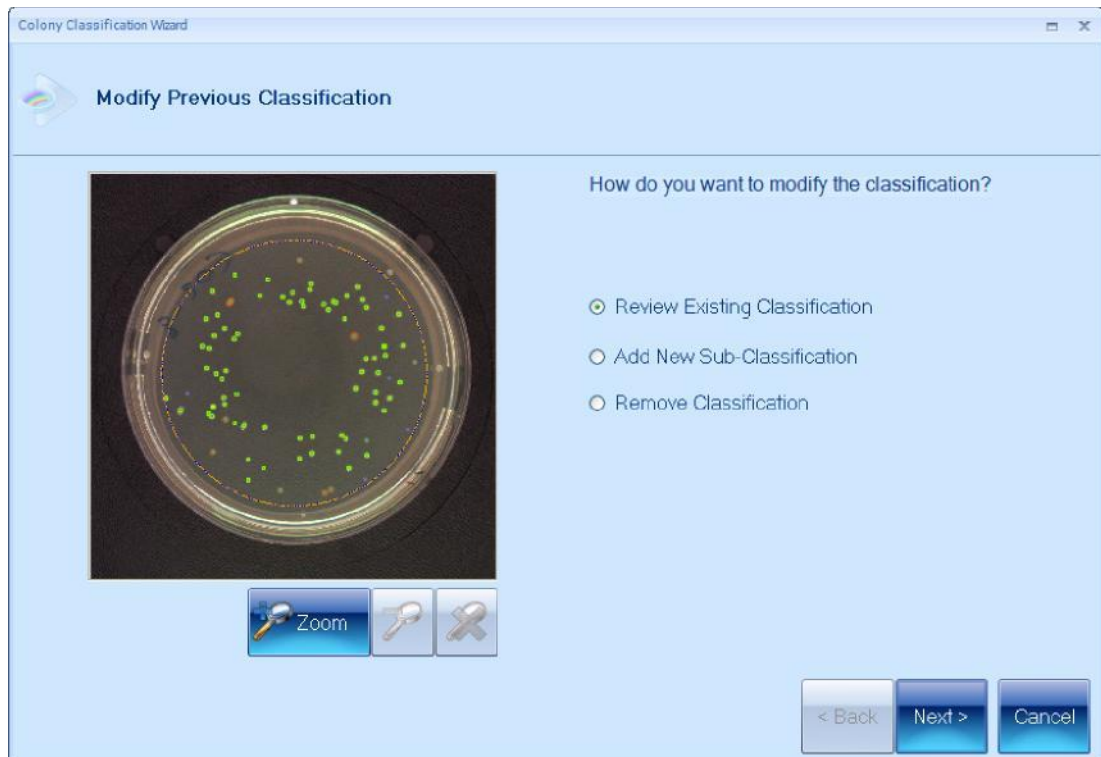
Modifying an existing classification

To modify an existing classification:

1. If required, replace the current image by loading a different plate (see *Loading plates into the instrument*, page 9) and capturing a new image (see *Capturing an image*, page 10).
2. Press



to display the **Modify Previous Classification** page of the **Colony Classification Wizard**:



Note If there are no sub-classifications, the final item in the list is **Repeat Color Classification** instead of **Remove Classification**.

3. Press:

- **Review Existing Classification** to change the classification details (you can also adjust the **Reject Small Particles** and colony splitter settings for a color classification, provided it has no sub-classifications)
- **Add New Sub-Classification** to add a new sub-classification
- **Remove Classification** to remove a (sub-)classification
- **Repeat Color Classification** to replace the existing color classification with a new one.

4. Press **Next** to go on to the next step, which depends on the selection you made in Step 3. For details, see:

- the next section, *Reviewing an existing classification*
- *Adding a new sub-classification*, on the facing page
- *Removing a (sub-)classification*, page 74
- *Repeating a color classification*, page 76.

Reviewing an existing classification

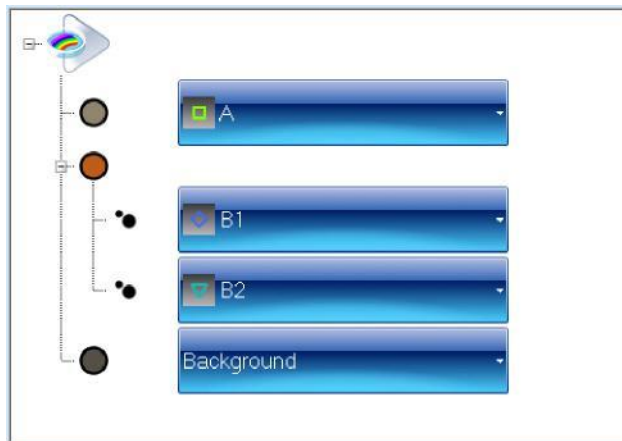
The next step after you select **Review Existing Classification** and press **Next** in the **Modify Previous Classification** page (see *Modifying an existing classification*, on the previous page) of the **Colony Classification Wizard** depends on whether the existing classification has any sub-classifications:

1. If the existing classification has any sub-classifications, the **Classification Details** page will be displayed immediately – go straight to Step 2.

If the existing classification has no sub-classifications, the **Review Color Classification** page of the **Colony Classification Wizard** is displayed again:

- a. If required, adjust the **Reject Small Particles**, **Remove Fringes Around Colonies**, **Complete Colonies Across Frame** and colony splitter settings – see *Review Color Classification*, page 57, for details.
- b. Press **Next** to display the **Classification Details** page.

2. The **Classification Details** page shows the current classification tree:



You can use the classification tree in the same way as in the **Colony Classification Wizard** (see *Classification Details*, page 60) to specify for each sub-classification:

- whether it corresponds to debris, background or a type of colony,

and for colony types:

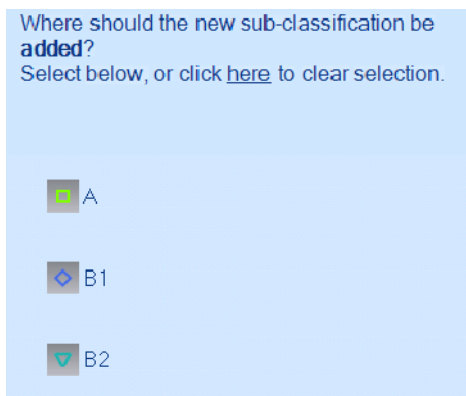
- the name to be used to identify it
- the marker used to identify it on the image
- the color used for the marker.

3. Press **Finish** to close the **Colony Classification Wizard** and return to the main ProtoCOL 3 window, which will show the updated classification details.

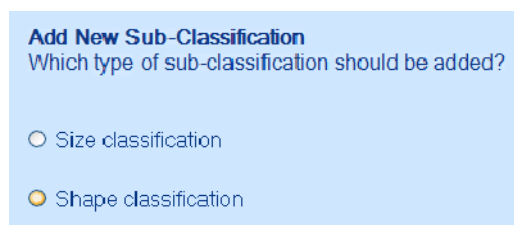
Adding a new sub-classification

Note The procedure described in this section is an alternative to the procedures described earlier in *Size classification*, page 63, and *Shape classification*, page 67.

The next step after you select **Add New Sub-classification** and press **Next** in the **Modify Previous Classification** page of the **Colony Classification Wizard** (see *Modifying an existing classification*, page 71) is to select where to create the new sub-classification using the classification tree in the **Add New Sub-Classification** page:



1. Click on the existing colony type that you want to subdivide with the new classification (see *Adding sub-classifications*, page 62, for more details).
2. Press **Next** to display the **Modify Previous Classification** page, which has buttons for selecting the type of classification to add:



3. Click on the radio button for the classification you want to add.
4. Press **Next** to create the new sub-classification.

Creating a new batch

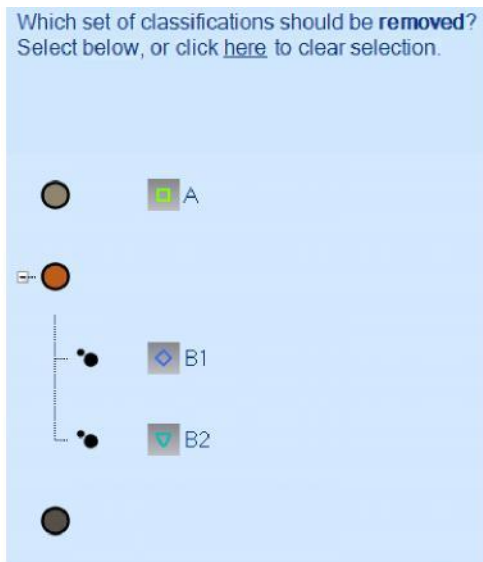
5. The remaining steps depend on which type of classification you selected in Step 3, and are exactly the same as the corresponding step in the procedures for adding sub-classifications using the classification buttons in the **Classification** tab – for details see:

- Size classification*, page 63
- Shape classification*, page 67.

Removing a (sub-)classification

Note The **Remove Classification** command is only available if the color classification already has one or more sub-classifications.

The next step after you select **Remove Classification** and press **Next** in the **Modify Previous Classification** page of the **Colony Classification Wizard** (see *Modifying an existing classification*, page 71) is to select which classification to remove using the classification tree in the **Remove Sub-Classification** page:



1. Click in the tree to select which classification to remove.

Note You select a classification by clicking on one of the results of the classification. For example: if you click on **B1**, the size sub-classification will be selected and both **B1** and **B2** will be highlighted; if you click on **A**, the color classification will be selected and the whole tree will be selected.

2. Press **Next**.

3. What happens next depends on whether in Step 1 you selected:

- A sub-classification:

The **Classification Details** page will be displayed showing the new classification tree with the selected sub-classification removed (you cannot change any of the details in the tree):

Press **Finish** to confirm the change and return to the main ProtoCOL 3 window.

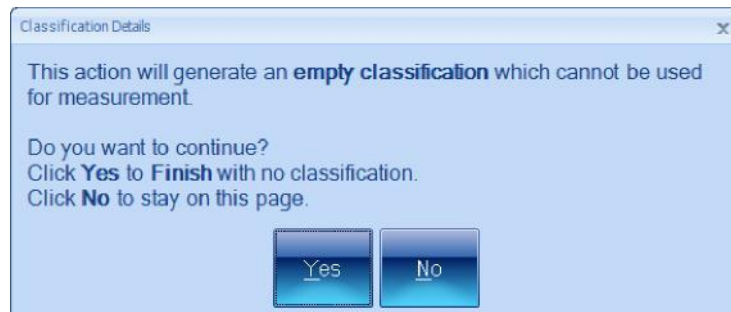
- The color classification:

A dialog box will be displayed:



- Press **Yes** if you want to replace the existing classification and its subclassifications with a new color classification. You will be taken to the **Configure Color Classification** page so that you can create the new color classification – see *Color classification*, page 43, for details.
- Press **No** if you want to remove the existing classification and its subclassifications without replacing it with a new color classification. The **Classification Details** page will be displayed with an empty classification tree.

If you then press **Finish** in the **Classification Details** page, another warning dialog box will be displayed:



- Press **Yes** if you want to continue with an empty classification – this will take you back to the situation you were in when you first created the batch – see *Creating the batch*, page 20; you will not be able to accept the batch until you have created a color classification – see *Accepting the batch design*, page 132.
- Press **No** if you have changed your mind about removing the color classification. You will be returned to the **Classification Details** page, where you can press **Cancel** to stop the classification removal procedure altogether or press **Back** to return to Step 1.

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Repeating a color classification

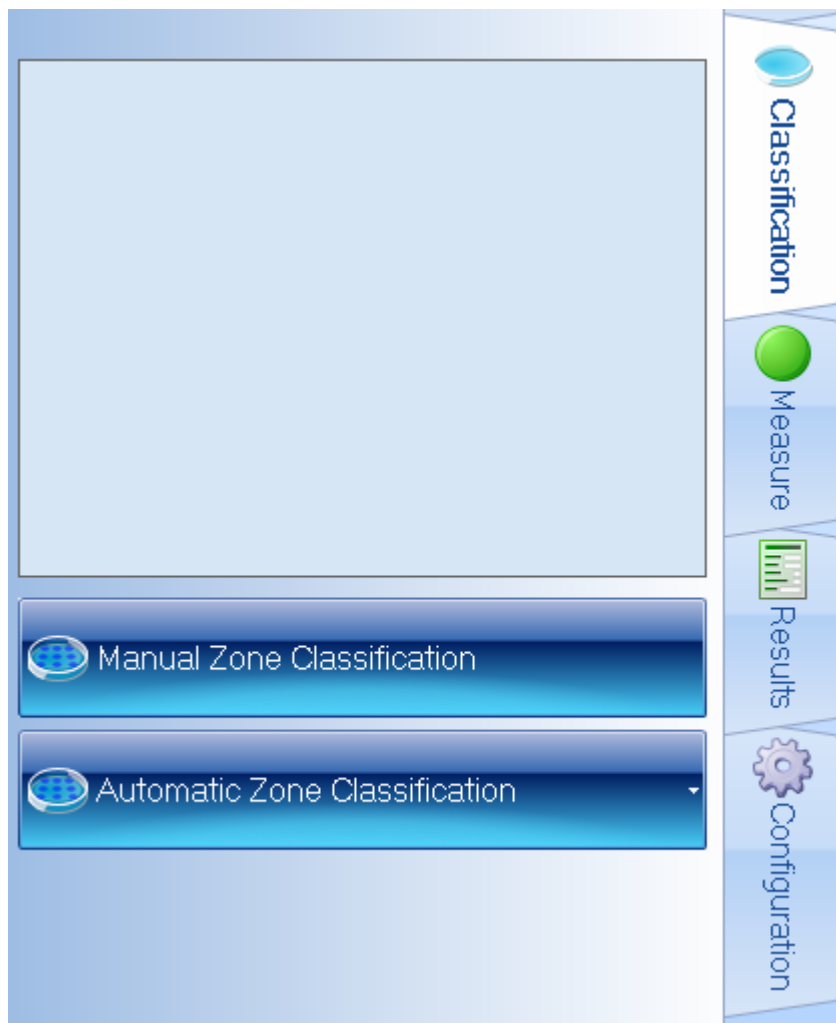
Note The **Repeat Color Classification** command is only available if the color classification has no sub-classifications.

The next step after you select **Repeat Color Classification** and press **Next** in the **Modify Previous Classification** page of the **Colony Classification Wizard** (see *Modifying an existing classification*, page 71) is to create the new color classification using the **Configure Color Classification** page – see *Color classification*, page 43, for details.

The new color classification will replace the existing one.

The Classification tab – zone measurement batches

In order to measure the antibiotic susceptibility or inhibition zones on a plate, ProtoCOL 3 has to distinguish between the colors of the zones, discs/wells (if there are discs/wells on the plates) and background. You enable it to do this by carrying out the process of **Zone Classification** using the **Classification** tab:



To display the classification controls in the Batch Designer:

Press the **Classification** tab:



The following sections take you through the zone classification procedure:

- *Starting up the zone classification wizards*, below
- *Specifying the number and arrangement of the zones*, on the next page
- *Type of analysis*, page 88
- *Identifying the zone colors*, page 91
- *Setting detection parameters manually*, page 94
- *Reviewing the zone measurements*, page 104

Notes If you base a new batch on an existing batch, by default it will use the same zone classification as the existing batch (though you can change the zone classification if required). If you are creating a completely new batch, you will not be able to accept it for taking measurements until it has been zone classified.

Starting up the zone classification wizards

To carry out a zone classification procedure:

1. Insert a 'typical' plate from the batch into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.

To be 'typical', the plate should have the full range of disc, zone and background colors appearing on plates in the batch. Also, if any of the plates in the batch have problems such as zones with indistinct edges or zones that overlap each other, you should pick a plate that has these problems – once you have set the zone classification to cope with the most difficult plates, it should be able to handle the others correctly.

2. Capture the image – see *Capturing an image*, page 10.
3. Press either manual or automatic zone classification



Press manual classification to start the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard**, depending on the sort of batch you are creating.

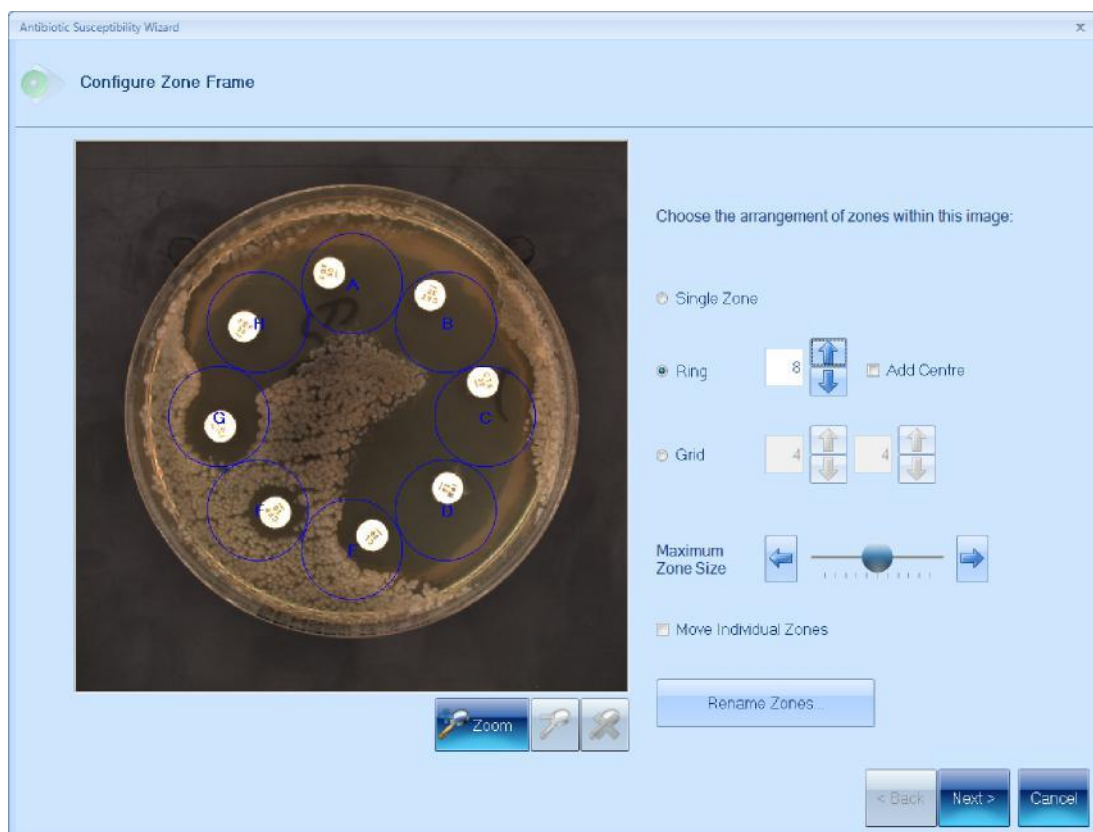
Creating a new batch

In fact, you use the same procedures in both wizards, which are described in the following sections – the same instructions apply to both application wizards.

The automatic classification option has to be selected before accepting the batch. Once selected, you can accept the batch, press 'Detect Discs' and then measure your plates.

Specifying the number and arrangement of the zones

When you start the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard**, the **Configure Zone Frame** page is displayed:



Note If there is no current image, an image will be automatically captured when you press the **Zone Classification** button. If the image shown in the wizard is too light or too dark, press **Cancel** to close the wizard and adjust the exposure accordingly (see *Setting the exposure*, page 10), then press the **Zone Classification** button again to restart the wizard.

To specify the number and arrangement of the zones on the plates:

1. If there is only one zone on each plate:

Press **Single Zone**.

If the zones are arranged in a ring (as in the example picture at the beginning of this section):

- a. Press **Ring** – the value box next to the dialog box will become enabled:



- b. Set the number of zones on each plate by typing directly into the value box or by pressing the up or down arrow button.

- c. If the plates in the batch have a zone at the center of the ring in addition to those at the circumference, check **Add Center**.

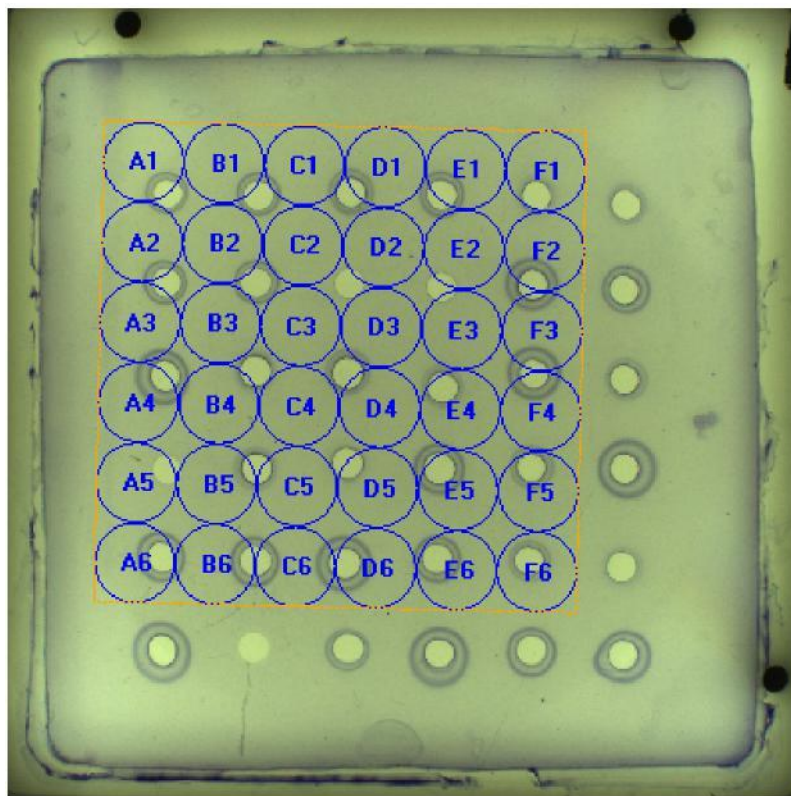
If the zones are arranged in a grid:

- a. Press **Grid** – the value boxes next to the dialog box will become enabled:



- b. Set the number of columns (first box) and rows (second box) in the grid of zones on each plate by typing directly into the value boxes or by pressing the up or down arrow button.

When you press on the radio buttons to select these options, a number of measurement frames (blue circles), corresponding to the selected number and arrangement of zones, will appear on the image – for example:



Note If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11.

2. ProtoCOL 3 uses the measurement frames to decide which areas of the image it should analyze when it is detecting and measuring the zones.

For a **Single Zone** frame, you should:

Adjust the frame in the **Configure Zone Frame** page so that it is centered over the zone in the image (see *Moving frames in the Configure Zone Frame page*, page 81) and large enough to measure the largest zone appearing on the plates (see *Resizing zone frames*, page 83).

Creating a new batch

For a ring or grid of frames, you should:

- a. Move, resize and rotate the ring or grid as a whole so that the center of each frame lies over the center of each of the zones in the image – see *Moving frames in the Configure Zone Frame page*, on the facing page, *Resizing zone frames*, page 83, and *Rotating a ring or grid of zone frames*, page 85, for instructions.

If required, you can also move the individual sub-frames within the grid – see *Moving frames in the Configure Zone Frame page*, on the facing page.

- b. Resize the sub-frames in the ring or grid so that they are large enough to measure the largest zone appearing on the plates – see *Resizing zone frames within a ring or grid of frames*, page 86.

ProtoCOL 3 provides some tolerance in the positioning of the frames to allow for inaccuracies in the positioning of the zones on the plates, but you should try to get the best fit you can.

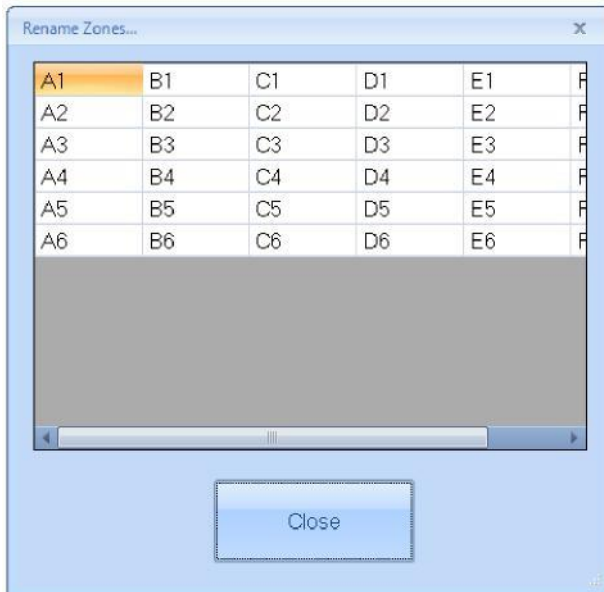
Note If the batch is set to allow zone adjustment (see *Setting batch restrictions*, page 133), you will be able to move a single frame or individual frames in a ring or grid in Measurement mode to allow for plate variation before taking a measurement. However, you cannot resize frames or move or rotate a ring or grid of frames as a group in Measurement mode.

3. To rename the frames if you are using a **Ring** or **Grid** of frames: a.

Press:



to display the **Rename Zones** dialog box:



- b. Click in the cell for the first frame you want to rename.
- c. Type in the new name.
- d. Repeat Steps **b** and **c** for the other frames.
- e. Press **Close** to confirm the changes and close the dialog box.

The new names will appear labeling the frames – these names will also be used to identify the results for the individual zones (see *Working with results*, page 157).

4. When you are satisfied with the arrangement and positioning of the frames, press **Next** to display the **Configure Type of Analysis** page – see *Type of analysis*, page 88.

Moving frames in the Configure Zone Frame page

Notes This section shows you how to move measurement frames in the **Configure Zone Frame** page of the zone classification wizards – see *Specifying the number and arrangement of the zones*, page 78. This sets the default zone position(s) for the batch when it is saved.

You can also adjust the frames temporarily in the main ProtoCOL 3 window in the Batch Designer (see *Test Measure Plate*, page 129), which you may want to do when you are taking test measurements. However, any changes you make to the settings there will be lost when you save the batch.

You can also adjust the frames temporarily in the main ProtoCOL 3 window in Measurement mode if the batch is set to allow zone adjustment (see *Setting batch restrictions*, page 133). Any changes you make to the settings in measurement mode will be lost when you close the batch – the default settings created in the **Configure Zone Frame** page of the zone classification wizard will be reloaded if you reopen the batch.

You use essentially the same techniques to adjust the frames in all cases.

This section shows you how to:

- Move a single zone frame so that it is centered over the zone in the image.

And for a ring or grid of frames:

- Move the ring or grid of frames as a whole.
- Move individual frames within the ring or grid of frames.

For a ring or grid of frames, you should arrange the ring or grid and/or individual frames in the ring or grid so that the center of each frame lies over the center of each of the zones in the image. To do this, you should use a combination of moving, resizing (see *Resizing zone frames*, page 83) and rotating (see *Rotating a ring or grid of zone frames*, page 85) the ring or grid as a whole, and then, if necessary, fine tune the positions of individual frames.

To move the frame(s):

1. If you are working with a ring or grid of frames and you want to move the ring or grid of frames together as a group:
 - Leave **Move Individual Zones** unchecked.

If you want to fine tune the position of individual frames within the group:

- a. Check **Move Individual Zones**.

The **Select Multiple Zones** button will be displayed under the check box – see the next step.

- b. To move a selection of the frames together (rather than individual frames one at a time):
 - i. Press:



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- ii. Select the required frames by clicking in each of them in turn – the selected frames will be highlighted in yellow.
- iii. To deselect a selected frame:

Click in it – the yellow highlighting will be removed.

To deselect all selected sub-frames:

Press:



2. Move the pointer near to the center of the frame, ring of frames or grid of frames, or one of the selected frames – an orange circular drag handle will appear in the center of the frame or group of frames, for example:



3. Drag the frame(s) and drop it (them) in the required position.

Resizing zone frames

Notes This section shows you how to resize the measurement frames in the **Configure Zone Frame** page of the zone classification wizards – see *Specifying the number and arrangement of the zones*, page 78. This sets the default frame size for the batch when it is saved.

You can also adjust the frames temporarily in the main ProtoCOL 3 window in the Batch Designer (see *Test Measure Plate*, page 129), which you may want to do when you are taking test measurements. However, any changes you make to the settings there will be lost when you save the batch.

You can also adjust the frames temporarily in the main ProtoCOL 3 window in Measurement mode if the batch is set to allow zone adjustment (see *Setting batch restrictions*, page 133). Any changes you make to the settings in measurement mode will be lost when you close the batch – the default settings created in the **Configure Zone Frame** page of the zone classification wizard will be reloaded if you reopen the batch.

You use essentially the same techniques to adjust the frames in all cases.

You can use the procedure described in this section to resize a single zone frame or a ring or grid of frames as a whole. However, the reason for doing so is different in each case:

- For a single zone frame, you should:
 - Resize the frame so that it is large enough to include the largest zone appearing on the plates in the batch.
- For a ring or grid of frames, you should:
 - Resize the ring or grid of frames as a whole so that the individual frames can be positioned over each of the zones in the ring or grid – to do this, you should use a combination of resizing, moving (see *Moving frames in the Configure Zone Frame page*, page 81), and rotating (see *Rotating a ring or grid of zone frames*, page 85) the ring or grid as a whole, and then, if necessary, fine tune the positions by moving individual frames.

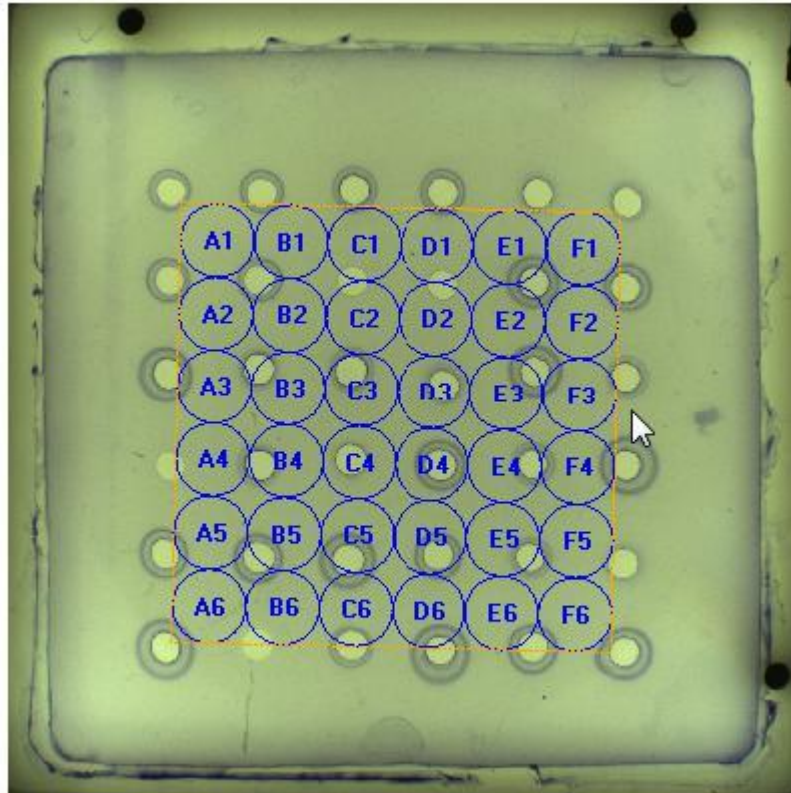
Note Resizing a ring or grid of frames as a whole also resizes the frames themselves by the same amount. However, once you have positioned the ring or grid of frames as a whole, you may also need to resize the frames without changing the ring or grid size to make sure they are large enough to include the largest zone appearing on the plates in the batch – see *Resizing zone frames within a ring or grid of frames*, page 86.

To resize an individual (**Single Zone** selected) frame or a ring or grid of frames as a whole:

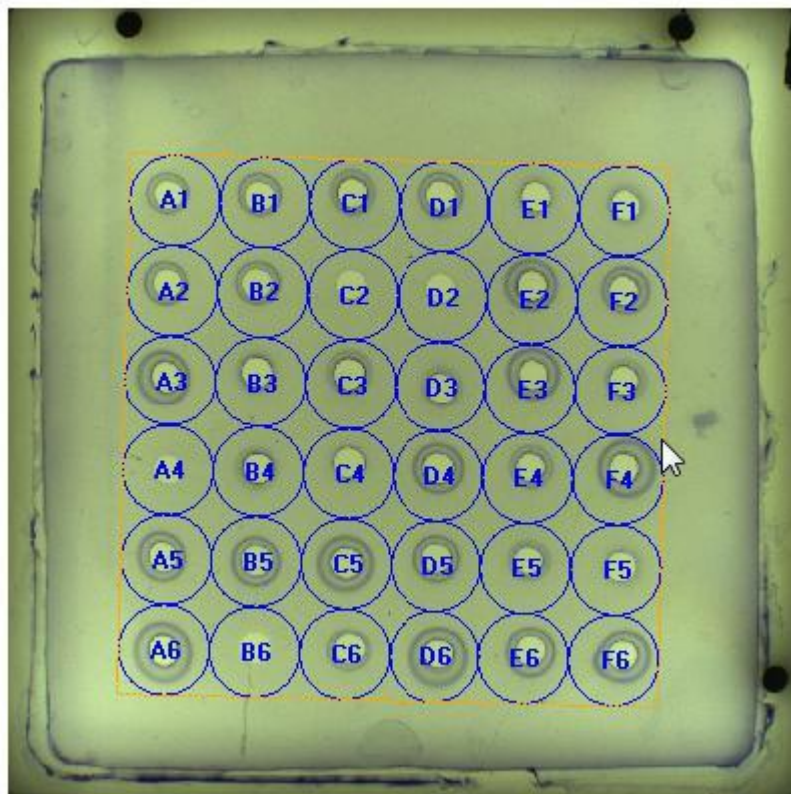
1. If you are resizing a ring or grid of frames as a whole, make sure **Move Individual Zones** is unchecked.
2. Move the pointer near to the single zone frame boundary or the boundary around a ring or grid of frames.

Creating a new batch

The frame(s) boundary will turn orange, showing that you can adjust its size, for example:



3. Drag the boundary to resize the frame(s), for example:



Rotating a ring or grid of zone frames

Notes This section shows you how to rotate a ring or grid of measurement frames in the **Configure Zone Frame** page of the zone classification wizards – see *Specifying the number and arrangement of the zones*, page 78. This sets the default arrangement for the batch when it is saved. You will probably need to use this procedure in combination with moving (see *Moving frames in the Configure Zone Frame page*, page 81) and resizing (see *Resizing zone frames*, page 83) the grid as a whole.

You can also adjust the frames temporarily in the main ProtoCOL 3 window in the Batch Designer (see *Test Measure Plate*, page 129), which you may want to do when you are taking test measurements. However, any changes you make to the settings there will be lost when you save the batch.

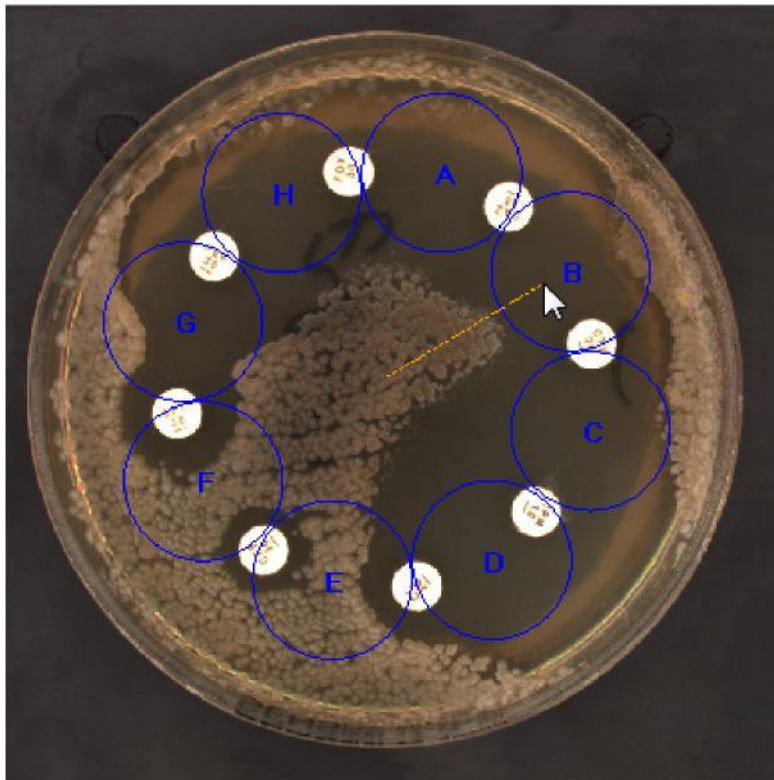
You can also adjust the frames temporarily in the main ProtoCOL 3 window in Measurement mode if the batch is set to allow zone adjustment (see *Setting batch restrictions*, page 133). Any changes you make to the settings in measurement mode will be lost when you close the batch – the default settings created in the **Configure Zone Frame** page of the zone classification wizard will be reloaded if you reopen the batch.

You use essentially the same techniques to adjust the frames in all cases.

To rotate a ring or grid of frames:

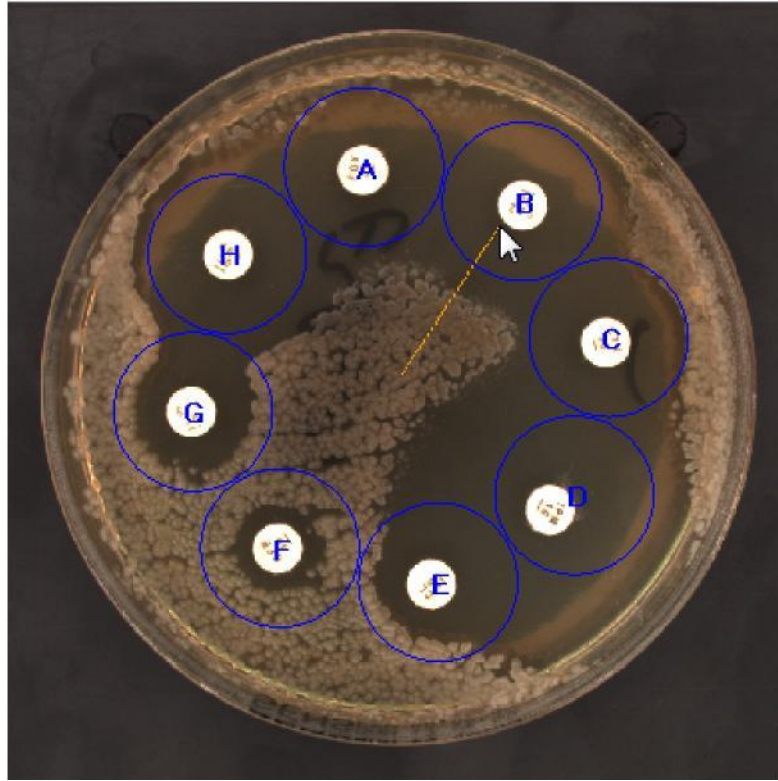
1. Make sure **Move Individual Zones** is unchecked.
2. Move the pointer to a point about half way between the center and edge of the ring or grid of frames.

An orange line will appear joining the pointer to the center, for example:



Creating a new batch

3. Drag the line around the center to rotate the ring or grid of frames so that the centers of the frames lie over the centers of the zones, for example:



Resizing zone frames within a ring or grid of frames

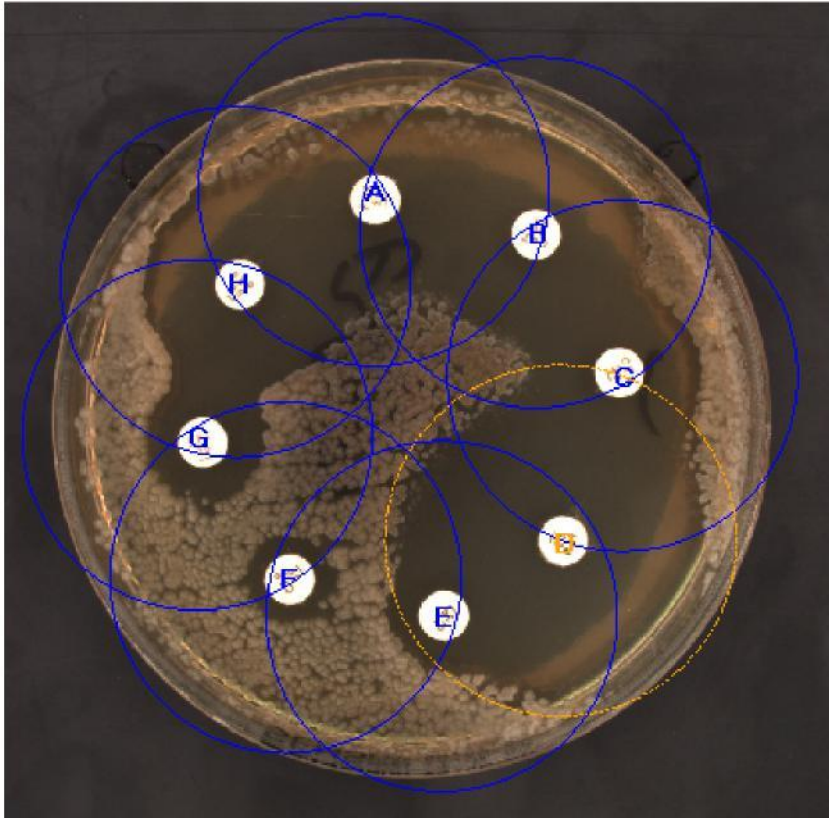
Once you have arranged the frames in a ring or grid over the zones in the image in the **Configure Zone Frame** page (see *Specifying the number and arrangement of the zones*, page 78), you may need to resize the frames themselves without moving them to make sure they are large enough to measure the largest zone appearing in the batch of plates.

To adjust the size of the frames in a ring or grid of frames without changing the size of the grid:

In the **Maximum Zone Size** controls, drag the bar or press the arrow buttons:

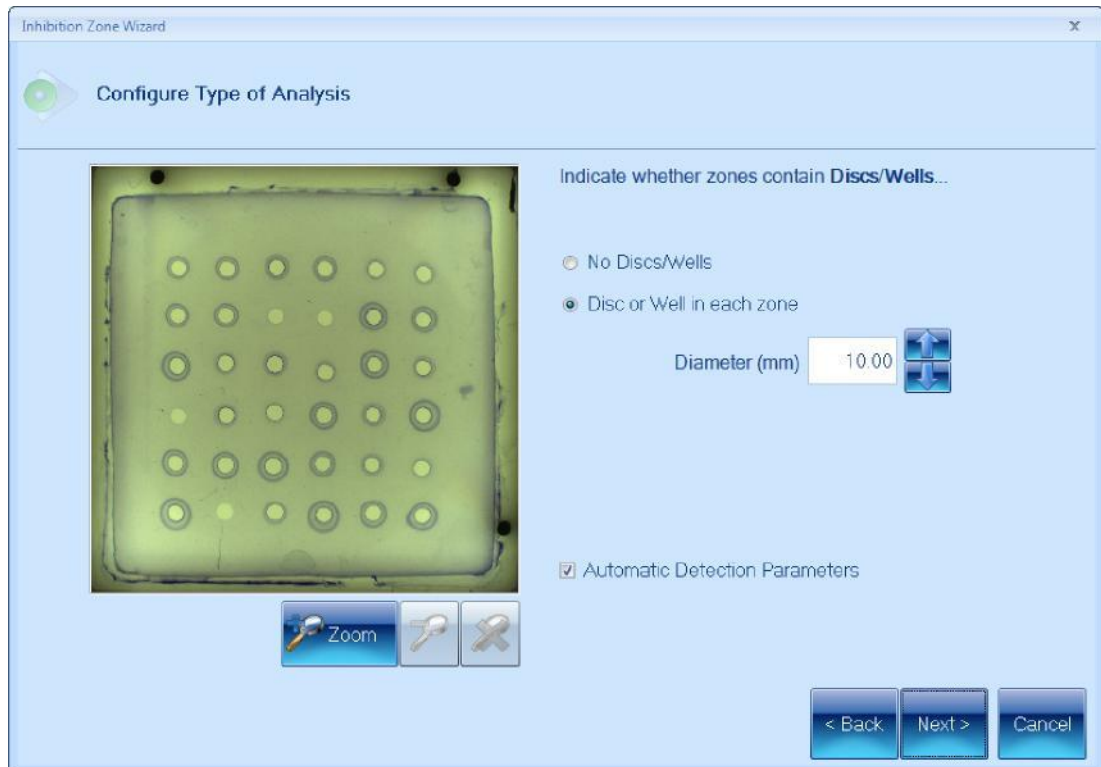


You should resize the frames so that they are large enough to enclose the largest zones appearing on the plates in the batch, but not so large that ProtoCOL 3 has to do a significant amount of unnecessary processing. Note that if you increase the size of the frames, you can make them overlap each other, and that you may need to do this if some of the zones on the plates in the batch are overlapping – for example:



Type of analysis

The **Configure Type of Analysis** page is displayed when you press **Next** in the **Configure Zone Frame** page of the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard** – see *Specifying the number and arrangement of the zones*, page 78.



To specify how ProtoCOL 3 should analyze the image:

1. If the plate images do not have visible discs or wells:

Press the **No Discs/Wells** radio button

Otherwise:

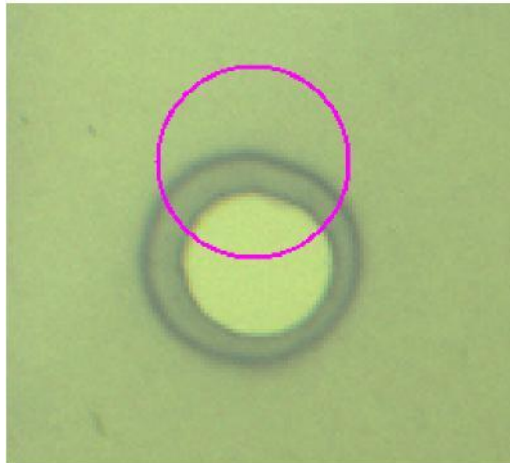
- a. Press the **Disc or Well in each zone** radio button.
- b. To set the diameter of the disc or well either:

Type directly into the **Diameter** box or pressing the up/down arrow buttons.

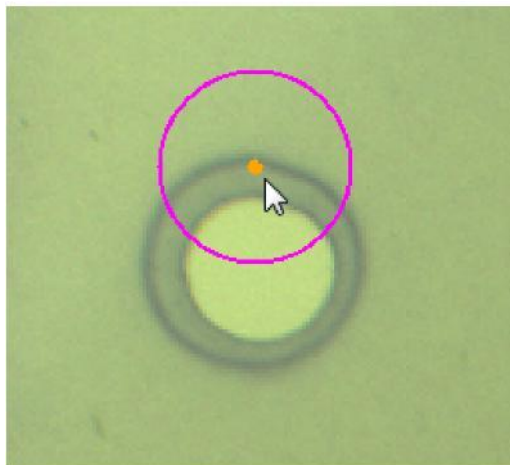
Or:

- i. Click in the **Diameter** box.

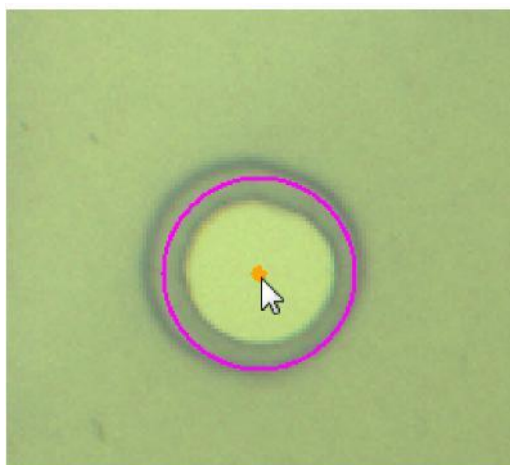
A marker circle will appear on the image:



- ii. Move the pointer to the center of the marker circle. A drag handle will appear at the center of the marker circle:



- iii. Drag the marker circle so that it is centered over a well/disc:



- iv. Move the pointer over the circumference of the marker circle.

Creating a new batch

The marker circle will become highlighted showing that you can resize it:



- v. Drag the circumference of the marker circle so that its diameter is just outside / barely touching the outside of the well/disc:



The **Diameter** box will be set to the new diameter of the marker circle.

2. To allow ProtoCOL 3 to select the zone detection parameters automatically:

Leave **Automatic Detection Parameters** checked.

To set the detection parameters manually:

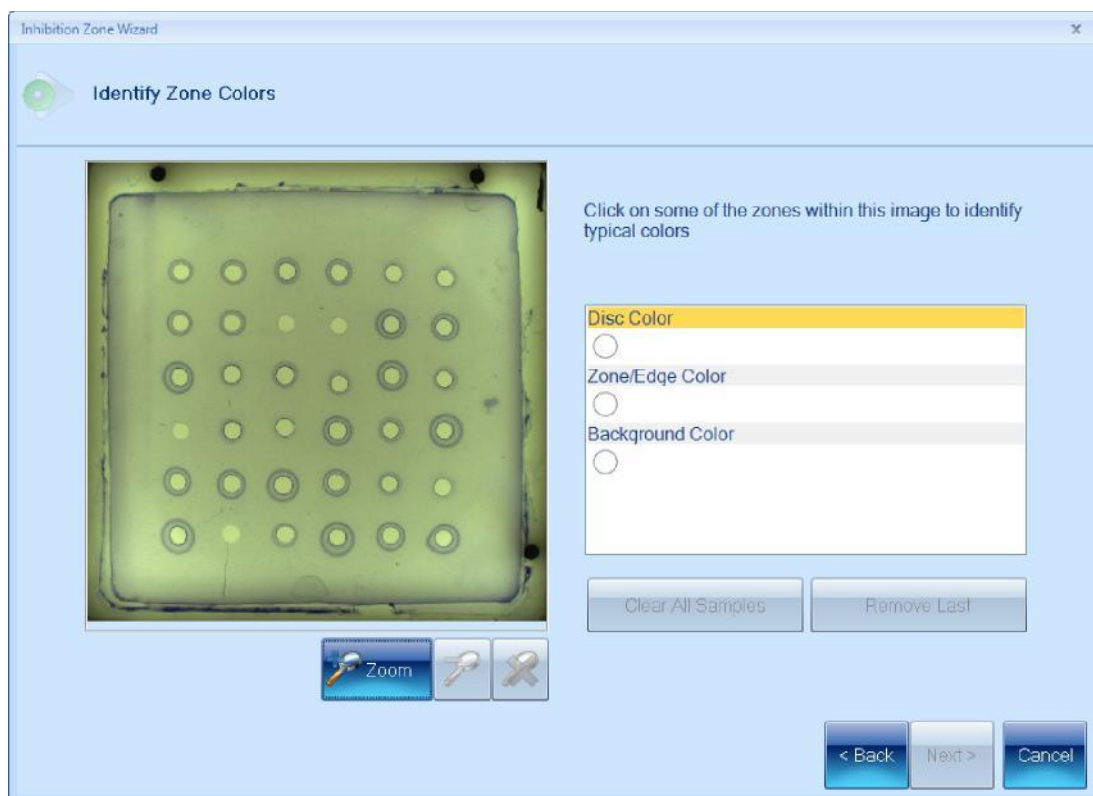
Press **Automatic Detection Parameters** to uncheck it.

Note At a first pass, you should leave **Automatic Detection Parameters** checked to see how well ProtoCOL 3's selection of the zone detection parameters works. If you then find that the zone detection is unsatisfactory, you will be able to press **Back** in the **Review Zone Measurements** page (see *Reviewing the zone measurements*, page 104) to return to this step so that you can try setting the parameters manually.

3. Press **Next** to display the **Identify Zones** page – see *Identifying the zone colors*, on the facing page.

Identifying the zone colors

The **Identify Zones** page is displayed when you press **Next** in the **Configure Type of Analysis** page of the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard** – see *Type of analysis*, page 88:

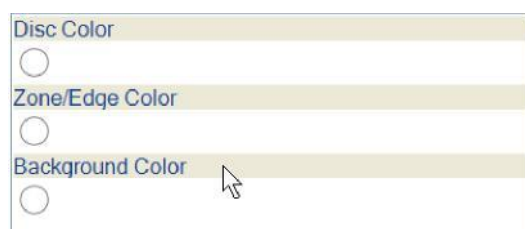


Note The **Disc Color** item will only appear if you selected **Disc or Well in each zone** option in the **Configure Type of Analysis** page – see *Type of analysis*, page 88.

You use the **Identify Zones** page to identify the colors in the plate image so that ProtoCOL 3 can detect and measure the zones. You do this by taking samples of the background, zone and, where relevant, disc/well colors in the image.

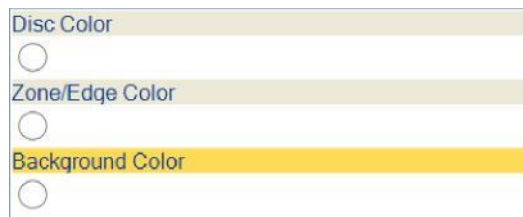
To identify the disc, zone and background colors for the plates in the batch:

1. Click on the **Disc Color**, **Zone/Edge Color** or **Background Color** item in the list. For example:

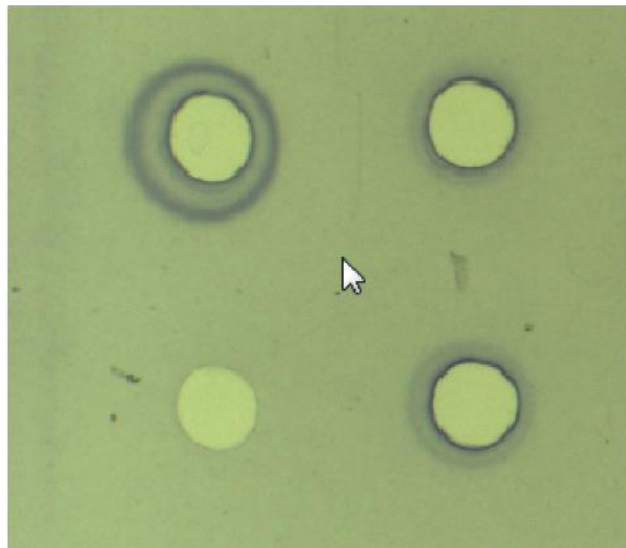


Creating a new batch

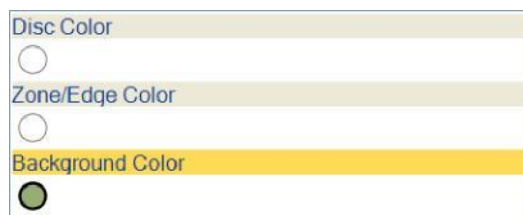
to select it. The selected item will be highlighted in orange:



2. In the image, click in the corresponding part of the image to sample its color:



When you click, a circle showing a sample of the color you clicked on will appear in the item:



Note If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11. Zooming the image may help you ensure that you click accurately on the appropriate part of the image when you are selecting color samples.

3. If you click at the wrong point and want to remove the last color sample added:

Press



Note This removes the last color sample from the *currently selected* item – if the required item is not currently selected, click on it first to select it.

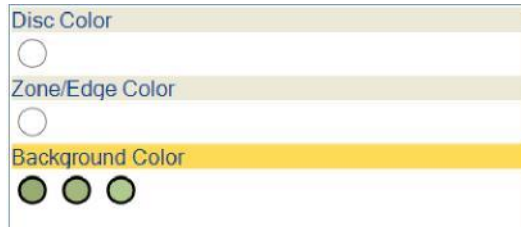
The Classification tab

If you want to remove *all* the samples from *all* items:

Press

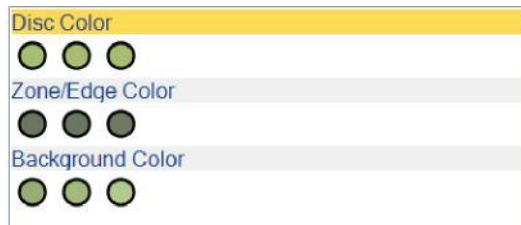


- Repeat Step 2 as required to select a representative sample covering the full range of colors for the selected item as it appears in the image:



Note For the **Zone/Edge Color** item, if the zone has a distinct edge with a different color, take samples from the edge as well as the body of the zone. The more color samples you choose, the longer it will take to analyze.

- Repeat Steps 1–4 for the other items:



- When you are satisfied that you have added a representative sample of colors for all the items, press **Next** (the **Next** button is disabled until you have added at least one sample for each item).

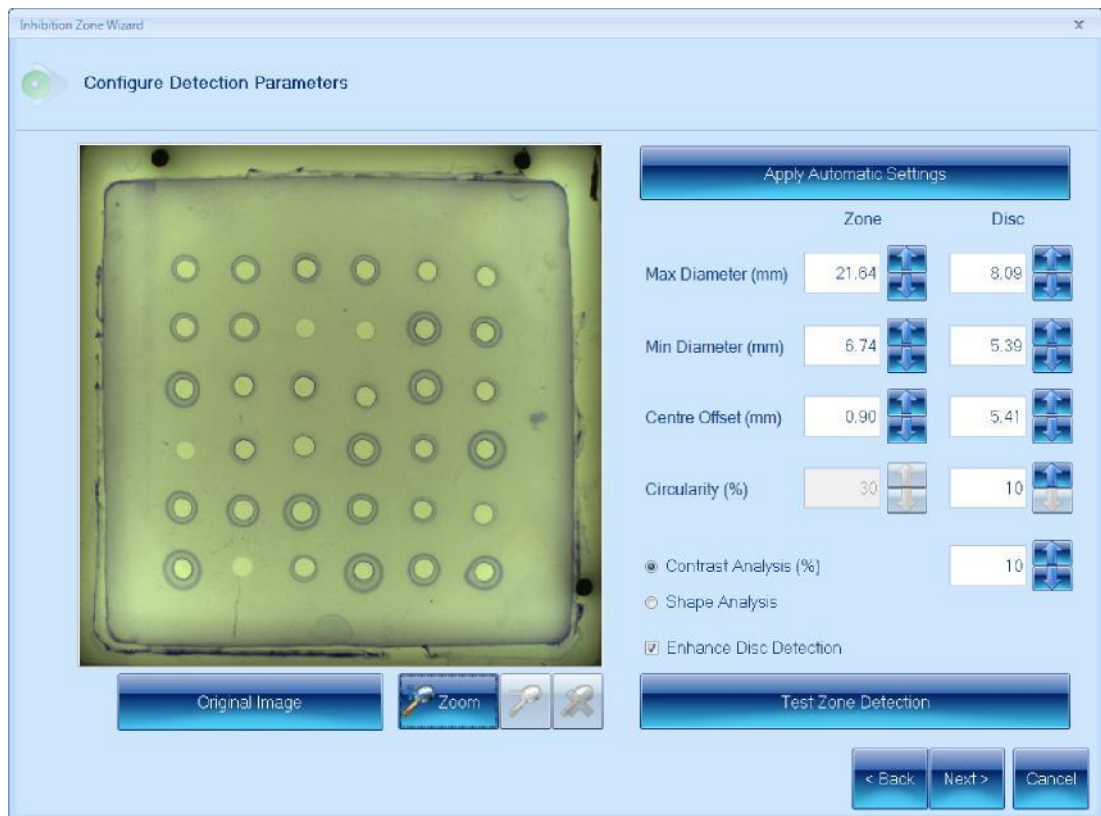
What happens next depends on whether you left **Automatic Detection Parameters** checked or unchecked in the **Configure Type of Analysis** page (see *Type of analysis*, page 88):

- If you chose to use **Automatic Detection Parameters**, ProtoCOL will use its automatically determined parameters to detect and measure the zones (this requires a considerable amount of processing, so there may be a short delay) and will display the results in the **Review Zone Measurements** page – see *Reviewing the zone measurements*, page 104.
- If you unchecked **Automatic Detection Parameters**, the **Configure Detection Parameters** page will be displayed so that you can set the detection parameters manually – see the next section, *Setting detection parameters manually*.

Setting detection parameters manually

The **Configure Detection Parameters** page is displayed when you press **Next** in the **Identify Zones** page of the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard** (see *Identifying the zone colors*, page 91) if you left **Automatic Detection Parameters** unchecked in the **Configure Type of Analysis** page (see *Type of analysis*, page 88). This page will not be displayed if you chose to use **Automatic Detection Parameters**, but instead you will be taken directly to the **Review Zone Measurements** page (see *Reviewing the zone measurements*, page 104).

If you selected **Disc or Well in each zone** in the **Configure Type of Analysis** page (see *Type of analysis*, page 88), the **Configure Detection Parameters** page includes both **Zone** and **Disc** controls:



But if you selected **No Discs or Wells** in the **Configure Type of Analysis** page, there are no **Disc** controls, and the **Enhance Disc Detection** check box is permanently disabled:

Apply Automatic Settings

Zone

Max Diameter (mm) 16.40

Min Diameter (mm) 10.00

Centre Offset (mm) 0.68

Circularity (%) 30

Contrast Analysis (%) 10

Shape Analysis

Enhance Disc Detection

Test Zone Detection

The following topics show you how to optimize the zone and disc detection parameters:

- *Checking the zone classification*, on the next page
- *Testing the zone and disc detection parameters*, page 98
- *Diameter and offset parameter values*, page 99
- *Shape (circularity) and contrast analysis*, page 102
- *Enhanced disc detection*, page 103
- *Resetting the parameters to the automatic settings*, page 104.

Note In general, making the detection parameter settings more restrictive makes the analysis of the image and the detection of zones and discs faster and more accurate. However, it also increases the risk that some zones and discs appearing on the image may not be detected.

When you are satisfied with the settings you have made:

Press **Next** to go on to the **Review Zone Measurements** page – see *Reviewing the zone measurements*, page 104.

Checking the zone classification

Before you adjust the detection settings on the **Configure Detection Parameters** page (see *Setting detection parameters manually*, page 94), it is a good idea to check the zone classification carried out in the **Identify Zones** page (see *Identifying the zone colors*, page 91).

To check the zone classification:

1. Press



to display the image display menu:

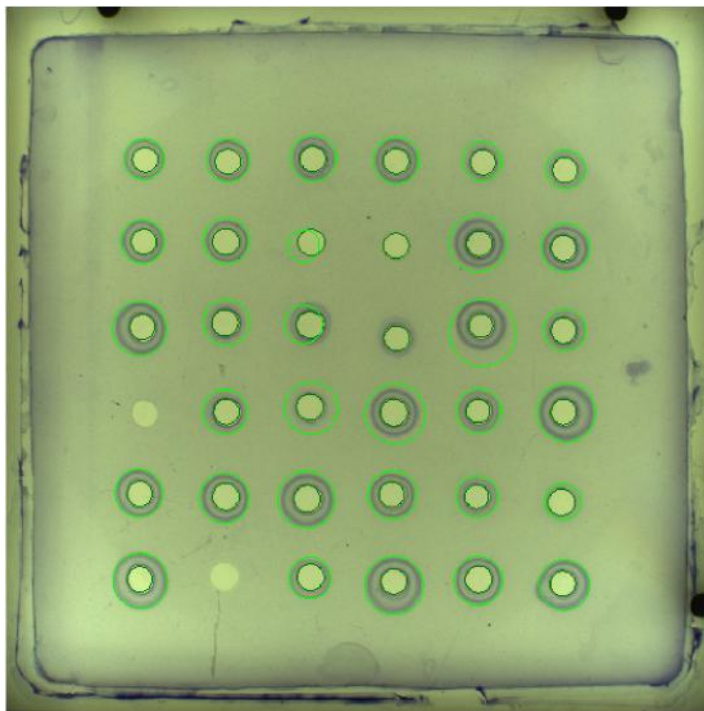


2. Choose **Display: Colormap Image**.

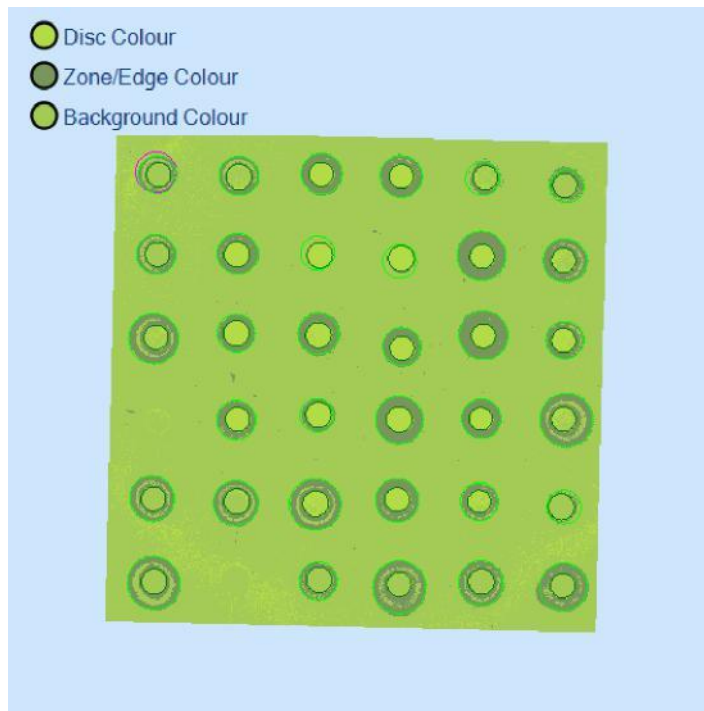
If the image has not been zone classified yet, the wizard will show a progress bar as it zone classifies the image, it will then switch from showing the original image to showing the colormap image.

For example:

- Original image



- Colormap image



The colormap image shows the parts of the image lying within the frames and detected as the discs, zones and background in different colors (see the key at the top left corner of the image area) so that you can check how well the detection process has been carried out. If there are any problems, you can press **Back** to return to the **Identify Zones** page (see *Identifying the zone colors*, page 91) to change the color sampling.

To switch back to the original image:

1. Press



to display the image display menu again.

2. Choose **Original Image**.

Note Once the zone classification has been carried out, there is no delay in switching between the two image displays. The only parameter change on this page of the wizard that requires a time-consuming reanalysis is the **Enhance Disc Detection** setting.

See also

- *Testing the zone and disc detection parameters*, on the next page
- *Diameter and offset parameter values*, page 99
- *Shape (circularity) and contrast analysis*, page 102
- *Enhanced disc detection*, page 103
- *Resetting the parameters to the automatic settings*, page 104.

Testing the zone and disc detection parameters

The following topics explain what the zone and disc detection parameters do, and how to change them. In order to see if you need to make any changes, and the effect of any changes that you do make, you can carry out a test.

To test the effect of the zone and disc detection parameter settings:

Press

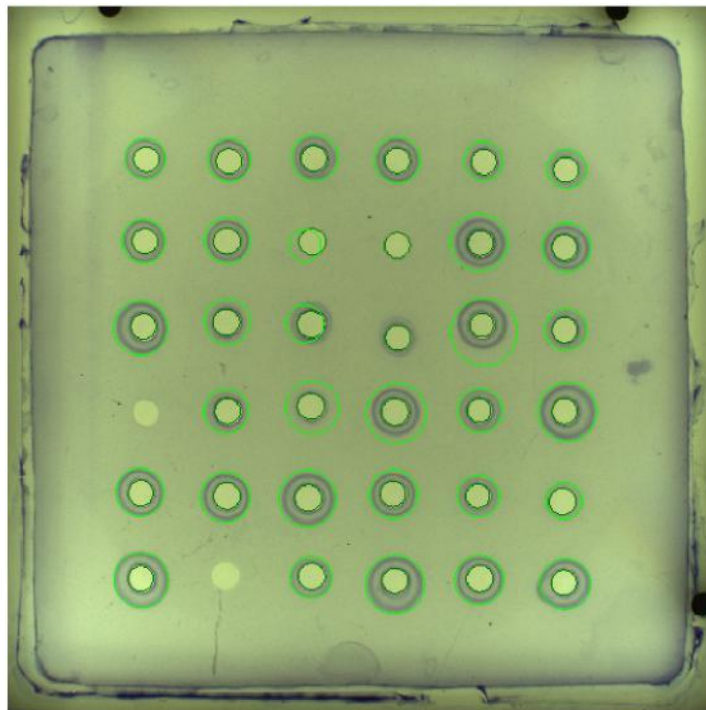


If you have not pressed this button or displayed the colormap image (see *Checking the zone classification*, page 96) since you sampled the colors in the **Identify Zones** page (see *Identifying the zone colors*, page 91), the wizard will carry out a color classification of the image (which may take a while as it requires a considerable amount of processing) as well as detect the location of the discs and zones.

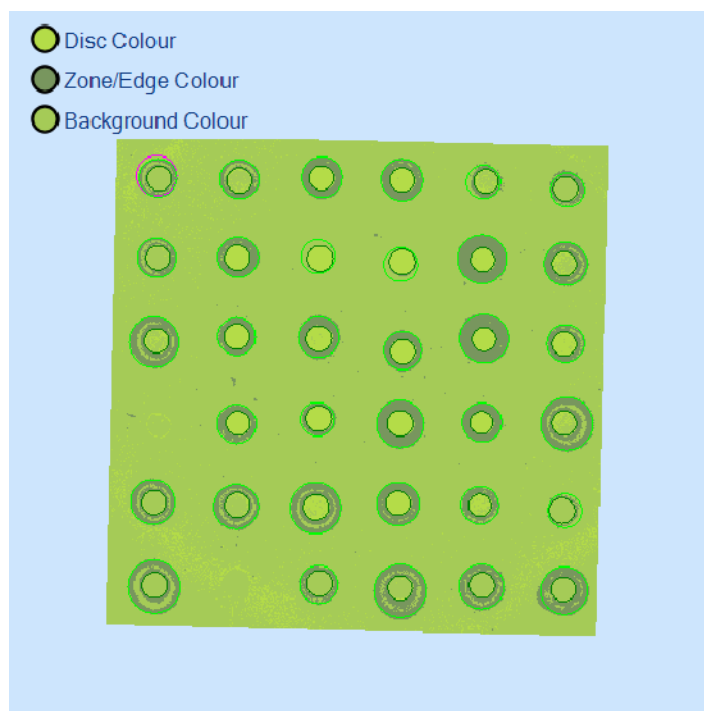
The boundaries of the detected zones and discs will be shown in green in either the original or Colormap image view (see *Checking the zone classification*, page 96, for how to switch between image views).

For example:

- Original image view:



- Colormap image view:



Note If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11. Zooming the image may help you make a more accurate appraisal of the results.

If you are satisfied with the results of the test:

Press **Next** to go on to the **Review Zone Measurements** page (see *Reviewing the zone measurements*, page 104).

On the other hand, if there are any problems, you can adjust the detection parameters manually. For details, see

- *Diameter and offset parameter values*, below
- *Shape (circularity) and contrast analysis*, page 102
- *Enhanced disc detection*, page 103
- *Resetting the parameters to the automatic settings*, page 104.

See also

- *Checking the zone classification*, page 96.

Diameter and offset parameter values

When ProtoCOL 3 is analyzing antibiotic susceptibility and inhibition zone plate images it searches for zones (and discs) with diameters lying between minimum and maximum values. It begins the search from the center of each of the measurement frames, but allows an offset tolerance in the positioning of the frames – the center offset is the maximum distance allowed between the center of the detected zone (or disc) and the center of the measurement frame.

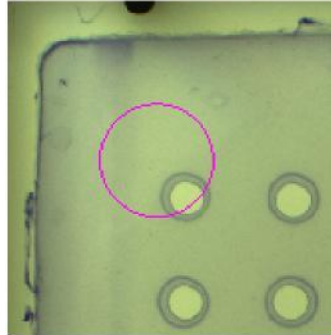
Creating a new batch

To set any of the diameter or offset parameter values:

1. Click in the corresponding value box to select it:



A reference circle of the currently set size will appear on the image:



(You can also use this circle to change the value – see the set of instructions below.)

2. Type directly into the value box or press the up or down arrow button to increase or decrease the value.

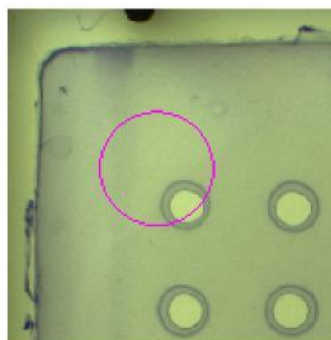
Using the reference circle to change a value

If you want to change one of the **Diameter** or **Center Offset** values, you can use the reference circle on the image as an alternative to the above procedure.

Note If required, you can zoom the image in the same way as in the main window – see *Zooming the image*, page 11. Zooming the image will help you make more accurate adjustments in the following procedures.

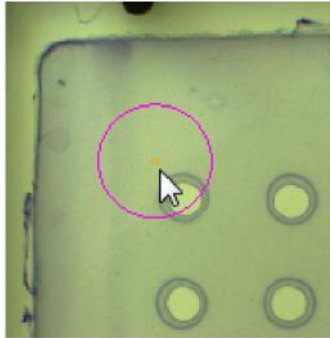
To resize a parameter using the reference circle:

1. Click in the parameter's value box to select it and display the reference circle on the image:

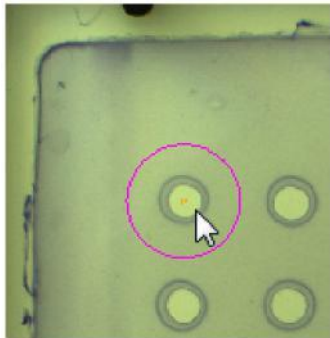


2. If required, to move the reference circle (for example, so that it is over a particular zone which you want to use as a model for the maximum zone size):

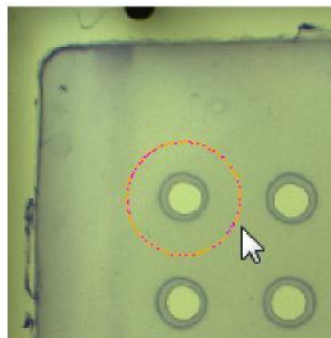
- a. Move the pointer to the middle of the reference circle. An orange drag handle will appear:



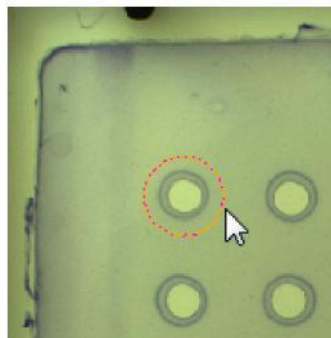
- b. Drag the reference circle to the required position on the image:



3. Move the pointer near to the circumference of the reference circle. The reference circle will turn orange to show that you can adjust it:



4. Drag the circumference to resize the reference circle:



Creating a new batch

As you drag, the setting in the value box will change to match the new size.

See also

- [Checking the zone classification](#), page 96
- [Testing the zone and disc detection parameters](#), page 98
- [Shape \(circularity\) and contrast analysis](#), below
- [Enhanced disc detection](#), on the facing page
- [Resetting the parameters to the automatic settings](#), page 104.

Shape (circularity) and contrast analysis

The **Circularity** boxes in the **Configure Detection Parameters** page (see *Setting detection parameters manually*, page 94) allow you to set circularity limits on the detected zones and/or discs. The higher the value you set, the greater the requirement for circularity – if you set a value of **100%**, the detected zone or disc must be circular and non-circular zones or discs will be rejected; the lower the value you set, the more tolerant the zone or disc detection will be to shape irregularity.

Alternatively, you can choose to use **Contrast Analysis** for zone detection – the higher the value you set, the greater the contrast must be between the zone and background for the zone to be detected; the lower the value you set, the more tolerant the zone detection will be to lack of contrast.

To set a **Circularity** or **Contrast Analysis** value:

1. Press the **Contrast Analysis** or **Shape Analysis** radio button to choose between using **Contrast Analysis** or **Shape Analysis** for *zone* detection.

When **Contrast Analysis** is selected, the **Circularity** controls for the **Zone** are disabled:

	Zone	Disc
Max Diameter (mm)	49.47	35.03
Min Diameter (mm)	29.19	23.36
Centre Offset (mm)	2.06	12.37
Circularity (%)	30	10
<input checked="" type="radio"/> Contrast Analysis (%)		10
<input type="radio"/> Shape Analysis		
<input checked="" type="checkbox"/> Enhance Disc Detection		

When **Shape Analysis** is selected, the **Contrast Analysis** controls are disabled:

	Zone	Disc
Max Diameter (mm)	49.47	35.03
Min Diameter (mm)	29.19	23.36
Centre Offset (mm)	2.06	12.37
Circularity (%)	30	10
<input type="radio"/> Contrast Analysis (%)		10
<input checked="" type="radio"/> Shape Analysis		
<input checked="" type="checkbox"/> Enhance Disc Detection		

Note If the **Disc** parameter boxes are displayed (ie if you selected **Disc** or **Well** in each zone in the **Configure Type of Analysis** page – see *Type of analysis*, page 88), the **Circularity** box in the **Disc** column is enabled even if the **Shape Analysis** radio button is deselected. The radio buttons choose what type of analysis is used for zone detection, they have no effect on the use of shape analysis (circularity) for disc detection.

2. Click in the value box for the **Circularity** or **Contrast Analysis** value you want to set to select it. For example:

Circularity (%)	30	10
<input type="radio"/> Contrast Analysis (%)		10
<input checked="" type="radio"/> Shape Analysis		

3. Type directly into the value box or press the up or down arrow button to increase or decrease the value.

See also

- Diameter and offset parameter values*, page 99
- Enhanced disc detection*, below
- Resetting the parameters to the automatic settings*, on the next page.

Enhanced disc detection

The **Enhance Disc Detection** check box on the **Configure Detection Parameters** page (see *Setting detection parameters manually*, page 94) is enabled if you selected **Disc** or **Well** in each zone in the **Configure Type of Analysis** page (see *Type of analysis*, page 88); it is permanently disabled if you selected **No Discs or Wells**.

Creating a new batch

You may want to select **Enhance Disc Detection** if the color of the disc/well is very similar to the color of the background. When **Enhance Disc Detection** is on, the colors of the disc/well and the background are combined to form a 'non-zone' color rather than separate disc/well and background colors, which can improve the accuracy of zone measurement. However, choosing **Enhance Disc Detection** may make the color classification process take a little longer.

To switch enhanced disc detection on or off:

Check or uncheck **Enhance Disc Detection**.

When you change the enhanced disc detection setting, ProtoCOL 3 will need to do a new color classification before it can detect and measure zones. It will do this immediately if the colormap image is displayed (see *Checking the zone classification*, page 96), when you next test the zone detection (see *Testing the zone and disc detection parameters*, page 98) or when you press **Next** to move on to the **Review Zone Measurements** page (see *Reviewing the zone measurements*, below). As the reanalysis requires a considerable amount of processing, there may be a short delay before the results are shown.

See also

- Diameter and offset parameter values*, page 99
- Shape (circularity) and contrast analysis*, page 102
- Resetting the parameters to the automatic settings*, below.

Resetting the parameters to the automatic settings

To reset the zone and disc detection parameters to the automatic settings:

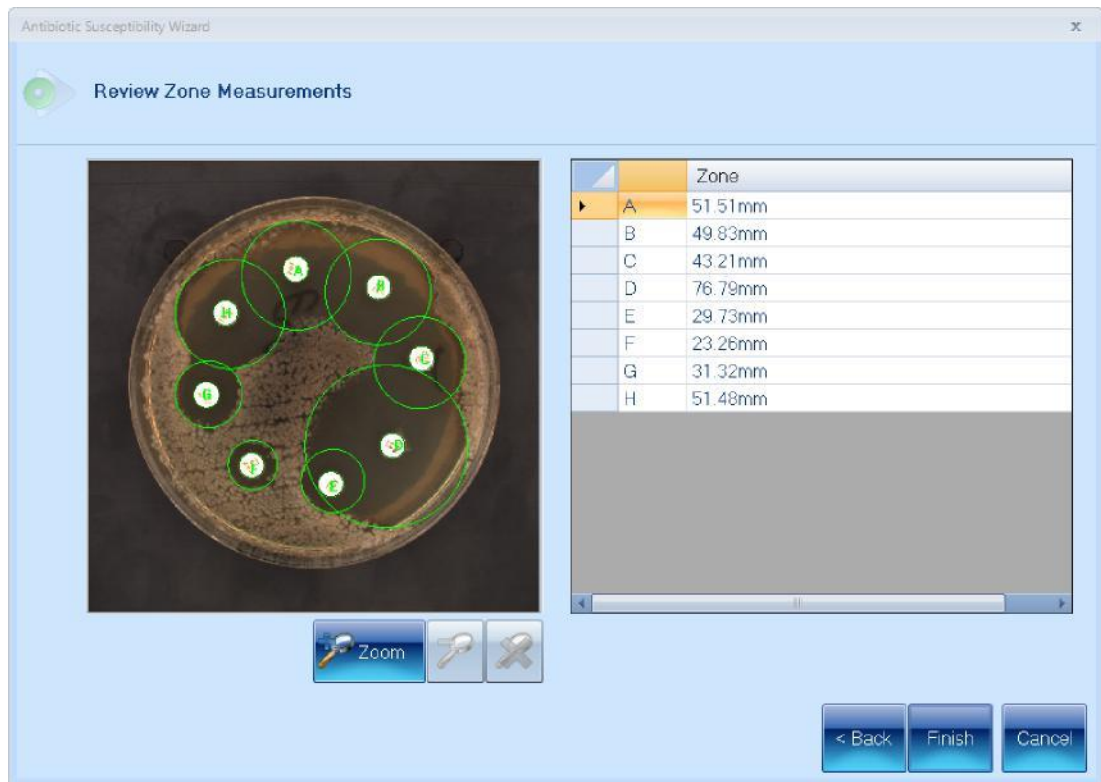
Press



Reviewing the zone measurements

The **Review Zone Measurements** page is displayed in the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard** after you have pressed **Next** in the **Identify Zones** page (see *Identifying the zone colors*, page 91) if you chose to carry out automatic zone and disc detection, or after you have pressed **Next** in the **Configure Detection Parameters** page if you chose to carry out manual zone and disc detection (see *Setting detection parameters manually*, page 94).

For example:



The left-hand pane shows the image with the boundaries of the detected zones and discs shown in green. The right-hand pane shows the diameters of the detected zones, or where there has been a problem in detecting a zone or disc, it gives an error message.

To review the zone classification:

1. Examine the detected zones and discs carefully to check that they have been detected accurately.
2. Look through the results table and check any error messages.

For example:

	Zone
A	43.74mm
B	45.75mm
C	38.27mm
D	No Zone Detected
E	28.20mm
F	No Disc Detected (offset > max)
G	30.97mm
H	46.35mm

Some error messages may not reflect a problem with the zone classification. For example, if there is no zone visible at a point in the image where ProtoCOL 3 is looking for one (ie within the area of a frame), the results may show the message offset > max – in effect, what this message means is that ProtoCOL 3 failed to find a zone even when it looked at the maximum offset from the center of the frame. This is not a problem, or a surprise, if in fact there isn't a zone visible. However, there would be a problem if there is a zone there that should be measured – the problem may be

Creating a new batch

caused by inaccurate positioning of the frames (in which case you would need to adjust the frames – see *Specifying the number and arrangement of the zones*, page 78, and the sections following it), by irregularities in the positioning of the discs or wells on the plates (in which case you might need to increase the offset parameter – see *Setting detection parameters manually*, page 94) or some other problem, such as the color classification (see *Identifying the zone colors*, page 91).

3. Either:

- If you wish to change the zone classification, press **Back** to return to the **Identify Zones** page (see *Identifying the zone colors*, page 91) or the **Configure Detection Parameters** page (see *Setting detection parameters manually*, page 94).
- If you are happy that the zone classification is as good as possible, press **Finish** to confirm your settings and return to the main ProtoCOL 3 window.

Note If you press **Back** after having carried out automatic zone and disc detection, you will be returned to the **Identify Zones** page. If you now want to try setting the detection parameters manually, press **Back** in the **Identify Zones** page (see *Identifying the zone colors*, page 91) to go back to the **Configure Type of Analysis** page (see *Type of analysis*, page 88), uncheck **Automatic Detection Parameters**, and press **Next** twice to display the **Configure Detection Parameters** page (see *Setting detection parameters manually*, page 94).

Modifying a zone classification

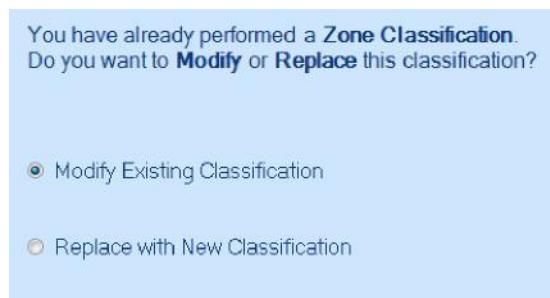
To modify an existing zone classification:

1. Press



to start the **Antibiotic Susceptibility Wizard** or **Inhibition Zone Wizard** again.

The **Modify or Replace Existing Classification** page will be displayed with the following controls:



2. If you want to create a completely new classification from scratch:

- a. Select **Replace with New Classification**.
- b. Press **Next** to display the **Configure Zone Frame** page.

See *Specifying the number and arrangement of the zones*, page 78, for how to use this page and following steps.

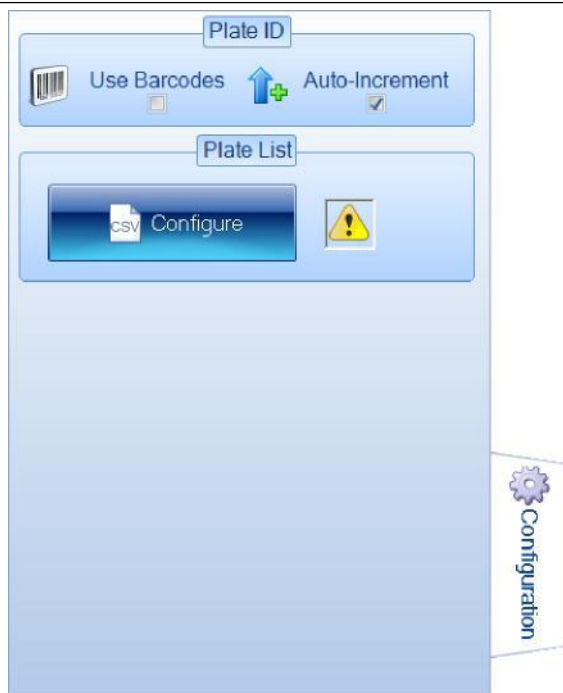
If you want to keep some features of the current classification:

- a. Select **Modify Existing Classification**.
- b. Press **Next** to display the **Configure Zone Frame** page, which initially will just have a single **Click to Choose New Frame** button.
- c. If you want to define a new frame for the batch:
 - i. Press **Click to Choose New Frame** to display the frame controls.
 - ii. Define the new frame – see *Specifying the number and arrangement of the zones*, page 78, for details.
- d. Press **Next** to display the **Configure Type of Analysis** page.

See *Type of analysis*, page 88, for how to use this page and the steps following it.

The Configuration tab

Note The **Configuration** tab does not appear in Measurement mode.



Note The **Use Barcodes** check box is disabled if there is no barcode reader attached to the PC.

To display the configuration controls:

Press the **Configuration** tab:



The **Configuration** tab in the Batch Designer allows you to specify how plate identifiers and, where relevant, dilutions should be assigned to plates in measurement mode.

Creating a new batch

If the batch you are creating is based on another batch, the **Configuration** tab shows the settings selected in the parent batch:



The green check marks show the settings selected in the parent batch – if the parent batch uses manually entered parent plate identifiers, the **Parent is using auto-increment** will have a red cross next to it, but its check box will be enabled.

To use the same configuration tab settings as in the parent batch:

Leave the **Parent Batch** check boxes unchanged.

Note If the **Parent is using a plate list**, you will need to create a new plate list for the new batch – see *Selecting auto-incrementing plate identifiers*, page 111, for instructions.

If you do not want to use the same configuration tab settings as in the parent batch:

Uncheck the **Parent Batch** check box(es) for the settings you do not want to use – see the references below for how to set independent configuration tab settings for the current batch.

If you are creating a new batch from scratch, or have chosen not to use the parent batch settings, you can choose to use:

Manual plate identifiers and dilutions

You will need to enter an identifier manually for each batch and, if required, manually change the dilution.

See *Manually entered plate identifiers*, on the facing page, for how to choose this option.

□ **Auto-incrementing plate identifiers and manual dilutions**

Automatic identifiers will be created for each plate as it is measured, and, if relevant, you can manually change the dilution.

See *Selecting auto-incrementing plate identifiers*, page 111, for details.

□ **A plate list of plate identifiers and, optionally, dilutions**

Each identifier (and dilution) in the list is assigned to each of the plates in turn as they are measured. You can enter a plate list by hand, import it from a file or take it from a LIMS system.

See *Using an external source for plate identifiers*, page 114, for details.

□ **A barcode reader**

The barcode reader can be used to read identifiers and, depending on the setup, dilutions directly from a barcode on the plate to enter identifiers/dilutions manually or in combination with a plate list or LIMS.

See *Using a barcode reader for plate identifiers*, page 113, for details.

Manually entered plate identifiers

To set up a batch to use manually entered plate identifiers and, where relevant, dilutions: 1.

Press



to display the **Configuration** tab:



Creating a new batch

2. If the new batch is based on an existing batch, there will be a set of **Parent Batch** controls below the **Plate List** controls. If the parent batch did not use manually entered plate identifiers, uncheck the **Parent Batch** check box(es) – see *The Configuration tab*, page 107, for details.
3. If the icon in the **Plate List** box is



go straight to Step 4.

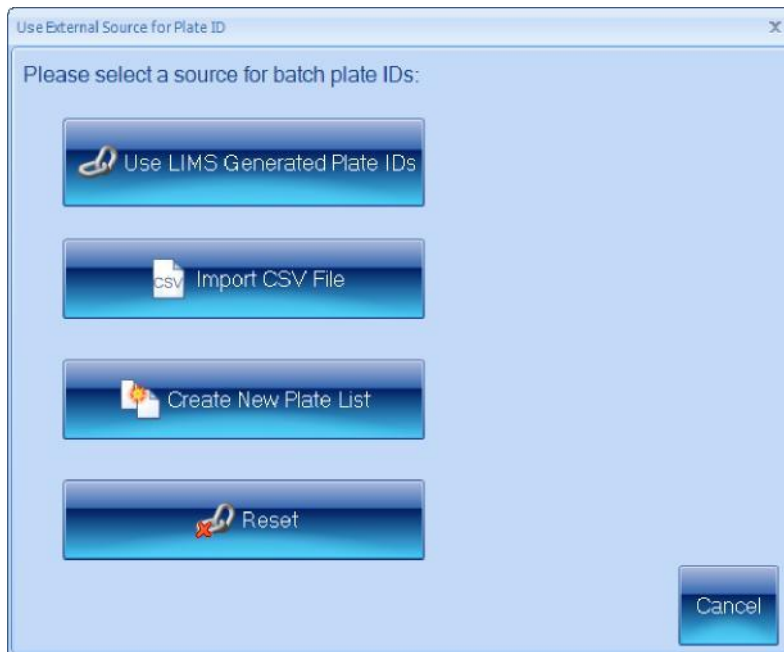
Otherwise, an external source of plate identifiers has been selected for the batch and the **Auto-Increment** control will be disabled.

To enable the **Auto-Increment** control and deselect the external source:

- a. Press



to display the **Use External Source for Plate ID** dialog box:



- b. Press



4. If **Auto-increment** is selected in the **Plate ID** box, click the check box to deselect it.
5. If you want to read the manually entered plate identifiers (and dilutions, depending on your setup) from a barcode on the plate, select **Use Barcodes** in the **Plate ID** box – see *Using a barcode reader for plate identifiers*, page 113, for details.

Selecting auto-incrementing plate identifiers

Note This option is selected by default when you create a new batch from scratch.

To use auto-incrementing plate identifiers:

1. Press



to display the **Configuration** tab:



2. If the new batch is based on an existing batch, there will be a set of **Parent Batch** controls below the **Plate List** controls. If the parent batch did not use manually entered plate identifiers, uncheck the **Parent Batch** check box(es) – see *The Configuration tab*, page 107, for details.
3. If the icon in the **Plate List** box is



go straight to Step 4.

Otherwise, an external source of plate identifiers has been selected for the batch and the **Auto-Increment** control will be disabled.

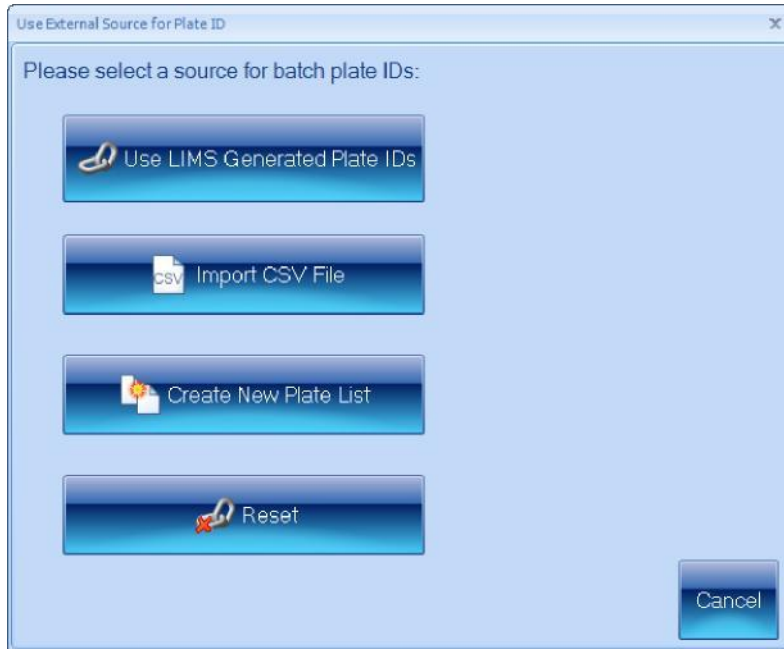
Creating a new batch

To enable the **Auto-Increment** control and deselect the external source:

- a. Press



to display the **Use External Source for Plate ID** dialog box:



- b. Press



4. If **Auto-increment** is not selected in the **Plate ID** box, click the check box to select it.

5. Press the **Measure** tab



to display the measure controls (see *The Measure tab*, page 123).

6. In the **Plate ID** box, type in text to act as a basis for the identifier.

If the text includes a sequence of digits, these will be used as the starting point for the auto-incrementing; if it includes more than one sequence, the rightmost sequence will be used as the starting point; if there are no digits in the text, auto-incrementing digits will be added to the end of the text. For example:

Template	First identifier	Second identifier	Third identifier
abc	abc1	abc2	abc3
abc18	abc19	abc20	abc21
abc 18def	abc 19def	abc20def	abc21def
66abc 18def	66abc 19def	66abc20def	66abc21def

Using a barcode reader for plate identifiers

Note Depending on how the barcode reader is programmed, the barcode reader may or may not be able to read dilution information.

When you are working in Measurement mode, you can use a barcode reader to read the identifier/dilution from the plate in combination with an external source of plate identifiers (see *Using an external source for plate identifiers*, on the next page) or to input manual identifiers (see *Manually entered plate identifiers*, page 143).

If you intend to use a barcode reader to read plate identifiers (and dilutions where relevant – see the note above):

1. Make sure there is a barcode reader attached to the PC – see the note in Step 2.
2. Press



to display the **Configuration** tab:



Note The **Use Barcodes** check box is disabled if there is no barcode reader attached to the PC.

3. Select **Use Barcodes**.

See *Using a barcode reader for identifiers and dilutions*, page 146, for more on using the barcode reader to input plate identifiers/dilutions.

Creating a new batch

Using an external source for plate identifiers

You can use the following external sources for plate identifiers:

- a LIMS
- a CSV file
- a list created in ProtoCOL 3.

To specify an external source for plate identifiers:

1. Press



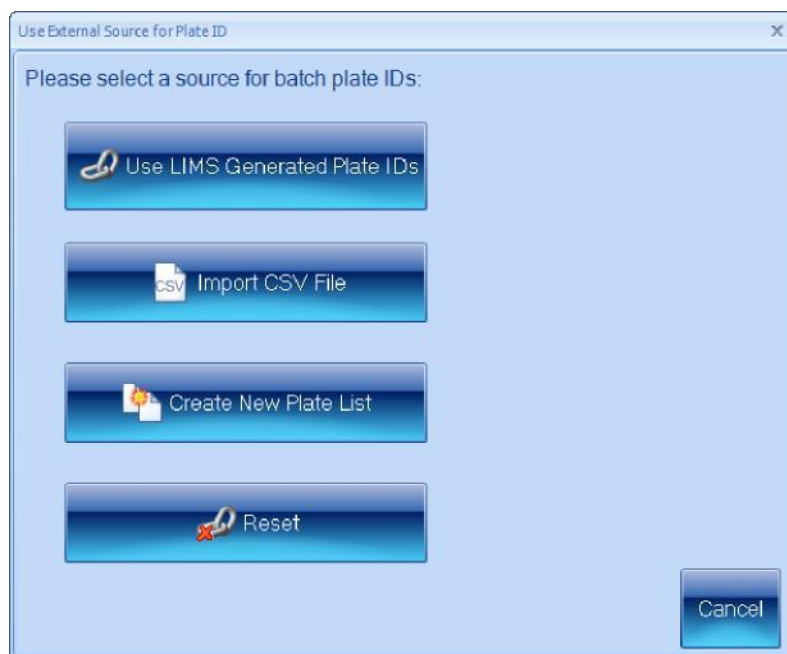
to display the **Configuration** tab:



2. Press



to display the **Use External Source for Plate ID** dialog box:



3. Press the button for the required option.

The following sections describe each of the options in turn:

- Linking to a LIMS*, on the next page
- Using a CSV list of identifiers*, page 117
- Creating a plate list*, page 121.

If one of the options is already selected, and you want to go back to using Auto-incrementing identifiers or manual identifiers:

Press



When you have selected an external source for plate identifiers, the icon in the **Plate List** box in the **Configuration** tab will show:



if you have selected to use a LIMS generated list



if you have selected to use an imported list or a list created in ProtoCOL 3



if you have chosen not to use an external source of plate identifiers.

Creating a new batch

Linking to a LIMS

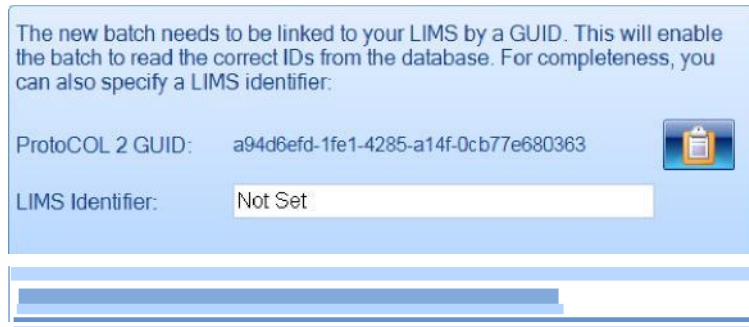
Note See *LIMS connection properties*, page 191, for how to set up ProtoCOL 3 to work with a LIMS.

To make a link between the batch and the LIMS generated plate identifiers:

1. Press



in the **Use External Source for Plate ID** dialog box (see *Using an external source for plate identifiers*, page 114) to display the LIMS link controls:



2. Make a note of the **ProtoCOL 3 GUID** shown – you can press



to copy it to the clipboard.

Note When the LIMS generated identifiers are added to the ProtoCOL 3 database they should be labeled with the ProtoCOL 3 GUID for the batch in order to link them to the batch – see the paragraphs following these instructions for more information about adding LIMS generated identifiers to the ProtoCOL 3 database.

3. If you wish to link the ProtoCOL 3 plate table back to the LIMS, you can add a **LIMS Identifier**.

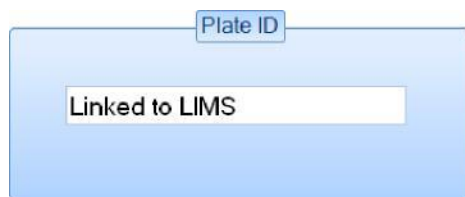
This is not essential for the operation of ProtoCOL 3, but can be used to form a link between the table in ProtoCOL 3 and a table in your LIMS database. The details of how to form the link and what identifier to use will depend on the LIMS you are using.

4. Press **OK** to close the **Use External Source for Plate ID** dialog box.

When you have selected an external source for plate identifiers, the icon in the **Plate List** box in the **Configuration** tab will show:



The **Plate ID** box on the **Measure tab** (see *The Measure tab*, page 123) will show that the plate ids will be linked to the LIMS:



The precise details of how you add the LIMS generated plate identifiers and dilutions to the ProtoCOL 3 database will depend on your LIMS. As a simple example, the following SQL code could be used to add the plate identifier 'Plate1' with a dilution of '1:10' to the ProtoCOL 3 database for a batch that has been given the ProtoCOL 3 GUID 'a94d6efd-1fe1-4285-a14f-0cb77e680363':

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

If required (see Step 3 above), you can add a LIMS identifier as well:

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

Notes ProtoCOL 3 does not require you to use unique identifiers/dilutions.

For Dilution Series batches, if an identifier in the LIMS list has a dilution, that dilution will be used and any of the dilution(s) set in ProtoCOL 3 (see *Dilution Series and Spiral Plate batches*, page 125) will be ignored. On the other hand, if the LIMS list does not include a dilution, each of the dilutions in the ProtoCOL 3 dilutions list will be used in turn for that identifier.

If you are using a LIMS as an external source of plate identifiers for an Ames batch (see *Using an external source for plate identifiers*, page 114), each plate identifier should appear twice in the plate list if you are going to perform a remeasurement (see *Repeating a measurement for an Ames batch plate*, page 154).

When you are working in Measurement mode, you can use a barcode reader in conjunction with a LIMS list to match each plate you are measuring to its identifier/dilution in the LIMS list – see *Using a barcode reader for plate identifiers*, page 113.

Using a CSV list of identifiers

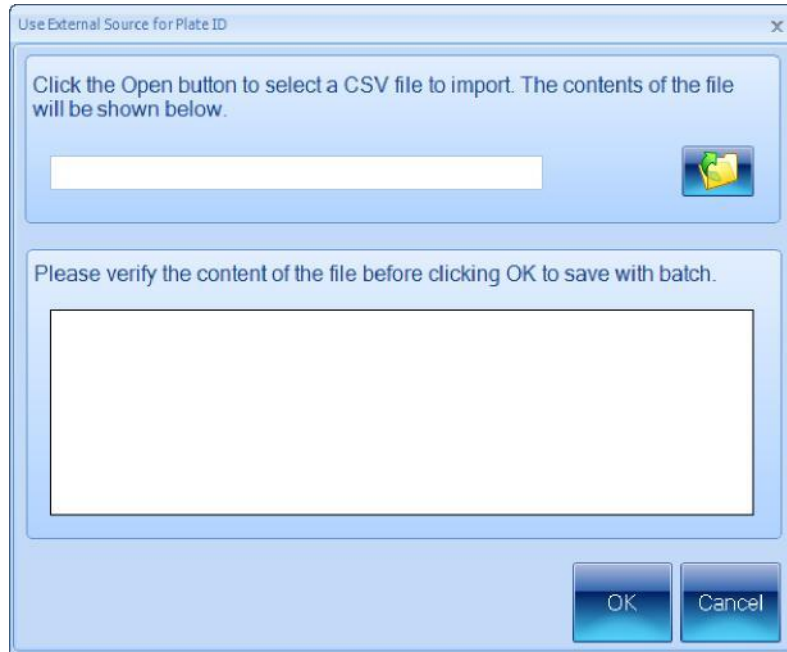
To use a CSV file containing a list of plate identifiers and dilutions (see the text following the instructions for the format to use for CSV files):

1. Press



Creating a new batch

in the **Use External Source for Plate ID** dialog box (see *Using an external source for plate identifiers*, page 114) to display controls for opening the CSV file:



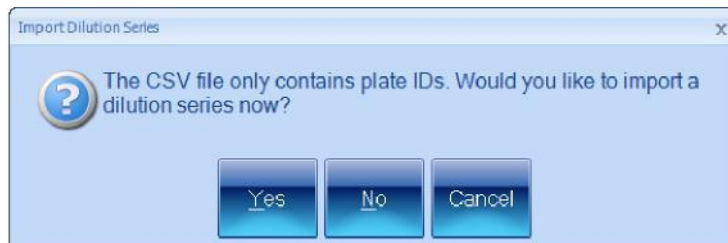
2. Press



to open a standard Windows **Open** dialog box.

3. Use the **Open** dialog box to locate and select the required CSV file.
4. Press **Open** to open the selected file.

If you are creating a *Dilution Series* batch and the CSV file does not contain *any* dilution information, you will be asked if you want to import a dilution series (otherwise, go straight to Step 5):

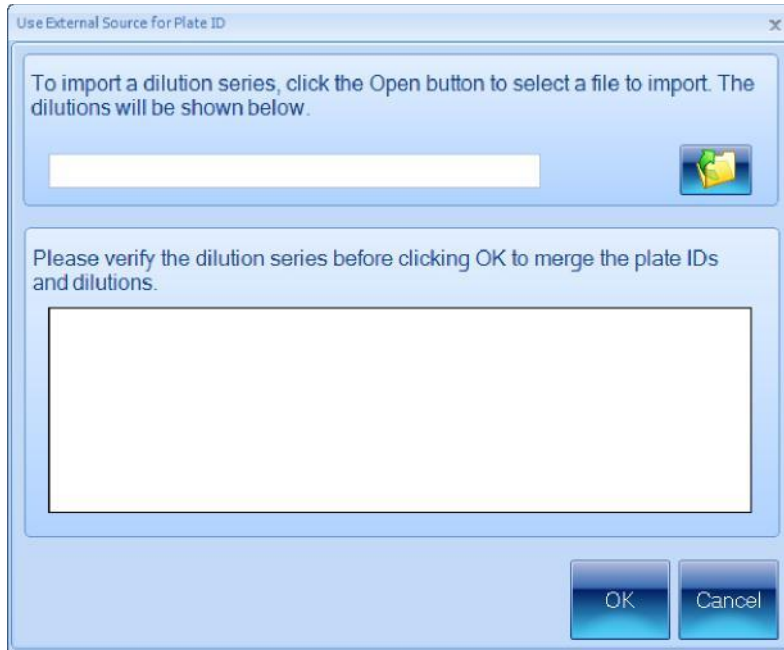


- a. If you want to import a csv file containing a dilution series, press **Yes**, and go to Step b.

If you do not want to import a dilution series, press **No** – the default dilution will be used for each of the imported plate identifiers. Go to Step 5.

If you want to abort the procedure for importing plate identifiers completely, press **Cancel**.

- b. If you pressed **Yes** in the previous step, controls will be displayed for you to import a second csv file containing the dilution series:



- c. Press



to open a standard Windows **Open** dialog box.

- d. Use the **Open** dialog box to locate and select the required CSV file.
- e. Press **Open** to open the selected file.

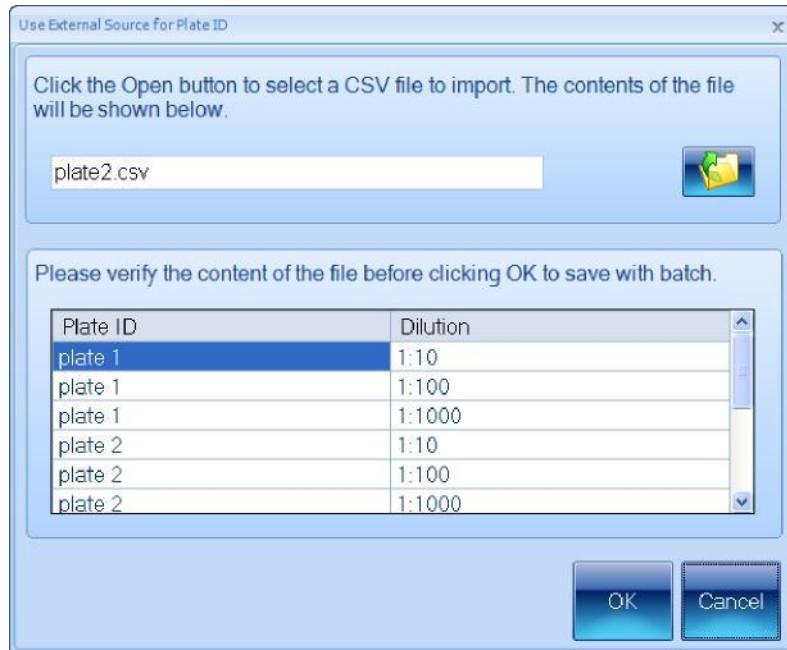
The contents of the file will be shown in the preview box:



Creating a new batch

- f. Check the preview box to make sure the contents are correct.

If the contents are correct, press **OK** to show the combined plate identifiers and dilutions:



Note If you are using a csv list as a source of plate identifiers for a Simple Ames batch (see *Using an external source for plate identifiers*, page 114), each plate identifier should appear twice in the plate list if you are going to perform a re-measurement (see *Repeating a measurement for an Ames batch plate*, page 154). For Ames Study Manager batches, please see the appendix.

5. The contents of the file will be shown in the preview box (see example above) – check the preview box to make sure the contents are correct (see the subsection following these instructions for the format to use for the CSV file).
6. If the contents are correct, press **OK** to close the **Use External Source for Plate ID** dialog box and load the plate list into the batch.

The **Plate ID** box on the **Measure tab** (see *The Measure tab*, page 123) will show that the plate identifiers have been taken from an external source:



Note When you are working in Measurement mode, you can use a barcode reader in conjunction with a CSV list to match each plate you are measuring to its identifier/dilution in the CSV list – see *Using a barcode reader for plate identifiers*, page 113.

CSV file format

Each line of the file should consist of two strings separated by a comma:

Identifier,Dilution

where:

Identifier is the plate identifier

Dilution is text describing the dilution (if the string is not recognized or no dilution is given, 'No Dilution' is used).

The order of the lines in the file defines the order of the plates in the list.

For example:

Test1,No Dilution

Test2,1:5

Test3,1 in 1000

Test4,1 in 10⁶

Note ProtoCOL 3 does not *require* you to use unique identifiers/dilutions.

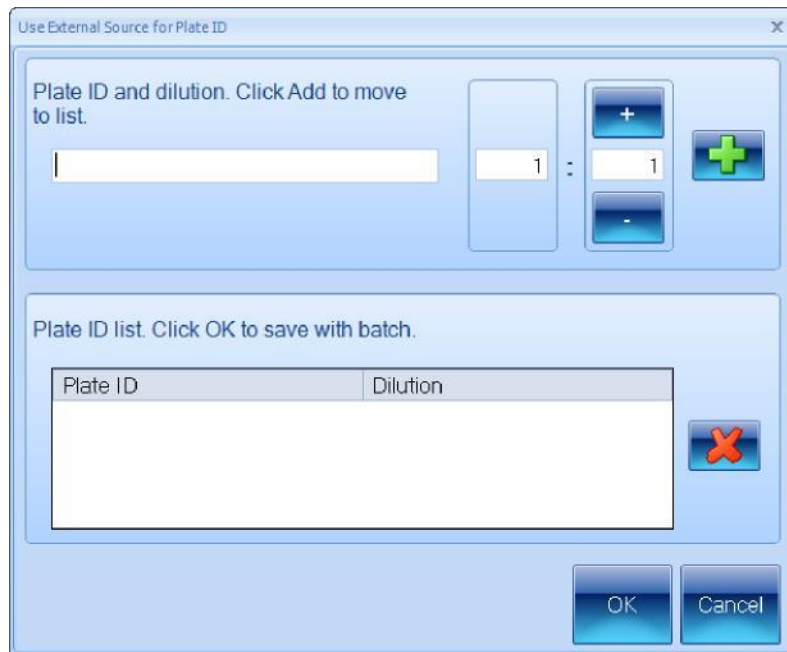
Creating a plate list

To create a plate list containing a list of plate identifiers and dilutions: 1.

Press



in the **Use External Source for Plate ID** dialog box (see *Using an external source for plate identifiers*, page 114) to display controls for creating a plate list:



Note The picture shows the **Use External Source for Plate ID** dialog box for a batch using dilutions. The dilution controls do not appear for Chromogenic, Multiwell, OPKA, SBA, Antibiotic Susceptibility or Inhibition Zone batches as the dilution is not relevant in these cases.

Creating a new batch

2. Type the identifier for the first plate into the edit box at the top of the dialog box.
3. For batch types using dilutions (see note above), use the dilution boxes to enter the dilution: you can type directly into the boxes or use the + and – buttons to increase or decrease the values.

Note The left-hand box is the sample proportion; the right-hand box is the total, so 1:10 means one part sample in ten parts total volume. This means that the right-hand number must not be smaller than the left-hand number.

4. Press



to add the identifier/dilution to the plate list.

5. Repeat Steps 2–4 for the remaining plates in the batch.

Note If you are using a plate list as the source of plate identifiers for an Ames batch (see *Using an external source for plate identifiers*, page 114), each plate identifier should appear twice in the plate list if you are going to perform a remeasurement (see *Repeating a measurement for an Ames batch plate*, page 154). For Ames Study Manager batches, please see the appendix.

6. If you change your mind about one of the plates in the list, click on it in the list to select it and press



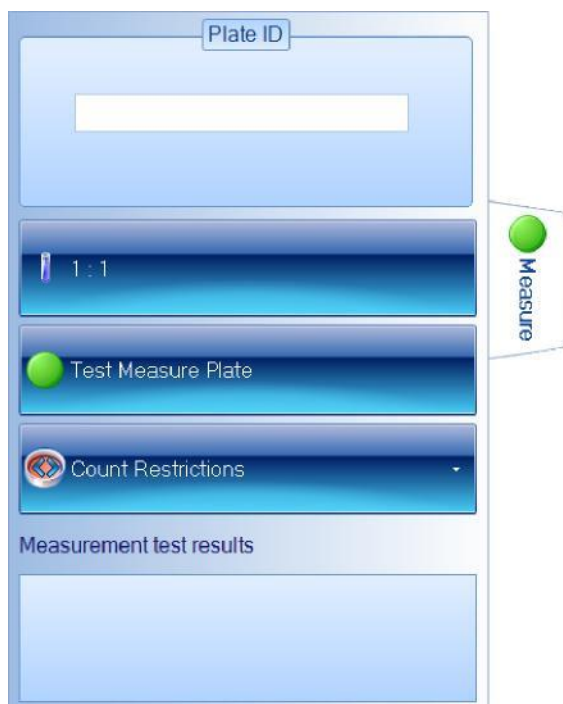
7. When you are satisfied that the list is complete and correct, press **OK** to close the **Use External Source for Plate ID** dialog box and load the plate list into the batch.

The **Plate ID** box on the **Measure tab** (see *The Measure tab*, on the facing page) will show that the plate identifiers have been taken from an external source:



Note When you are working in Measurement mode, you can use a barcode reader in conjunction with a custom plate list to match each plate you are measuring to its identifier/dilution in the plate list – see *Using a barcode reader for plate identifiers*, page 113.

The Measure tab



Notes The dilution control does not appear for Multiwell, SBA, OPKA, Chromogenic, Antibiotic Susceptibility or Inhibition Zone batches and has a different form for Dilution Series and Spiral batches.

The **Count Restrictions** button does not appear for Spiral, Chromogenic, Antibiotic Susceptibility or Inhibition Zone batches.

To display the measure controls in the Batch Designer:

Press the **Measure** tab:



The **Measure** tab in the Batch Designer allows you to:

- enter text to act as a template for use with Auto-incrementing identifiers (see *Selecting auto-incrementing plate identifiers*, page 111)
- set the dilution ratio when relevant (see *Dilution*, on the next page)
- set a count limit for plates (not Spiral plate, Chromogenic, Antibiotic Susceptibility or Inhibition Zone batches) – see *Setting Count Restrictions*, page 128
- carry out a test count of the current plate (see *Test Measure Plate*, page 129) so that you can check the batch settings – if you are using a color classification (see *Color classification*, page 43), you must carry out a test measurement before you can accept the batch for Measurement mode.

Creating a new batch

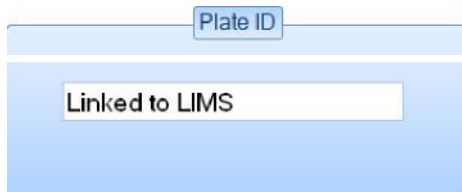
Plate ID



The **Plate ID** control on the **Measure** tab in the Batch Designer allows you to specify a template for auto-incrementing plate identifiers – see *Selecting auto-incrementing plate identifiers*, page 111, for details.

Note If you choose to use auto-incrementing plate identifiers, you will not be able to accept the batch design until you have entered a template for the identifiers (see *Accepting the batch design*, page 132).

If you have chosen to use a LIMS as the source for the plate identifiers (see *Using an external source for plate identifiers*, page 114), the **Plate ID** control will show



but if you have chosen to use an imported plate list or a plate list created in ProtoCOL 3, it will show



Dilution

Notes Dilutions are not relevant for OPKA, SBA, Chromogenic, Multiwell, Antibiotic Susceptibility or Inhibition Zone batches.

If you have chosen to use a LIMS, CSV file or a custom plate list as the source of the plate identifiers for use in Measurement mode (see *Using an external source for plate identifiers*, page 114), you can include individual dilutions with each plate identifier – see the previous section, *Plate ID*. If no dilution is supplied in the lists, 'No dilution' is assumed.

To specify the dilution to use in calculating results:

Press the **Measure** tab



to display the dilution controls.

The dilution controls used for Dilution Series and Spiral Plate batches are different from those for other types of batch – for details, see:

- *Dilution Series and Spiral Plate batches*, below
- *Dilution for other batch types*, page 127.

Dilution Series and Spiral Plate batches

Note See *Dilution for other batch types*, page 127, for how to set the dilutions for Ames, Multi-Sector, Multiwell and Pour Plate batches; dilutions are not relevant for OPKA, SBA, Chromogenic, Antibiotic Susceptibility or Inhibition Zone batches.

To set the dilutions for a Dilution Series or Spiral Plate batch:

1. Press the dilutions button to display the dilutions menu (the button shows the current dilution):

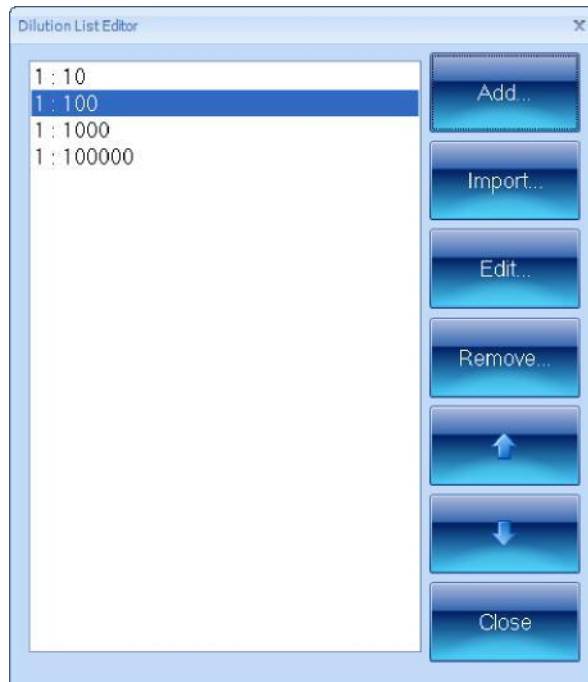


Notes For a Dilution Series batch, there are left and right arrow buttons below the dilutions button. These can be used to change the dilution for a plate as an alternative to selecting the dilution from the dilutions menu, though selecting the dilution in the Batch Designer has no effect for a Dilution Series batch.

However, when you carry out a test measurement for a Spiral Plate batch in the Batch Designer, the dilution selected in the dilutions menu does have an effect, as the results show a count/ml value – see *Test Measure Plate*, page 129.

Creating a new batch

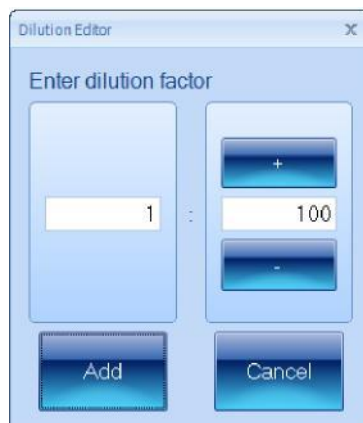
2. Select **Edit Dilution List** from the menu to open the **Dilution List Editor**:



3. If required, to import a list of dilutions (see *Dilutions file format* after these instructions for details):
 - a. Press **Import** to open a standard Windows **Open** dialog box.
 - b. Use the **Open** dialog box to locate and select the required file.
 - c. Press **Open** to open the selected file.

The dilution list in the file will be added to the **Dilution List Editor**, replacing any existing entries.

4. To add a new dilution to the list:
 - a. Click on the dilution at the point you want to add the new dilution.
 - b. Press **Add** to open the **Dilution Editor**:



- c. Use the dilution boxes to enter the dilution: you can type directly into the boxes or use the + and – buttons to increase or decrease the values.

Note The left-hand box is the sample proportion; the right-hand box is the total, so 1:10 means one part sample in ten parts total volume. This means that the right-hand number must not be smaller than the left-hand number.

- d. Press **Add** to close the **Dilution Editor** and add the dilution to the list.
5. To change a dilution in the list:
 - a. Click on the dilution in the list to select it.
 - b. Press **Edit** to open the **Dilution Editor**.
 - c. Use the dilution boxes to edit the dilution: you can type directly into the boxes or use the + and – buttons to increase or decrease the values.
 - d. Press **Update** to close the **Dilution Editor** and confirm the change to the dilution.
 6. To remove a dilution from the list:
 - a. Click on the dilution in the list to select it.
 - b. Press **Remove**.

A confirmation dialog box will be displayed.
 - c. Press **Yes** to remove the dilution.
 7. To move a dilution up or down in the list in order to change the order of dilutions:
 - a. Click on the dilution in the list to select it.
 - b. Press the up or down arrow button.
 8. When you have finished editing the dilutions list, press **Close** to return to the main ProtoCOL 3 window.

Dilutions file format

Each line of the file should consist of a string representing the dilution (if the string is not recognized or no dilution is given, 'No Dilution' is used).

The order of the lines in the file defines the order of the dilutions in the list.

For example:

No Dilution

1:5

1 in 1000

1 in 10⁶

Note ProtoCOL 3 does not *require* you to use unique dilutions.

Dilution for other batch types

Note See *Dilution Series and Spiral Plate batches*, page 125, for how to set the dilutions for Dilution Series and Spiral Plate batches; dilutions are not relevant for OPKA, SBA, Chromogenic, Antibiotic Susceptibility or Inhibition Zone batches.

Creating a new batch

To set the dilution for an Ames, Multi-Sector, Multiwell or Pour Plate batch:

1. Press



(the button shows the current dilution) to open the dilution editor:



2. Use the dilution boxes to enter the dilution: you can type directly into the boxes or use the + and – buttons to increase or decrease the values.
3. Press **Update** to confirm the dilution you have set and return to the main ProtoCOL 3 window.

Setting Count Restrictions

Note The **Count Restrictions** button does not appear for Spiral Plate, Chromogenic, Antibiotic Susceptibility or Inhibition Zone batches. This will only appear when setting up a batch and cannot be changed once a batch has been accepted.

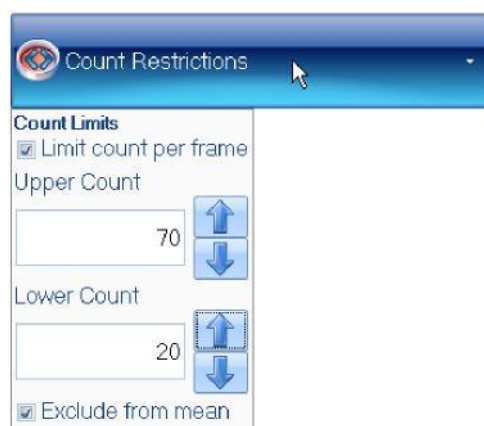
To place restrictions on the count result:

1. Press the **Measure** tab



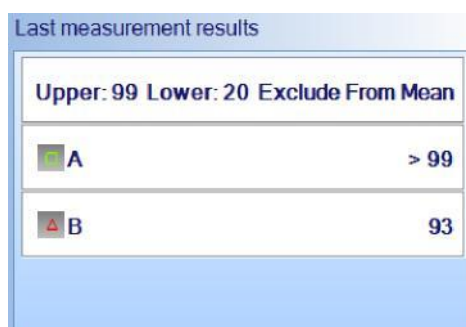
to display the measure controls, including the **Count Restrictions** button.

2. Press the **Count Restrictions** button to display the **Count Restrictions** controls:



3. Check **Limit count per frame** to enable count restrictions.
4. Either type the required limits directly into the **Upper Count** and **Lower Count** edit boxes or press the arrow buttons to increase or decrease the values.
5. Check **Exclude from mean** if you want any results violating the count restrictions to be ignored when calculating the mean results; leave it unchecked to include all results in mean calculations.

When count restrictions have been set and you carry out a test measurement in the Batch Designer (see *Test Measure Plate*, below) or perform a measurement in Measurement mode, the **Measurement test results** or **Last measurement results** box, respectively, will show whether the limit has been breached, for example:



As this example shows, if there is more than one colony type, the count limits (99 and 20 in this case) are applied to each of them separately.

Test Measure Plate

If you are using a color classification (see *Color classification*, page 43), you will not be allowed to accept the new batch (see *Accepting the batch design*, page 132) until you have carried out a separate test measurement.

Note The **Total Plate Count** procedure (see *Total Plate Count*, page 38) includes a test measurement, so you are not required to carry out another one if you are using that instead of a color classification.

Creating a new batch

To carry out a test measurement:

1. Press the **Measure** tab



to display the measure controls.

2. Press



Note If you are using an uncalibrated imported image, a warning dialog box will be displayed asking you to calibrate the image – see *Calibrating the image*, page 13, for details.

The results of the measurement will be shown in the **Measurement test results** panel.

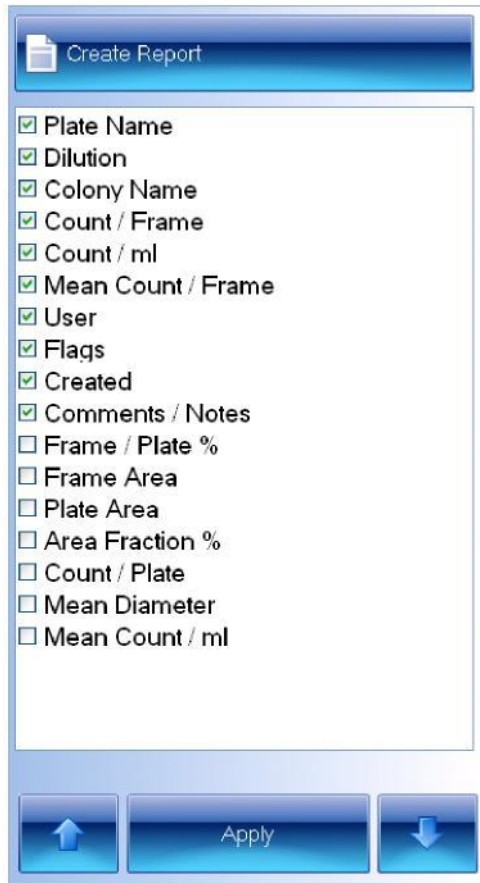
For colony counting batches, if the button below the image is labeled **All Colony Markers**, the detected colonies will also be marked on the image using the colors and markers selected for each colony type (if the button is labeled **No Colony Markers**, press the button and select **All Colony Markers** to display the detected colonies on the image).

3. Check the image to make sure that:
 - for a colony counting batch, the colonies have been correctly detected and distinguished from the background and any debris on the plate.
 - for a zone measuring batch, the zones and discs, if present, have been correctly detected.
4. If there are any problems with the colony/zone detection, you will need to go back to change the batch settings using one of the procedures described earlier in *Setting up batches*, page 27.

If you are happy with the measurement results, you can proceed to accept the batch for Measurement mode – see *Accepting the batch design*, page 132.

The Results tab

The **Results** tab allows you to specify what results should be included in the Results table and the order of the columns:



Notes The picture shows the **Results** tab in Measurement mode; the **Apply** button does not appear in the Batch Designer.

The results included in the list depend on the type of plate used in the batch.

The reports can be opened in Open Office, Excel or PDF. With PDF the maximum number of result options is ten. If you have too many options, a warning message will appear.

To display the results controls:

Press the **Results** tab:



You can set a default selection of results in the Batch Designer, but you can also make a different selection at any time in Measurement mode as required.

Results with a selected check box will be included in the Results table; unselected results will not be included. The order of the results in the list is the order they will appear in the Results table.

Creating a new batch

To change whether a result will be included in the Results table:

1. Click on the result to select it.
2. Click on the result again to select/deselect the check box.
3. To carry out the change when working in Measurement mode, press:



To move a result up or down the list to change the order of results in the Results table:

1. Click on the result to select it.
2. To move the result up the list, press



To move the result down the list, press



3. To carry out the change when working in Measurement mode, press:



Accepting the batch design

To confirm that you have completed the setup procedure and want to proceed to taking measurements:

Press



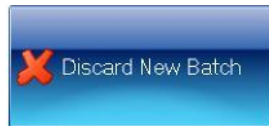
Notes The button will be disabled until you have carried out all steps required for setting up the batch. However, even if the button is enabled, you will be prevented from accepting the batch if you have not given the batch a name (see *Giving the batch a name*, page 28) or, if you are using auto-incrementing plate identifiers (see *Selecting auto-incrementing plate identifiers*, page 111), you have not set a template plate id (see *Plate ID*, page 124).

The **Accept New Batch** button is enabled to show that you *can* proceed to taking measurements if you wish – you can still make further changes to the setup if required before pressing the **Accept New Batch** button.

The next chapter in the Manual, *Using Measurement Mode to count colonies and measure zones*, shows you how to use ProtoCOL to carry out colony counts and zone measurements; the chapter following that, *Working with results*, page 157, shows you how to view and edit the results, and then compile a report.

If you change your mind about creating the new batch:

Press



to abort the new batch – you will be asked to confirm that you want to do this.

Managing batches

Exporting batch details

To export the details of a batch to an XML file:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Press:



to display the **Export Data** dialog box – this is a standard Windows **Save As** dialog box.

3. Use the **Export Data** dialog box to select a location and enter a filename for the exported batch details.
4. Press **Save** to save the batch details file.

If required, you can move the XML file to another PC and use it as the basis for a new batch on that PC – see *Creating a new batch based on an existing batch or an exported batch file*, page 24.

Setting batch restrictions

Batch restrictions enable you to protect some batch properties against accidental changes. Batch restrictions can be changed at any time – in particular, they can be changed both before and after the batch has been accepted (see *Accepting the batch design*, on the previous page), and apply to the batch in both the Batch Designer and Measurement mode.

Note The 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 – see *Moving frames*, page 33.

Creating a new batch

To set restrictions on what changes can be made to a batch:

1. If you are working in Measurement mode and the batch is not already open or is not selected, open/select it – see *Opening and selecting batches*, page 139.
2. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



3. Press:



to display the **Batch Management** submenu.

4. Press:



to display the **Edit Batch Restrictions** dialog box:



The items in the dialog box depend on the type of batch.

5. Check the boxes for items you want users to be able to change when they are working with the batch in Measurement mode; unchecked items will be locked so that users

cannot change them accidentally.

6. Press **Save** to confirm the settings and close the dialog box.

Deleting batches

To delete a batch from the database:

1. If the batch you want to delete is currently open, close it – see *Closing batches*, page 155.
2. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



3. Press:



to display the **Batch Management** submenu.

4. Press:



Creating a new batch

to display the **Delete Batch** dialog box listing **The 10 most recent batches**:



5. If the required batch is not in the list of most recent batches:

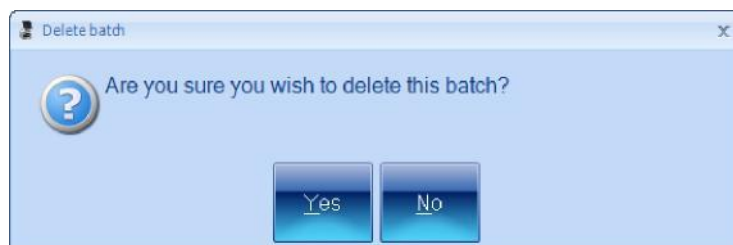
- a. Type the name, or a part of the name, of the required batch into the **Search criteria** box.
- b. Press



Only batches with names that contain the text you entered will be listed.

6. Select the required batch in the list – you will only be able to delete batches if they are ticked in the **Available** column; open batches will not be available.
7. Press **Delete** to delete the batch.

You will be asked to confirm that you want to delete the batch:



8. Press **Yes** to delete the batch.

You will be asked to give a reason for deleting the batch:



9. Enter the reason. You can enter your own reason or choose from the drop down menu.
10. Press **OK** to delete the batch.

Note Although the batch can no longer be viewed in ProtoCOL 3, it is not actually removed from the ProtoCOL 3 database and can be viewed there if required for audit purposes.

Using Measurement Mode to count colonies and measure zones

Once you have created and accepted a new batch (see *Creating a new batch*, page 19, and *Accepting the batch design*, page 132), the general procedure for counting colonies or measuring zones on plates is:

1. Open or select the batch – see the next section, *Opening and selecting batches*.
2. Load the first plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
3. Capture an image – see *Capturing an image*, page 10.
4. Adjust the measurement frame(s) if required – see *Adjusting frame settings in Measurement mode*, page 141.
5. Enter a plate identifier if it has not been entered automatically, or change an automatically entered identifier and dilution if required – see *Entering plate identifiers and dilutions*, page 142.
6. Carry out the measurement – see *Counting colonies*, page 147, and *Measuring inhibition zones*, page 154.
7. Repeat Steps 2–6 for all the other plates in the batch.
8. Check the results and edit them if necessary, then create a report for the batch – see the following chapter, *Working with results*, page 157.
9. Close the batch – see *Closing batches*, page 155.

Note For Ames batches, you may need to count the plates twice – see *Repeating a measurement for an Ames batch plate*, page 154, for details. For more information on Ames, please see the appendix at the end of this document.

Steps 2 and 3 are also required when creating and setting up the batch – the cross references take you back to earlier sections in the Manual. Step 8 is described in the next chapter, *Working with results*, page 157.

Instructions for the remaining steps are given in this chapter (though much of Step 4 is common to creating and setting up the batch and is covered by references to the *Creating a new batch*, chapter).

Opening and selecting batches

If you have just created and accepted a new batch (see *Creating a new batch*, page 19, and *Accepting the batch design*, page 132), it will be automatically open and selected in Measurement mode. However, you can also open a previously created batch if there is no batch open currently, or if you want to open another batch in addition to any currently open batches (you can have several batches open at the same time – see later in this section for how to select which open batch to work with).

Using Measurement Mode to count colonies and measure zones

To open a batch in Measurement mode:

1. Press:



to display the **Open Batch** dialog box listing **The 10 most recent batches**:



Open batches are marked with a tick in the **Available** column.

2. If the required batch is not in the list of most recent batches:
 - a. Type the name, or a part of the name, of the required batch into the **Search criteria** box.
 - b. Press



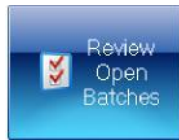
Only batches with names that contain the text you entered will be listed.

3. Select the required batch in the list.
4. Press **Open** to open the batch.

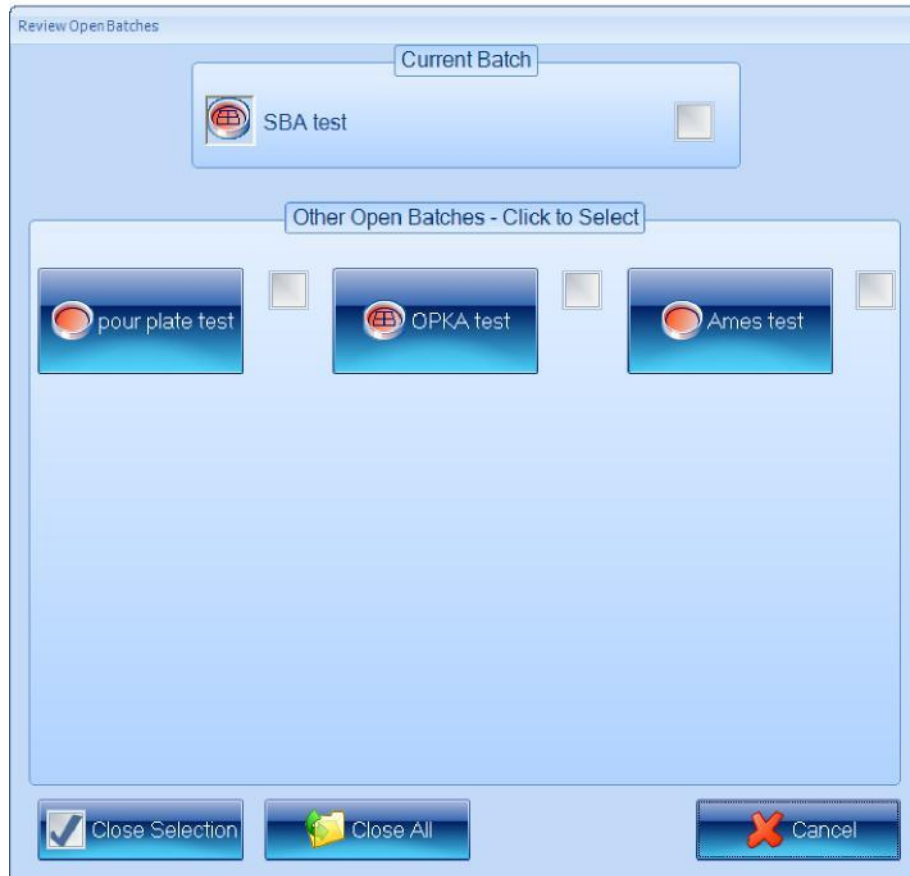
If there is more than one batch currently open in ProtoCOL 3, you can select which one to work on at any time (unless you are in the process of creating a new batch).

To select an open batch:

1. Press:



to display the **Review Open Batches** dialog box:



The currently selected batch is listed with a check box in the **Current Batch** box, and there are buttons and check boxes for the **Other Open Batches** in the box below it.

2. Press the button for the required batch in the **Other Open Batches** box.

The **Review Open Batches** dialog box will close and the selected batch shown in Measurement mode.

Adjusting frame settings in Measurement mode

Providing access to the relevant operation has not been restricted (see *Setting batch restrictions*, page 133), you can make temporary adjustments to the frame settings in Measurement mode.

In all cases, you use essentially the same techniques as when you are setting up a batch.

Using Measurement Mode to count colonies and measure zones

Note Any changes you make to the settings in measurement mode will be lost when you close the batch – the default settings set in the **Classification** tab (see *The Classification tab*, page 28) when you created the batch will be reloaded the next time you reopen the batch.

For zone measurement batches, you can:

- Move the frame(s) – see *Moving frames in the Configure Zone Frame page*, page 81
- Resize the frame or the overall size of a ring or grid of zone – see *Resizing zone frames*, page 83
- *Rotating a ring or grid of zone frames*, page 85,

For colony counting batches, you can change the frame type, shape, size, position and orientation – see the following for details:

- Frame shape – see *Choosing the type of frame to use*, page 30
- Frame size, position and orientation – see *Adjusting the position and size of frames*, page 33.

Entering plate identifiers and dilutions

Each plate in the batch must be given a plate identifier, but the procedure required depends on the options selected when the batch was created.

Note If several plates are given the same identifier (for example, with a Dilution Series), they will be grouped together under the same item in the Results table – see *Working with results*, page 157, for details.

For details, see:

- the next section, *Auto-incrementing plate identifiers*
- *Manually entered plate identifiers*, on the facing page
- *External source – LIMS, CSV file or Custom plate list*, page 145
- *Using a barcode reader for identifiers and dilutions*, page 146.

Auto-incrementing plate identifiers

See *Selecting auto-incrementing plate identifiers*, page 111, for details of how to set up a batch to use auto-incrementing plate identifiers, including the rules used to generate the new identifier.

When any type of batch with auto-incrementing plate identifiers is first opened, the template identifier set when the batch was created (see *Selecting auto-incrementing plate identifiers*, page 111) will be set automatically in the **Next Plate Id** box as the first identifier for the batch. For example:



After each measurement:

- For non-Dilution Series batches, a new plate identifier will be set ready for the next plate by auto-incrementing the previous identifier.
- For Dilution Series batches, if there are still some dilutions on the next dilution list, the plate identifier will be unchanged and the next dilution in the dilution list will be set. When there are no more dilutions in the list, the plate identifier will be auto-incremented and the first dilution in the dilution list will be set.

If required, you can edit the identifier in the **Next Plate Id** box. That identifier will then be used for the next measurement, and will be auto-incremented for subsequent measurements according to the normal auto-incrementing rules. If you enter a new identifier for a Dilution Series batch, the measurement will be used as the first measurement of a new dilution series.

Manually entered plate identifiers

To enter a manual plate identifier:

Type the identifier into the **Next Plate ID** box:

A screenshot of a software interface showing a text input field. The field is labeled 'Next Plate ID' in a light blue header bar. Below the header is a white text box with a thin blue border, currently empty.

You can also use a barcode reader to enter a manual plate identifier (and dilution if the barcode reader is programmed to read dilutions).

Changing the dilution set for a plate

Note Dilutions are not relevant for OPKA, SBA, Antibiotic Susceptibility or Inhibition Zone batches.

If required, you can change the dilution set for the current colony counting plate. The procedure required depends on the type of batch.

Ames, Multi-Sector, Multiwell and Pour Plate batches

To change the dilution for the current plate in a non-dilution series batch: 1.

Press the dilution button, for example:



Using Measurement Mode to count colonies and measure zones

to open the **Dilution Editor**:



2. Use the dilution boxes to enter the dilution: you can type directly into the boxes or use the + and – buttons to increase or decrease the values.

Note The left-hand box is the sample proportion; the right-hand box is the total, so 1:10 means one part sample in ten parts total volume. This means that the right-hand number must not be smaller than the left-hand number.

3. Press **Update** to confirm the dilution you have set and return to the main ProtoCOL 3 window.

Dilution Series and Spiral Plate batches

To select one of the other dilutions in the series, either:

Press one of the arrow buttons below the dilution button to select the next or previous dilution in the series, for example:



Or:

1. Press the dilution button, for example:



2. Select the required dilution from the **Dilution List**.

To edit the dilution list and choose a dilution from the new list:

1. Press the dilution button, for example:



2. Press **Edit Dilution List** to display the **Dilution List Editor**.
3. Use the **Dilution List Editor** to edit the dilution list – see *Dilution Series and Spiral Plate batches*, page 125, for how to use this dialog box.
4. Press the dilution button again and select the required dilution from the **Dilution List**.

Note The new dilution list will be used for future plates as well as the current one.

External source – LIMS, CSV file or Custom plate list

When the batch is set to use an external source of plate identifiers (see *Using an external source for plate identifiers*, page 114), ProtoCOL 3 creates a list of plate identifiers and, if relevant, dilutions from the external source.

When you first open the batch, the **Next Plate ID** box shows the first plate identifier on the list and, where relevant, the dilution control shows the dilution, for example:



Note The **Next Plate ID** text box is disabled for a batch using an external source for its plate identifiers – you cannot edit the identifiers.

After you have performed the measurement, the identifier (and dilution where relevant) for that measurement will be removed from the plate list and the next identifier (and dilution) will be shown. You can then repeat this procedure until the list is exhausted. When the list is exhausted, no more measurements can be made and an error message will be displayed if you try to perform a count, but see the following note.

Using Measurement Mode to count colonies and measure zones

Note Once you have accepted a batch for use in Measurement mode you cannot add plate identifiers to the batch's identifier list if it has been created from a CSV file (see *Using a CSV list of identifiers*, page 117) or a custom plate list (see *Creating a plate list*, page 121). However, you can add LIMS generated plate identifiers to the ProtoCOL 3 database at any time, so, for example, if you have measured the plates for all the identifiers currently loaded into the ProtoCOL 3 database from the LIMS, you can generate a new list from the LIMS and add those to the ProtoCOL 3 database using the same ProtoCOL 3 GUID as you used for the first set (see *Linking to a LIMS*, page 116), and then take the measurements from those plates.

If the plates are not arranged in the same order as the plate list, you can choose another identifier/dilution from the list – see the following subsection.

Selecting a different plate from the plate list

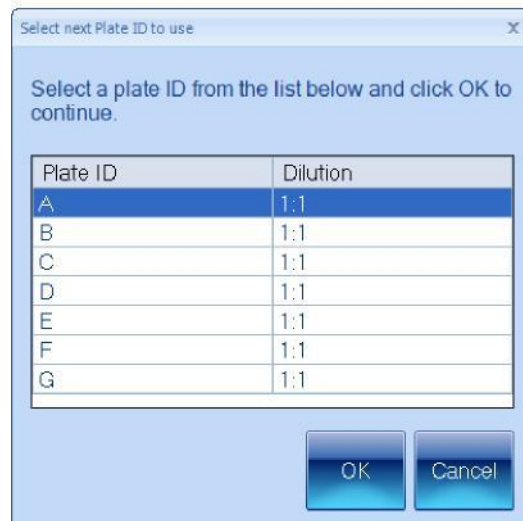
If the batch has been set up for use with a barcode reader (see *Using a barcode reader for plate identifiers*, page 113), you can use the barcode reader to read the plate identifier (and dilution where relevant and if the barcode reader is programmed to read dilutions) from the plate – ProtoCOL 3 will give an error if the identifier/dilution is not on its plate list for the batch.

Alternatively, if you need to choose a different plate identifier/dilution from the plate list: 1.

Press



to display the **Select next Plate ID to use** dialog box:



2. Click in the row containing the required plate identifier/dilution to select it.
3. Press **OK** to close the dialog box – the selected identifier will appear in the **Next Plate Id** box and the dilution will appear on the dilution button.

Using a barcode reader for identifiers and dilutions

You can use a barcode reader to enter a manual plate identifier (and dilution if the barcode reader is programmed to read dilutions) or pick a plate identifier and dilution from the plate list created from an external source (see *Using an external source for plate identifiers*, page 114).

Counting colonies

This section of the Manual describes how to count the colonies on a plate in Measurement mode.

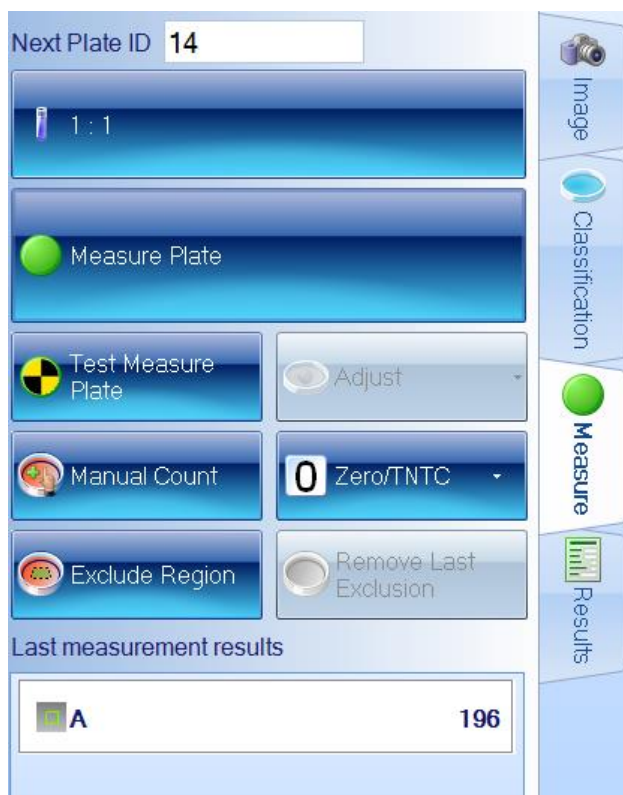
In most cases, you should be able to carry out an automatic colony count by simply pressing a button – for details, see *Counting the colonies on a plate automatically*, on the next page.

However, some plates or batches may require individual attention. In particular, you can:

- Create exclude regions to avoid areas of the plate that might cause problems – see *Exclude regions*, page 149
- Carry out a test measurement and adjust the colony detection settings – see *Test measurement and adjusting settings*, page 151
 - Carry out a manual colony count – see *Manual colony counting*, page 153
 - Enter a zero result for a blank plate – see *Entering a zero count*, page 154
- Repeat a measurement for an Ames plate – see *Repeating a measurement for an Ames batch plate*, page 154.

Note Some of the above procedures can only be applied to certain batch types – all exceptions are noted in the instructions.

All the above colony counting procedures use the **Measure** tab:



Using Measurement Mode to count colonies and measure zones

To display the **Measure** tab in Measurement mode:

Press:



Counting the colonies on a plate automatically

To count the colonies on a plate automatically:

Press



The results will be added to the Results table (see *Working with results*, page 157, for details) and shown in the **Last Measurement Results** panel:

Last measurement results	
A	50

If you have created a classification with more than one colony type, the results for each type will be shown:

Last measurement results	
A	68
B	88

For a Spiral Plate batch, the **Last Measurement Results** will also show the concentration:

Last measurement results	
A	108
Count/ml	21600

And for Multi-sector, Multiwell, OPKA and SBA batches, the **Last Measurement Results** can show either the count for:

- All Sectors:**



- or a selected sector:



You select **All sectors** or the required sector from the drop-down list at the top of the **Last Measurement Results** panel; you can also select an individual sector by clicking in its frame in the image.

See also:

Counting colonies, page 147, for a summary of how to modify the way automatic colony counts are performed and how to carry out colony counts using other methods.

Exclude regions

Note You cannot place exclude regions on Spiral Plate batches.

On occasions, you may want to avoid counting colonies on part of a plate because of some problem with it. One way of doing this is to create one or more exclude regions, which will be ignored when ProtoCOL 3 counts the colonies – the area of the exclude regions will be deducted from the area of the frame to ensure that results such as count/ml are correct.

Note If the batch allows you to change and/or adjust the frame (see *Setting batch restrictions*, page 133), an alternative for some batch types might be to choose a Partial Plate frame and/or to rotate the frame so that the problem area is not counted.

To define an exclude region on an image:

1. Press



to select it:



Note You may find it helpful to zoom the image before placing the exclude region on the image – see *Zooming the image*, page 11.

Using Measurement Mode to count colonies and measure zones

2. You define the exclude region in the same way as when you add exclude regions in the Colony Classification wizard – see *Excluding regions during color classification*, page 47, for detailed instructions.

To remove the last exclude region you added to the image (you can repeat the procedure to remove each of the regions in turn):

Press



Spiral plate properties

When you create a new batch you specify the properties of the plates you will be using – see *Creating a completely new batch*, page 20. In most cases these settings are fixed and cannot be changed once the batch has been created. However, provided you have the required permissions (see *User permissions*, page 189), you can change some of the properties for Spiral Plate batches.

To change the spiral plate properties for the current Spiral Plate batch:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



- Choose **Spiral Plate Properties** from the **Settings** submenu to display the **Edit Spiral Plate Properties** dialog box:



- Press the button for the **Spiral Volume** you are using.
- Set the **Spiral Count Rule** by typing in a value directly or by pressing the **+** or **-** button to increase or decrease the current value.

If you are using a two-sector counting frame and the number of colonies within the first sector is less than the threshold, the whole frame will be used instead.
- Press **OK** to confirm the settings and close the dialog box.

Test measurement and adjusting settings

If required, you can carry out a test measurement to check the colony detection for an individual plate without recording the result, and if the batch was based on a **Total Plate Count** (see *Total Plate Count*, page 38), you will be able to modify some of the batch colony detection settings if there are any problems.

To carry out a test colony count and adjust the detection settings:

- Press



Using Measurement Mode to count colonies and measure zones

The result of the test measurement will appear in the **Last Measurement Results** panel and if the batch is based on a **Total Plate Count**, the **Adjust** button will be enabled:

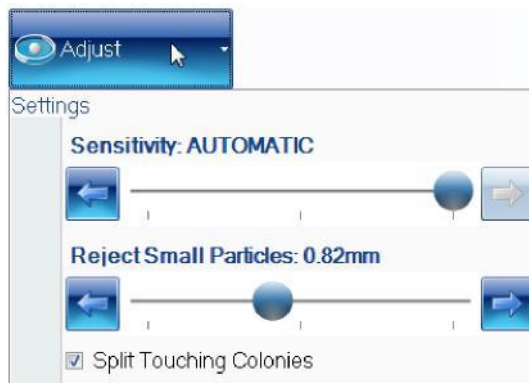


The result of the test measurement will **not** be added to the Results table (see *The Results table*, page 157).

If the button below the image is labeled **All Colony Markers**, the detected colonies will also be marked on the image using the colors and markers selected for each colony type (if the button is labeled **No Colony Markers**, press the button and select **All Colony Markers** to display the detected colonies on the image).

2. Check the image to make sure that the colonies have been correctly detected.
3. If you are satisfied with the colony detection, go straight to Step 4.

Otherwise, if there are problems with the colony detection and the batch is based on a **Total Plate Count**, press **Adjust** to display the **Settings** menu:



The controls are used in exactly the same way as when you are setting up a batch using a **Total Plate Count** – see *Total Plate Count*, page 38, for details.

Note If required, you will also be able to change the **Sensitivity**, **Reject Small Particles** and **Split Touching Colonies** settings after you have performed and recorded the count – see *Rejecting small particles in a result*, page 166, *Changing the Sensitivity setting for a result*, page 166, and *Splitting colonies in a result*, page 167, for details.

4. To carry out the measurement and record the results in the Results table, press



Note If you make any changes to the settings using the procedures described in this section, the new settings will be applied to the batch until you close the batch. If you wish to revert to the settings made when the batch was created, either make a note of the values and re-enter them using the above procedure, or close the batch (see *Closing batches*, page 155) and reopen it again (see *Opening and selecting batches*, page 139).

Manual colony counting

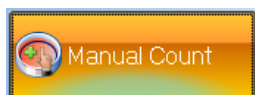
Note You cannot carry out a manual colony count for a Spiral Plate batch or batches with more than one type of colony (color, size or shape classification).

To carry out a manual colony count:

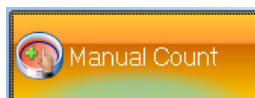
1. Press



The button will become selected showing that a manual count is in progress:



2. Click on the image to count each of the colonies in turn – as you click, the total in the **Last measurement results** panel will be incremented and the image will be marked to show that the colony has been counted.
3. When the count is complete, press



to record the result in the results table.

Notes Once you have begun a manual count, the only way to cancel it is to log out of or close ProtoCOL 3.

You can only add to the total by clicking on the image; you cannot decrease the count.

See also

Manually adding and removing colonies, page 164, for how to edit a result in the results table by adding or removing colonies **after** the count has been made and recorded.

Entering a zero count or too numerous to count

To enter a zero result for a plate:

Press the drop down menu



To enter a too numerous to count result for a plate:

Press the drop down menu



A new result will be added to the Results table with a 0 count or TNTC.

Note The image will not be saved with the result, even if **Save images to file** is selected in the **Settings** dialog box – see *System settings*, page 178.

See also

Zeroing the count, page 169, for how to zero a result in the results table **after** the count has been made and recorded.

Repeating a measurement for an Ames batch plate

Depending on the procedure you are following, you may need to take a second measurement for an Ames batch plate after an interval of time has elapsed.

To repeat a measurement for a plate in an Ames batch:

1. Insert the plate to be re-measured into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
2. Capture the image – see *Capturing an image*, page 10.
3. Enter or select the same plate identifier as you did for the first measurement – see *Entering plate identifiers and dilutions*, page 142.

Note If you are using an external source of plate identifiers for an Ames batch (see *Using an external source for plate identifiers*, page 114), each plate identifier should appear twice in the plate list if you are going to perform a re-measurement.

4. Press measure plate

The original measurement for the plate will be moved from the **Count** columns to the **Original Count** columns for the result in the Results table and the new count added to the Count columns – the **Difference/ml** column will show how much the count has increased.

If you are using Ames Study Manager, please see appendix one at the end of this document.

Measuring inhibition zones

To carry out a test measurement of the inhibition zones on a plate without recording the results:

Press



To measure the inhibition zones on a plate and record the results:

Press

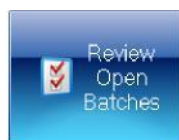


The results will be added to the Results table – see *Working with results*, page 157, for details.

Closing batches

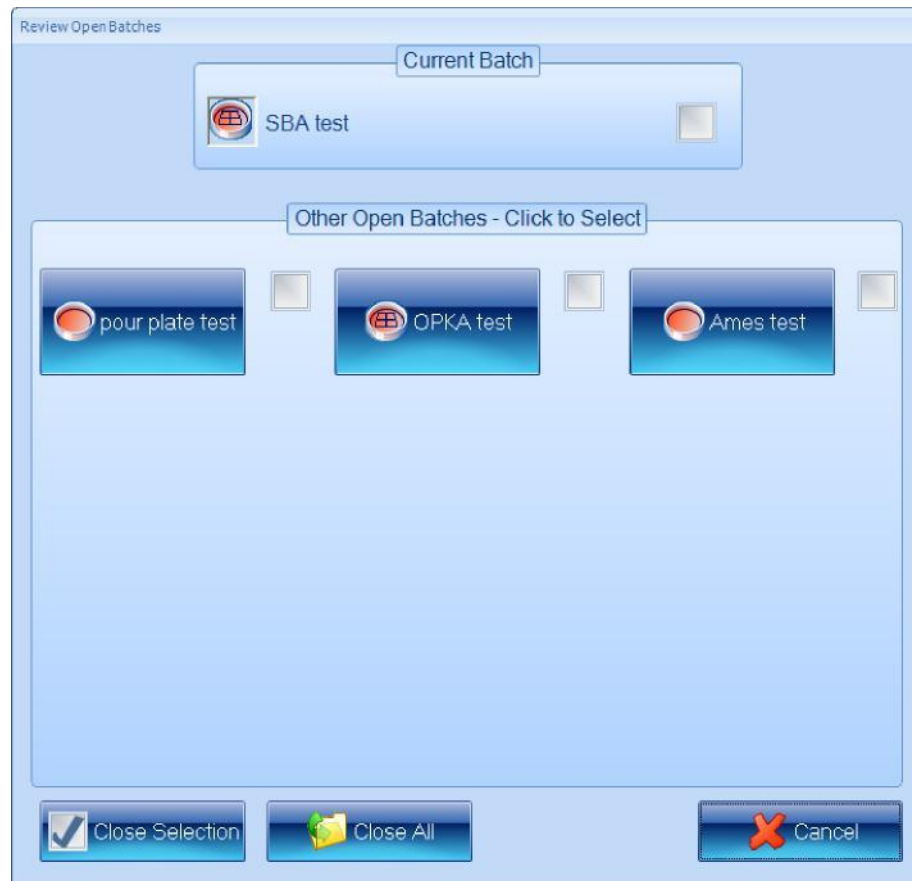
To close one or more open batches:

1. Press:



Using Measurement Mode to count colonies and measure zones

to display the **Review Open Batches** dialog box:



The currently selected batch is listed with a check box in the **Current Batch** box, and there are buttons and check boxes for the **Other Open Batches** in the box below it.

2. Either, to close a selection of batches:

- a. Click in the check boxes for the batches you want to close to select them.
- b. Press



Or, to close all the open batches:

Press



The **Review Open Batches** dialog box will close and either the selected batches or all the batches will be closed.

If there is more than one result with the same plate identifier (for example, the results for a Dilution Series), they will appear as separate plate results within the same identifier item and below the Mean result:



Plate Name	Dilution	Count / Frame	Count / ml
test1	1 : 5	59	279
test1	1 : 2	122	231
test1	1 : 1	290	275

For Multi-Sector, Multiwell, OPKA and SBA batches, the results for each sector are shown as a separate item within each plate item:

Sector	Colony Name	Count / Frame	Count / ml
Sector 1	A	2	6
	B	16	50
Sector 2	A	6	18
	B	16	50
Sector 3			
Sector 4			

You can select a row in the Results table by clicking in it, and move to the next or previous line by pressing the down or up arrow key on the keyboard.

To select the next or previous *plate identifier* in the Results table (there may be several plates with the same plate identifier – in a Dilution Series batch, for example):

Press  or  on the right-hand edge of the Results table.

Editing plate measurements

ProtoCOL 3 allows you to edit measurements in a variety of ways using the **Edit Result** tab.

Notes You can only edit results if the image was saved with the result – see *System settings*, page 178, for how to choose to save images with results.

If the image was not saved with the result, a dialog box will be displayed:



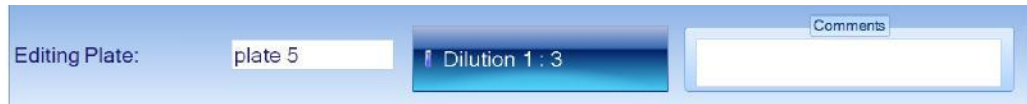
Press **OK** to continue.

To edit a measurement:

1. Select the measurement you want to edit by clicking in the plate's result in the Results table – see *The Results table*, page 157.
2. Press the **Edit/Review** tab



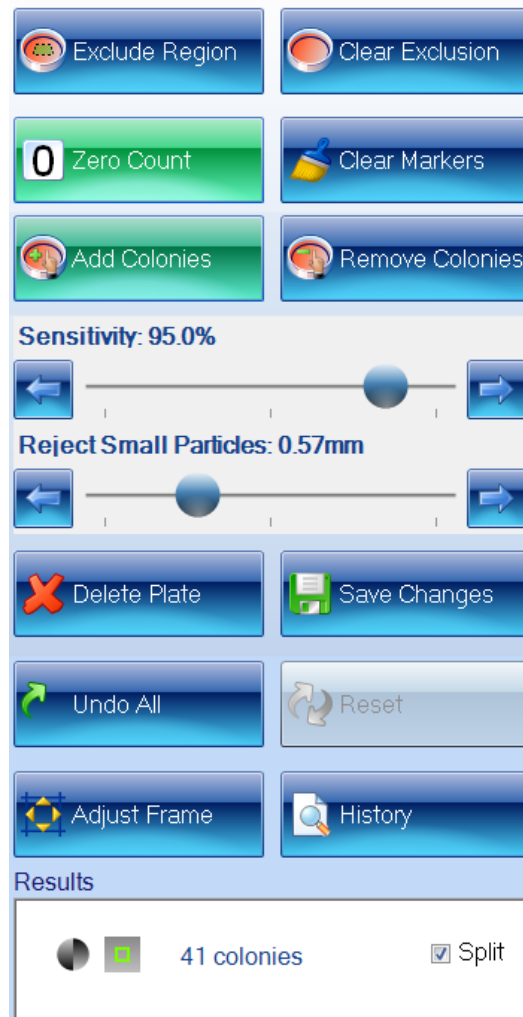
to display the **Editing Plate** panel at the bottom of the ProtoCOL 3 window:



and the **Edit Result** controls in the **Edit Result** tab.

The controls included on the **Edit Result** tab depend on whether the selected batch is:

- a colony counting Batch & if the review/approve feature has been selected in the permissions tab. When review/approve has been selected the save button will become a dropdown box allowing the plate to be marked as reviewed or approved.



Working with results

Notes The **Exclude Region**, **Clear Exclusion** and **Adjust Frame** controls are hidden for Spiral Plate batches.

For batches based on a **Total Plate Count** (see *Total Plate Count*, page 38), a **Sensitivity** slider appears above the **Reject Small Particles** slider. For counting outside of the frame please refer to the quick guide provided with your instrument.

- a zone measuring Batch



The controls on the **Edit/Review** tab and the **Editing Plate** panel allow you to carry out a wide range of editing operations, which are described in the following sections:

- Any batch type:
 - *Editing the plate identifier, comments and dilution*, on the facing page
 - *Saving changes to results*, page 162
 - *Undoing and resetting changes*, page 162
 - *Deleting results*, page 163
 - *Viewing the audit history of changes*, page 163.
- Colony counting batch:
 - *Manually adding and removing colonies*, page 164
 - *Adjusting the frame for a result*, page 165
 - *Rejecting small particles in a result*, page 166
 - *Changing the Sensitivity setting for a result*, page 166

- *Splitting colonies in a result*, page 167
- *Adding exclude regions to a result*, page 168
- *Zeroing the count*, page 169.
- Zone measuring batch:
 - *Manual zone measurement*, page 169
 - *Repeating an automatic zone measurement*, page 173.

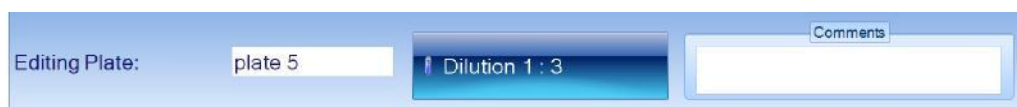
All batch types

Editing the plate identifier, comments and dilution

To add or edit a comment and / or edit the plate identifier and/or, where relevant, edit the dilution for a result:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.

The **Editing Plate** panel will be displayed at the bottom of the ProtoCOL 3 window:



2. Edit the plate identifier in the left-hand edit box as required.
3. Add to/edit the **Comments** in the right-hand edit box as required.
4. To edit the dilution, where relevant:
 - a. Press the dilution button, for example:



to open the **Dilution Editor**:



- b. Use the dilution boxes to enter the dilution: you can type directly into the boxes or use the + and – buttons to increase or decrease the values.

Notes The left-hand box is the sample proportion; the right-hand box is the total, so 1:10 means one part sample in ten parts total volume. This means that the right-hand number must not be smaller than the left-hand number.

- c. Press **OK** to confirm the dilution you have set and return to the **Editing Plate** panel.
5. Save the change to the result (see *Saving changes to results*, below) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, on the facing page, for how to view the reasons for changes to results.

The identifier, comment and/or dilution will be updated for the result and if you have changed the dilution, values depending on the dilution will also be updated. An **E** flag will appear in the **Flags** column showing the result has been edited.

Saving changes to results

To save any changes you have made to a result on the **Edit/Review** tab and/or the **Editing Plate** panel (see *Editing plate measurements*, page 158):

1. Press



The **Edit Count** dialog box will be displayed if the ProtoCOL 3 administrator has selected **Requires Audit Reason** for **Edit Results** (see *User permissions*, page 189):



2. Select the reason for the change from the drop-down list or enter your own reason (there is a minimum limit on the length of the text you can add – see *System settings*, page 178).
3. Press **OK**.

Note Any changes you have made to a result will be lost if you move away from the **Edit/Review** tab before saving them.

If required, you can view an audit history listing the reasons for the changes you have made to the result – see *Viewing the audit history of changes*, on the facing page.

Undoing and resetting changes

To undo all the changes made to a result on the **Edit/Review** tab and the **Editing Plate** panel (see *Editing plate measurements*, page 158) since the result was last saved (see *Saving changes to results*, above):

Press



For a colony counting batch, to reset the result to the original measurement value before any changes were made to it and saved using the **Edit Result** tab:

Press



Note Any changes made to the plate identifier, dilution or comment in the **Editing Plate** panel will not be reset.

Deleting results

To delete the result(s) for a plate:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Press



3. Save the change to the result (see *Saving changes to results*, on the previous page) – you may be asked to give a reason for the change.

The result will be deleted from the Results table. Refer to quick guide for re-using a plate name within a plate list.

Note Although the result is deleted from the Results table and can no longer be viewed in ProtoCOL 3, it is not removed from the ProtoCOL 3 database and can be viewed there if required for audit purposes.

Viewing the audit history of changes

When you make any changes to a result, ProtoCOL 3 makes an entry in the audit history for that plate.

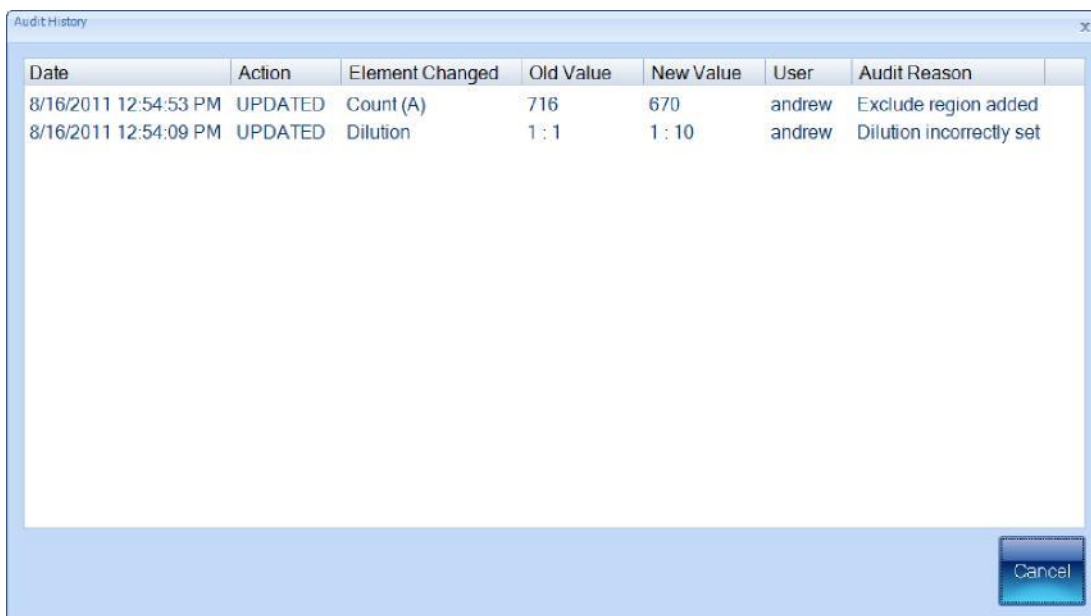
To view the audit history for a plate in the Results table:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Press



Working with results

to display the **Audit History** dialog box:



The screenshot shows a dialog box titled "Audit History" with a table of changes. The table has the following columns: Date, Action, Element Changed, Old Value, New Value, User, and Audit Reason. There are two rows of data.

Date	Action	Element Changed	Old Value	New Value	User	Audit Reason
8/16/2011 12:54:53 PM	UPDATED	Count (A)	716	670	andrew	Exclude region added
8/16/2011 12:54:09 PM	UPDATED	Dilution	1 : 1	1 : 10	andrew	Dilution incorrectly set

A "Cancel" button is located in the bottom right corner of the dialog box.

3. When you have finished viewing the audit history, press **Cancel** to close the dialog box.

Editing colony counting results

Manually adding and removing colonies

To add colonies to or remove colonies from the detected colonies for a result:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. If you want to remove all colony markers currently on the image so that you start the count from scratch, press



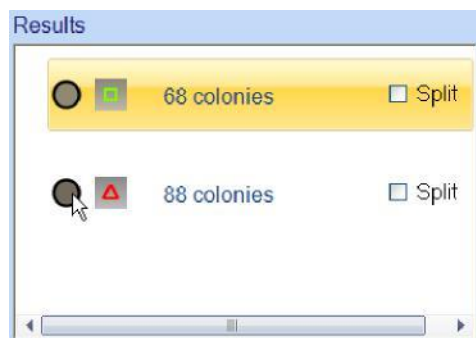
3. If you want to add colonies, press



If you want to remove colonies, press



- If you have used a color, size and/or shape classification to define more than one colony type, the first type will be selected by default. To select another type to add or remove, click on its row in the **Results** box at the bottom of the **Edit/Review** tab (avoid clicking on the actual result text – for example, **88 colonies** in the following picture):



- If you have chosen to add colonies, click on the image at the point you want to add the colony – you cannot add colonies outside the frame.

If you have chosen to remove colonies, click on the colony markers you want to remove.

The count will be updated in the **Results** box as you click in the image.

- Repeat Steps 4–5 for any other colony types.
- Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

The count(s) will be updated in the result together with any values depending on the count(s). An **M** flag will appear in the **Flags** column showing you have manually added or removed colonies.

Adjusting the frame for a result

Note You cannot adjust the frame for a Spiral Plate batch.

To adjust the frame for a plate result:

- Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
- Press



- Adjust the frame using the same techniques as when you create a batch or before taking a measurement – see *Adjusting the position and size of frames*, page 33.
- Press



again.

The plate will be re-measured using the adjusted frame.

5. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

Rejecting small particles in a result

When you create a batch, you can adjust the setting of the **Reject Small Particles** filter to remove the effect of small colored regions of the image that may be wrongly interpreted as colonies – see *Total Plate Count*, page 38, and *Review Color Classification*, page 57. For a batch based on a **Total Plate Count**, you can also change the **Reject Small Particles** filter in **Measurement Mode** before taking a measurement (see *Test measurement and adjusting settings*, page 151).

This section shows you how to change the **Reject Small Particles** setting for an individual plate after it has been measured. In this case, the batch can be based on a color classification or a **Total Plate Count**.

To change the **Reject Small Particles** filter setting for an individual plate result:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Drag the



slider; click the arrow buttons at the ends of the slider to adjust the slider by a single step; or press anywhere on the slider bar to set the slider to that position.

The plate will be re-measured with the new setting.

3. Check that the real colonies are still being counted while the small particles you want to reject are being excluded, and repeat Step 2 if the result is still not satisfactory.

Note You may find it helpful to zoom the image – see *Zooming the image*, page 11.

4. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

Changing the Sensitivity setting for a result

Note This section is only relevant for colony counting batches based on a **Total Plate Count** – see *Total Plate Count*, page 38. The **Sensitivity** control is hidden for batches based on a color classification.

When you create a batch based on a **Total Plate Count**, you can adjust the detection **Sensitivity** – see *Total Plate Count*, page 38. After you have accepted the batch, you can also change the **Sensitivity** setting in Measurement mode before taking a measurement (see *Test measurement and adjusting settings*, page 151).

This section shows you how to change the **Sensitivity** setting for an individual plate after it has been measured.

To change the detection **Sensitivity** setting for an individual plate result:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Drag the



slider; click the arrow buttons at the ends of the slider to adjust the slider by a single step; or press anywhere on the slider bar to set the slider to that position.

The plate will be re-measured with the new setting.

3. Check that the real colonies are being counted and the background and any debris are being excluded, and repeat Step 2 if the result is still not satisfactory.

Note You may find it helpful to zoom the image – see *Zooming the image*, page 11.

4. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

Splitting colonies in a result

When you create a batch, you can choose whether to use the colony splitter to split overlapping colonies so that they are counted separately – see *Total Plate Count*, page 38, and *Review Color Classification*, page 57. For a batch based on a **Total Plate Count**, you can also change the colony splitter setting in **Measurement Mode** before taking a measurement (see *Test measurement and adjusting settings*, page 151).

This section shows you how to change the splitter setting for an individual plate *after* it has been measured. In this case, the batch can be based on a color classification or a **Total Plate Count**.

To change the colony splitter setting(s) for an individual plate result:

1. Select the **Edit Result** tab for the plate result – see *Editing plate measurements*, page 158.
2. Click on the **Split** check box in the **Results** box at the bottom of the **Edit/Review** tab to change the setting (if you have used a color, size and /or shape classification to define more than one colony type, there are separate check boxes for each type and you can change the **Split** setting for each of them independently):



The plate will be re-measured with the new split setting.

Working with results

3. Check that overlapping colonies are being split satisfactorily and single colonies are not being split.

Note You may find it helpful to zoom the image when checking the effect of the colony splitter – see *Zooming the image*, page 11.

4. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

Adding exclude regions to a result

Note You cannot place exclude regions on Spiral Plate batch results.

If required, you can add exclude regions to a plate image before you take a measurement from it – see *Exclude regions*, page 149. However, you can also add and remove exclude regions after you have performed the measurement – when you remove exclude regions after the measurement, you can clear exclude regions that were added before the measurement as well as those added to the result after the measurement.

To define exclude regions for a plate result:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Press



to select it:



Note You may find it helpful to zoom the image before placing the exclude region on the image – see *Zooming the image*, page 11.

3. You define the exclude region in the same way as when you add exclude regions in the Colony Classification wizard – see *Excluding regions during color classification*, page 47,

The plate will be re-measured with the new exclude region in place.

4. To remove the last exclude region you added to the image (you can repeat the procedure to remove each of the regions in turn, including any added before the measurement was made):

Press



The plate will be re-measured with the last exclude region removed.

5. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

Zeroing the count

If the result for a plate is faulty in some way and you wish to exclude it, you can zero the count. If you zero a result, it will still be shown in the Results table (see *The Results table*, page 157) with count(s) equal to 0.

Notes This section shows you how to set the count(s) for a result to zero to record a zero result. If you just want to remove all the colony markers from the image and set the counts to zero so that you can count the colonies manually, press **Clear Markers** – see *Manually adding and removing colonies*, page 164. If you want to delete a faulty result, press **Delete Plate** – see *Deleting results*, page 163.

An alternative way to deal with a faulty result is to delete it (see *Deleting results*, page 163), but then it would be removed from the results table completely, and you could not reset the change later if you change your mind about excluding the result (see *Undoing and resetting changes*, page 162).

To set the count(s) for a plate to zero:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Press



All other result editing controls will be disabled apart from **Save Changes**, **Undo All**, **Reset** (if there are previously saved changes) and the controls in the **Editing Plate** panel at the bottom of the ProtoCOL 3 window.

3. Save the change to the result (see *Saving changes to results*, page 162) – you will be asked to give a reason for the change.

The result will be zeroed in the Results table.

Note When you zero a result that contributes to a mean (for example, in a dilution series), the result will still contribute to the mean (with count 0). If you want to remove the result's contribution to the mean, you can change its plate identifier (see *Editing the plate identifier, comments and dilution*, page 161) or delete the result (see *Deleting results*, page 163).

Editing zone measuring results

Manual zone measurement

To edit a result for an Inhibition Zone or Antibiotic Susceptibility batch by manually measuring the zones:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.

Working with results

2. Click in the image on the first zone you want to edit to select it. The current result will be:

- Shown as a circular zone on the image:



- Shown numerically in the **Selected Zone** box:



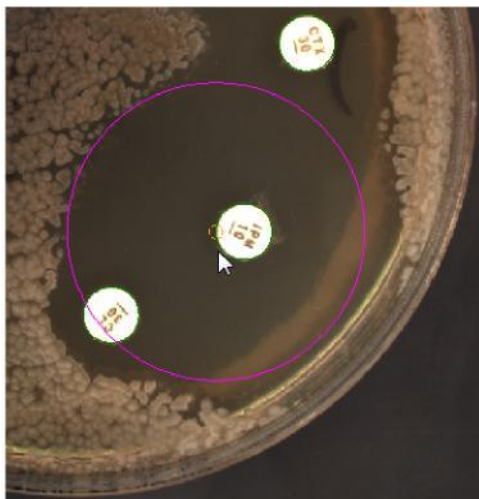
- Highlighted in the results table in the **Edit/Review** tab:

A	58.96 mm
B	48.44 mm
C	40.5 mm
D	62.53 mm
E	24.72 mm
F	20.09 mm
G	28.21 mm

You edit the zone result by dragging and resizing the result zone on the image.

3. If required, to move the result zone on the image:

- a. Move the pointer near to the center of the zone. An orange circular drag handle will appear at the center:

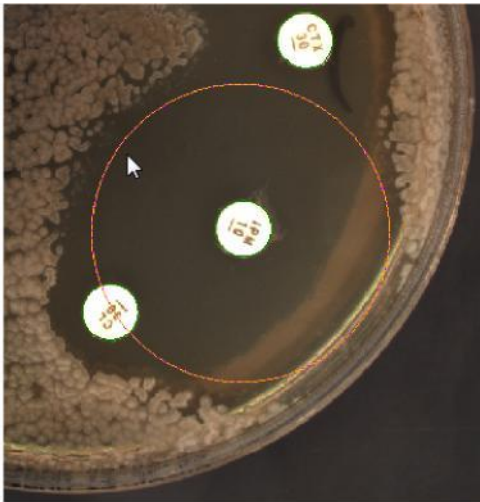


- b. Drag the zone and drop it at the required position:

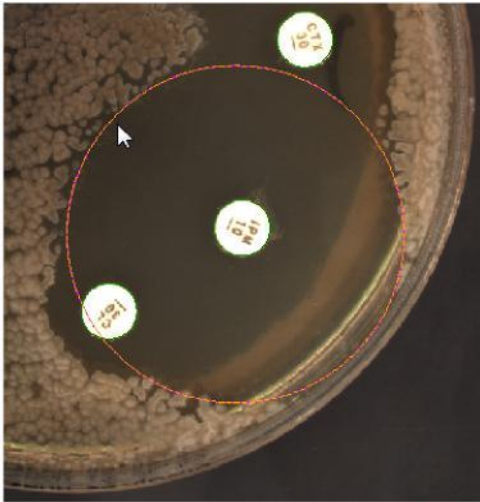


Working with results

- 4. To resize the result zone on the image:
 - a. Move the pointer near to the circumference of the zone. The circumference will be highlighted in orange:



- b. Drag the circumference to the required size:



The new result will be shown in the **Selected Zone** box and the results table:



5. Repeat Steps 2–4 for any other zones you want to edit.
6. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

Repeating an automatic zone measurement

To repeat an automatic zone measurement:

1. Select the **Edit/Review** tab for the plate result – see *Editing plate measurements*, page 158.
2. Press



The detected zones will be shown as circles on the image and the new results listed in the results table in the **Edit/Review** tab.

3. Save the change to the result (see *Saving changes to results*, page 162) – you may be asked to give a reason for the change; see *Viewing the audit history of changes*, page 163, for how to view the reasons for changes to results.

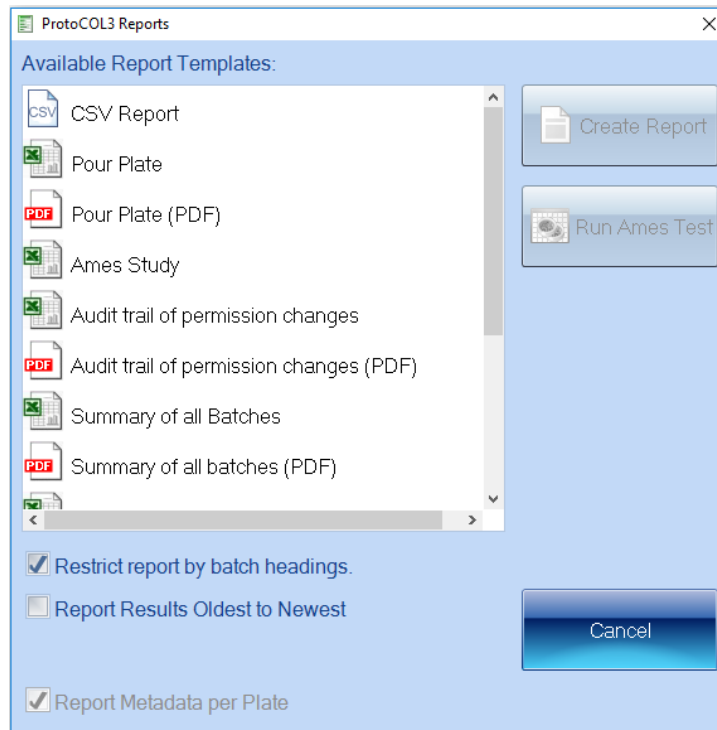
Compiling reports

To compile a results report:

1. Press



in the **Results** tab to display the **ProtoCOL 3 Reports** dialog box:



Note The Setup Statistical Analysis button only appears for installations that include the Unistat statistics module.

2. Click on the template for:

- **The current batch** (labeled by the batch type e.g. Pour Plate) to create a detailed report for the current batch
- **Summary of all Batches** to create a summary report covering all the batches in the ProtoCOL 3 database
- **Audit trail of all batches** to create an audit report covering all the batches in the ProtoCOL 3 database
- **Audit trail of the current batch** to create an audit report for the current batch.
- **Audit trail of logins** to create a report of login / logout activity.
- **Audit trail of permissions changes** to create a report which shows any changes made to user permission

3. To limit the information given for each result in the current batch report to the columns currently shown in the Results table (the default):

Leave **Restrict report by batch headings** checked.

To include all the data for each result, including columns not selected for the Results table (see *The Results tab*, page 131):

Click **Restrict report by batch headings** so that it is not checked.

Note The **Restrict report by batch headings** setting has no effect on summary or audit trail reports.

4. Check or uncheck **Report Results Oldest to Newest** to set the ordering of results in the report.
5. Press



Microsoft Excel will open with a worksheet containing the selected report.

Configuring ProtoCOL 3

This chapter shows ProtoCOL 3 administrators how to configure ProtoCOL 3 in a variety of ways. You will find out about:

- Displaying information about the program version you are using – see *About ProtoCOL 3*, below
- Using the **Settings** dialog box to configure the program – see *System settings*, on the next page
- Changing the color of the frame – see *Managing ProtoCOL 3 users*, page 184
- Setting the default inhibition zone diameters used to judge antibiotic sensitivity and resistance – see *Default Antibiotic Sensitivity*, page 182
- Selecting a scanner – see *Selecting a scanner*, page 182
- Setting the configuration for an external camera – see *Configuring an external camera*, page 183
- Setting up user accounts and permissions – see *Managing ProtoCOL 3 users*, page 184
- Configuring ProtoCOL 3 to work with a LIMS – see *LIMS connection properties*, page 191
- Specifying the location of the ProtoCOL 3 database on the PC or a network– see *Database location*, page 191
- Calibrating the instrument – see *Calibration*, page 192.

About ProtoCOL 3

To display information about the version of ProtoCOL 3 you are running:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Press



Configuring ProtoCOL 3

to display the **About ProtoCOL 3** dialog box:



3. Press **Save Details** to display a standard Windows **Save As** dialog box if you want to save the program version details in a text file.
4. Press **Close** to close the dialog box.

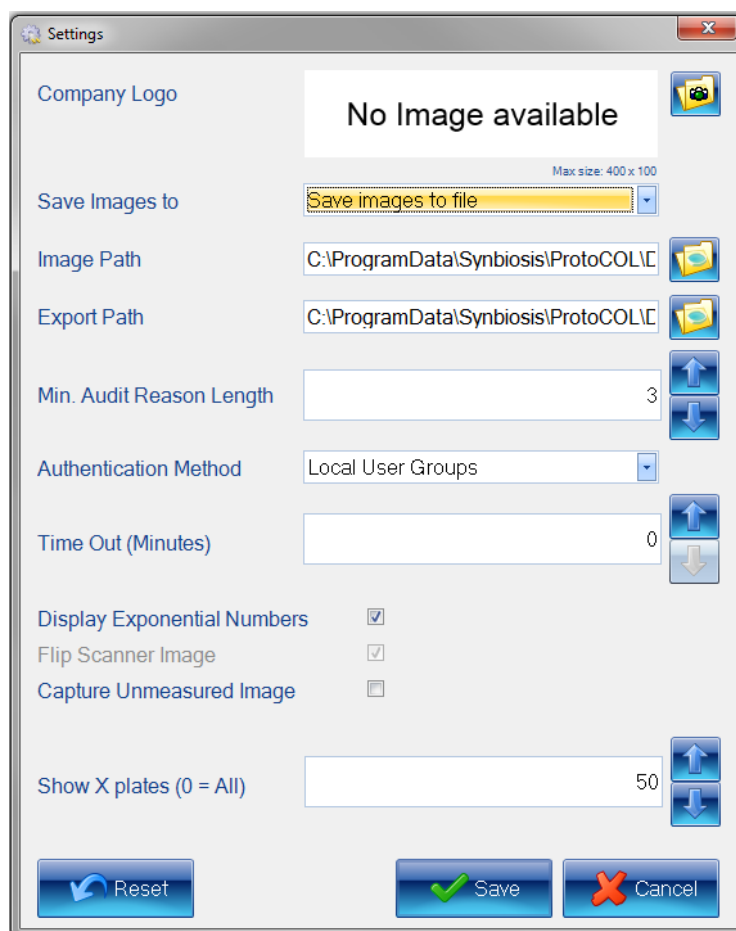
System settings

To view or change the system settings:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



- Choose **System Settings** from the **Settings** submenu to display the **Settings** dialog box:



- Enter the name of your organization under **Company Name**. The **Company Name** is included in reports – see *Compiling reports*, page 174.
- In the **Save Images to** box, choose whether to **Save Images to file** when you carry out a measurement; choose **Do not save images** if you do not want the images saved.

Note You will only be able to edit results after you have performed a measurement if the image is saved with the results – see *Editing plate measurements*, page 158, for details.

- Set the **Image Path** for the folder used to save images by typing the path directly into the box, or press



to display a standard Windows **Browse For Folder** dialog box so that you can select the required folder.

- Set the **Export Path** for the folder used to save exported batch details (see *Exporting batch details*, page 133) by typing the path directly into the box, or press



to display a standard Windows **Browse For Folder** dialog box so that you can select the required folder.

- In the **Min Audit reason length**, set the minimum number of characters for the text

entered by the user when giving the reason for a change (see *Saving changes to results*, page 162) – you can type directly into the edit box or use the arrow buttons to increase or decrease the number.

8. Select the **Authentication Method** you want to use – you can choose:

- None**
- Windows**
- Local User Groups**
- Network User Groups.**

See *Managing ProtoCOL 3 users*, page 184, for full details of what these mean and for how to set up Local and Network User Groups.

9. Set the **Time out** period – if an **Authentication Method** has been set (ie you did not set **None** in the previous step) and ProtoCOL 3 is not used for the set time out period, the current user will be logged out and the **Please log on to ProtoCOL 3** dialog box displayed so that the user, or another user, can log back in again. Set the **Time out** to **0** if you do not want to use this feature.
10. Check **Display Exponential Numb...** if you want to display results in exponential format.
11. Check **Flip Scanner Image** if you want scanned images flipped left to right. This setting corrects for the fact that plates are typically scanned from below and thus appear inverted.
12. Set a value for **Show X Plates** to choose how many plates to include in the Results table (see *The Results table*, page 157).
13. Press



to reset all settings to the values they had when you opened the dialog box.

14. Press



to confirm the new settings and close the dialog box.

To add a company logo to the PDF reports the maximum size should be 400x100. Adding this is done so using the Company Logo function in the System Settings.

Display properties

To set the color of the frame, frame border (see the paragraph following the instructions), zone marker or disc marker:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Choose **Frame Color**, **Frame Color (border)**, **Zone Marker Color (zone)** or **Zone Marker Color (disc)** from the **Settings** ▾ **Display Properties** submenu to display the Color palette, for example:



3. Either click on one of the **Standard Colors** in the palette to select it or press **More Colors** to display a color picker allowing you to choose from a wider range of **Standard** colors or to create your own **Custom** color.

Note The border color only applies to spiral frames. In particular, it is the color of the parts of the spiral that are not included in the two sectors of a two-sector frame, and it is the color of the inner boundary of an annular frame.

Default Antibiotic Sensitivity

The Default Antibiotic Sensitivity is only used when you use ProtoCOL 3 to measure zones.

To set the default boundaries for the measurements representing antibiotic sensitivity and resistance when you use ProtoCOL 3 to measure zones:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Choose **Edit Default Antibiotic Sensitivity** from the **Settings** submenu to display the **Default Antibiotic Susceptibility Values** dialog box:



3. Click in the **Resistant if <** box and set the diameter (in mm) such that smaller zones than this will be described as resistant – you can set the diameter by typing directly into the box or by clicking on the arrow buttons.
4. Click in the **Sensitive if >=** box and set the diameter (in mm) of the smallest zone diameter you want to describe as sensitive

The **Sensitive if >=** number should be larger than the **Resistant if <** number.

5. Press **OK** to confirm the new settings and close the dialog box.

Selecting a scanner

To select the required scanner if there is more than one TWAIN device attached to the PC:

1. Make sure a suitable TWAIN scanner has been installed on the PC and is currently connected to the PC and switched on.

2. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



3. Choose **Configure External TWAIN Source** from the **Settings** submenu to display the **Select Source** dialog box listing all the connected TWAIN devices.
4. Click on the required scanner in the list to select it.
5. Press **Select** to confirm the selection and close the dialog box.

Configuring an external camera

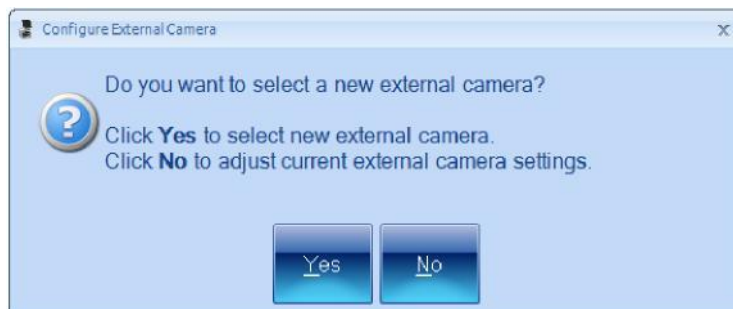
Note This topic is only relevant if you have purchased the option to use ProtoCOL 3 with an external camera.

To configure ProtoCOL 3 to work with an external camera:

1. Press the camera button at the top of the Image tab (see *The Image tab*, page 7) to display the source menu:



2. Choose **Configure External Camera** from the **Settings** submenu.
3. If you have already configured ProtoCOL 3 to work with an external camera, you will be asked if you want to select a new one (otherwise go to Step 4):



Press **Yes** if you want to create a new configuration; press **No** to abort the configuration procedure and continue to use the existing configuration.

4. The **Capture Configuration** dialog box will be displayed – see the separate documentation for using ProtoCOL 3 with an external camera for how to use this dialog box.

Managing ProtoCOL 3 users

You use the **Settings** dialog box (see *System settings*, page 178) to specify the **Authentication Method** used by ProtoCOL 3 to control access to the program. The options are:

- **None** – in this case there is no access control and:
 - anybody will be able to use ProtoCOL 3 without having to log on to the program
 - all users will have access to all ProtoCOL 3 functions
 - audit trails will record that changes were made by the user who was logged in to Windows at the time the change was made.

If you choose **None** as the **Authentication Method**, there is nothing more to do after you have made the selection.

- **Windows** – in this case:
 - users will have to log on to ProtoCOL 3 using their Windows user name and password (it does not have to be the user currently logged on to the PC, provided they have a user name and password to log on to that PC)
 - all users will have access to all ProtoCOL 3 functions
 - audit trails will record that changes were made by the user who was logged in to ProtoCOL 3 at the time the changes were made.

If you choose **Windows** as the **Authentication Method**, you will need to add user names and passwords to Windows for all the users who are going to use the ProtoCOL 3 installation – see *Windows password security*, on the facing page.

- **Local User Groups** – In this case:
 - users must be members of a ProtoCOL local user group on the PC
 - users will have to log on to ProtoCOL 3 using their Windows user name and password (it does not have to be the user currently logged on to the PC, provided they are able to log on to that PC)
 - the members of different ProtoCOL groups can be given different access rights to some ProtoCOL 3 functions
 - users may be required to enter reasons for changes they make to batches or results
 - audit trails will record which ProtoCOL user made any changes.

If you choose **Local User Groups**, you will need to:

- add Windows user names and passwords for all the users who are going to use the ProtoCOL 3 installation – see *Windows password security*, on the facing page
- define the user groups on the PC – see *ProtoCOL 3 user groups*, on the facing page
- define what permissions each user group should have – see *User permissions*, page 189.

- **Network User Groups** – this is the same as the previous option, except the user groups are defined at the Network level – see the same set up references as for **Local User Groups**.

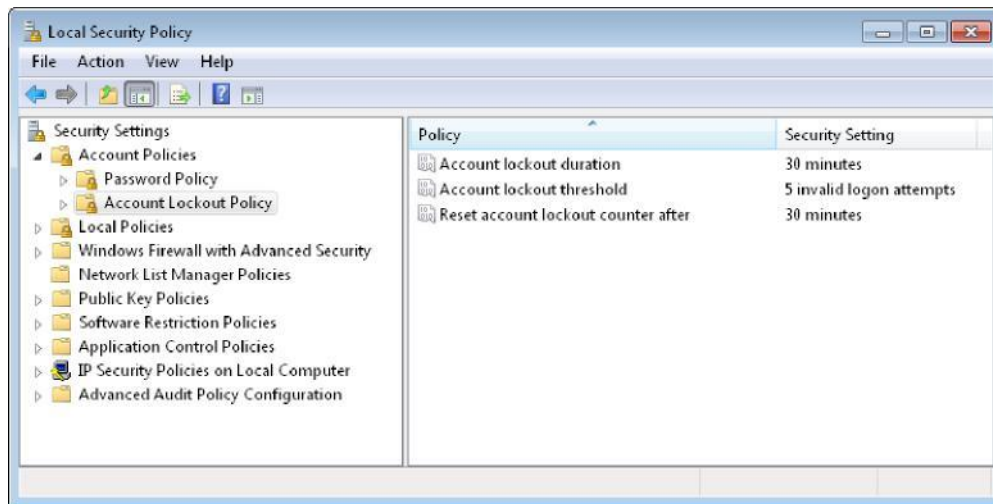
For details of how to view audit trails, see *Viewing the audit history of changes*, page 163.

Windows password security

Unless you choose to allow completely free access to the program (**Authentication Method** set to **None** in the **Settings** dialog box – see *System settings*, page 178), ProtoCOL 3 uses Windows password authentication to control access. This means that in order to log on to ProtoCOL 3, the user must enter a valid Windows user name and password for the PC – note that it does not have to be the user name and password of the currently logged on Windows user.

The ProtoCOL 3 PC is supplied from the factory with the single user, P3Admin, and the default password for this user is the serial number of the instrument, for example, P3\xxxx (note that these are case sensitive). This user has administrator privileges for both the PC and ProtoCOL 3 (see *Local groups*, on the next page, for more on the different types of ProtoCOL 3 user groups). The PC administrator will have to add user names and passwords for any other users required to use ProtoCOL 3 – see the Windows documentation for how to add Windows users.

The fact that ProtoCOL 3 uses Windows password authentication also means that the password security policy is determined by Windows, and can be set by the Windows administrator. For example, for a stand-alone ProtoCOL 3 system, running secpol.msc displays the **Local Security Settings** window:



These settings determine the password policy for ProtoCOL 3 on the PC, so, for example, setting the **Account lockout threshold** to **5 invalid logon attempts** would mean that if the user made five invalid log on attempts, nobody will be able to log on to ProtoCOL 3 on the PC during the **Account lockout duration**.

ProtoCOL 3 user groups

If the **Authentication Method** is set to **Local User Groups** or **Network User Groups** in the **Settings** dialog box (see *System settings*, page 178), in order to gain access to ProtoCOL 3, a user must:

- a. have a valid user name and password to log on to Windows on the ProtoCOL 3 PC – see *Windows password security*, above, for further details

- b. be a member of a ProtoCOL local or network user group – see the next section, *Local and Domain/Security groups* for details.

Local and Domain/Security groups

There are three ProtoCOL 3 user groups: ProtoCOL User, ProtoCOL Advanced User and ProtoCOL Admins.

You can set up ProtoCOL 3 user groups using:

- Local groups** on each ProtoCOL 3 PC.

Generally speaking, you would choose to use Local groups if you have one or more independent ProtoCOL 3 installations. You will need to set up the user groups separately on each of the ProtoCOL 3 PCs – see the next section, *Local groups*.

Or:

- Domain/Security groups** defined across a Windows Server domain.

If the ProtoCOL 3 PCs are connected to a network arranged in a Windows Server domain, you can use Windows Domain/Security groups to define the user groups. In this case the user groups are set up centrally on a domain controller rather than on the individual ProtoCOL 3 PCs – see *Domain/Security groups*, page 189.

When the ProtoCOL 3 PCs are arranged in a Windows Server domain network, it is also usually convenient for them all to be connected to a central ProtoCOL 3 database server rather than having separate independent databases on each PC – see *Database location*, page 191, for how to link a ProtoCOL 3 installation to a central database.

The following sections show you how to set up and use *Local groups* and *Domain/Security groups*, page 189.

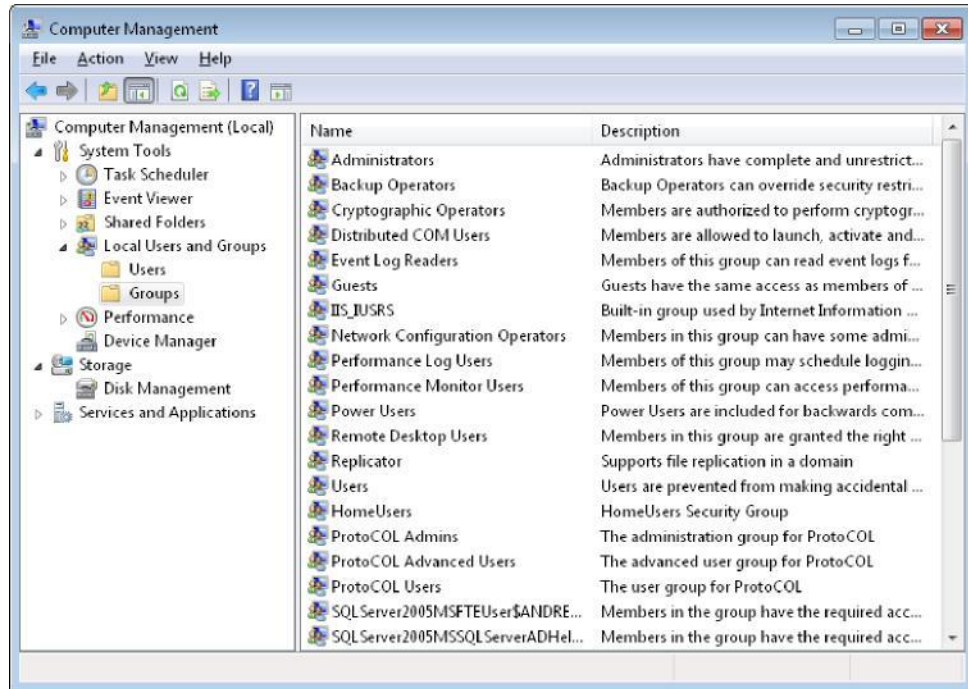
Local groups

You need to set up Local groups separately on each ProtoCOL 3 PC – see *Domain/Security groups*, page 189, for how to set up Domain/Security groups for use across a network.

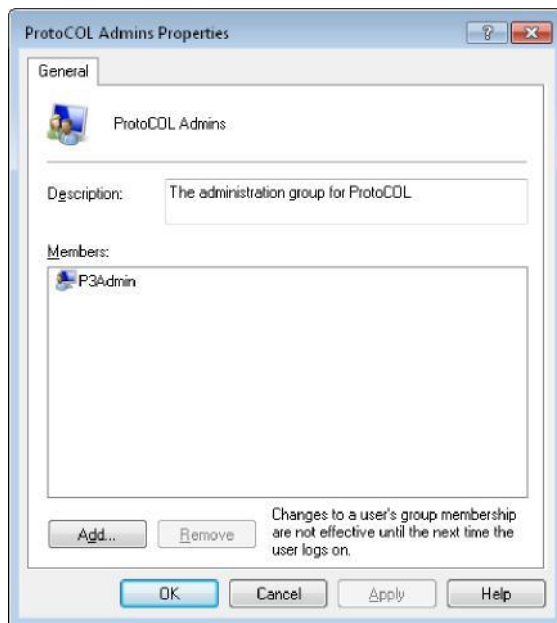
To set up Local user groups:

1. Open the Windows Control Panel.
2. Open the **Administrative Tools**.

3. Open **Computer Management**, expand **Local Users and Groups** and select **Groups**:



- 4. To add users to a group:
 - a. Right-click on the required group in the right-hand pane to display a context menu.
 - b. Choose **Add to Group** to display the **Group Properties** dialog box:



The example shows the **ProtoCOL Admins** group, which contains the **P3Admin** user by default. This means that the ProtoCOL 3 administrator can use the instrument with full permissions from the start. You can add other users to the ProtoCOL Admins group if required.

Configuring ProtoCOL 3

Note By default, members of the **ProtoCOL Admins** group have the full set of permissions for all ProtoCOL 3 functions. However, this is not required as ProtoCOL 3 administrators can restrict their own permissions – see *User permissions*, on the facing page.

c. Press **Add** to display the **Select Users** dialog box:

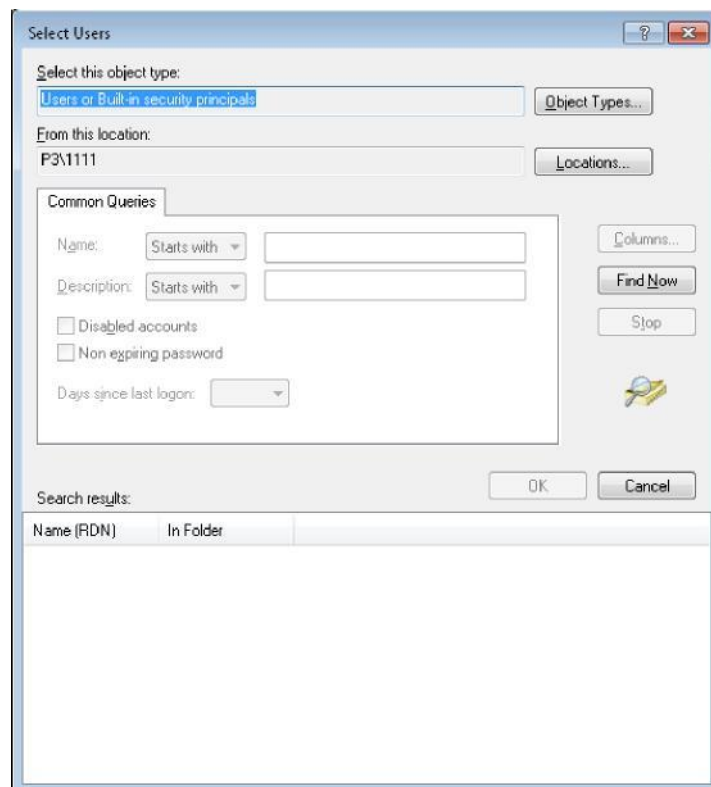


Either:

- i. Type the user name into the box; if required, you can enter multiple user names by typing them on the same line but separated by semi-colons.
- ii. Press **Check Names** to check that the user names are valid Windows log on user names – if a user name is not found, and you entered it correctly, you will need to create a new user account with this user name.

Or:

- i. Press **Advanced** to expand the **Select Users** dialog box:



- ii. Press **Find Now** to list the Windows users for this PC.
- iii. Select the required users to add to the group.

- iv. Press **OK** to close the dialog box and add the users to the box in the simple **Select Users** dialog box.
- d. Press **OK** to close the **Select Users** dialog box and add the users to the group in the **Properties** dialog box.

Note If required, you can also use the **Properties** dialog box to remove users from the group by selecting them and pressing **Remove**.

- e. Press **OK** to close the **Properties** dialog box.

The link between the Windows Local groups and ProtoCOL 3 permissions is defined in the **Edit User Permissions** dialog box – see *User permissions*, below, for details.

Domain/Security groups

The principles and procedures for creating and populating ProtoCOL 3 Domain/Security groups are similar to those described for *Local groups*, page 186, but using the Active Directory Users and Computers console instead of the Computer Management application.

The link between the Windows Domain/Security groups and ProtoCOL 3 permissions is defined in the **Edit User Permissions** dialog box – see *User permissions*, below, for details.

User permissions

If you are using **Local User Groups** or **Network User Groups** as the **Authentication Method** for ProtoCOL 3 (see *Managing ProtoCOL 3 users*, page 184), you can assign different permissions to users for some ProtoCOL 3 operations according to the type of user group they belong to (see *Local groups*, page 186, and *Domain/Security groups*, above, for how to assign users to user groups).

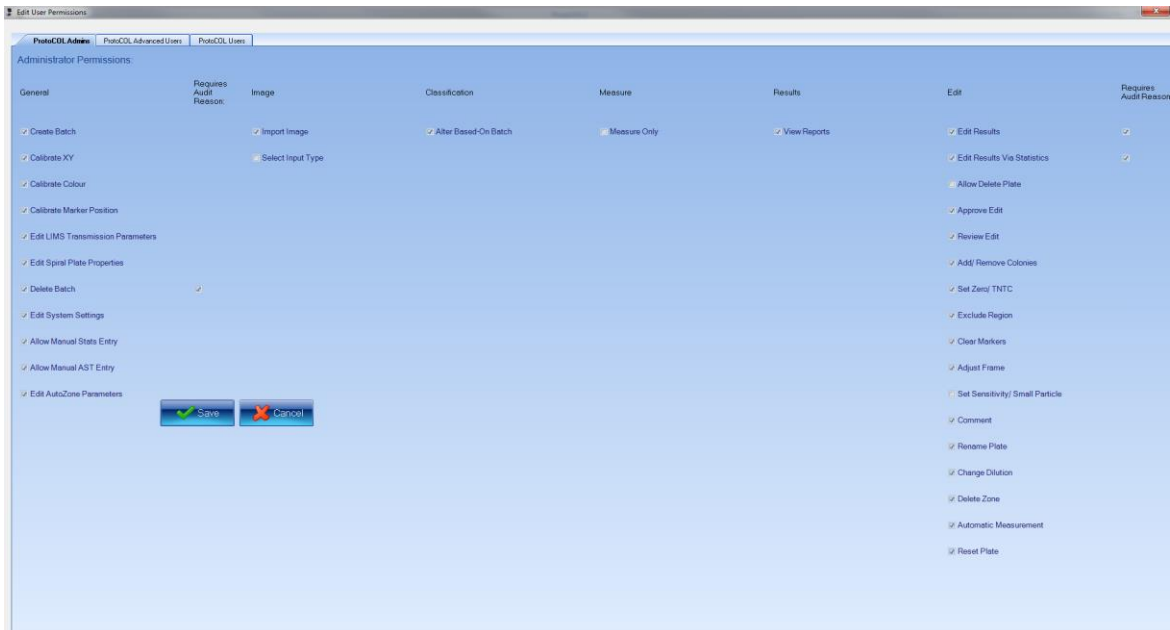
To set the permissions for different types of user group:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



Configuring ProtoCOL 3

2. Choose **Edit User Permissions** from the **Settings** submenu to display the **Edit User Permissions** dialog box:



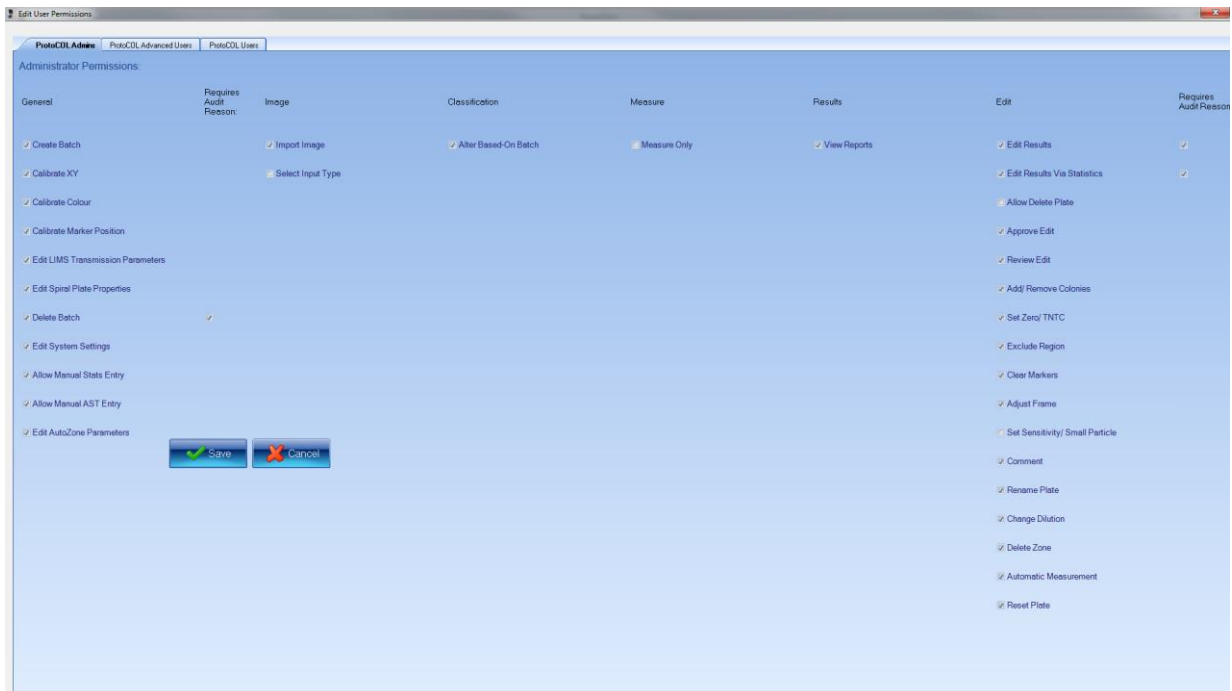
The dialog box has separate tabs for each of the three classes of ProtoCOL 3 users: ProtoCOL Administrators; ProtoCOL Advanced Users; and ProtoCOL Users. The contents are identical, except that the **Requires Audit Reason** check boxes only appear on the **ProtoCOL Admins** tab.

3. On the **ProtoCOL Admins** tab, check the **Requires Audit Reason** check boxes if you want users to be required to enter a reason for carrying out that change – a dialog box will be displayed if they make the change and they will not be able to proceed unless they enter a reason (see *Saving changes to results*, page 162; see also *System settings*, page 178, for the **Min Audit reason length** setting).
4. On each tab, check the **Permissions** check box for those operations you wish to allow that type of user group to carry out.
5. Press



to confirm the settings and close the dialog box.

Permissions



The administrator of the software can assign differing permissions to each user group using the software, this can help avoid groups of users performing unwanted actions of the system.

The permissions are organized into groups; General, Image, Classification, Measure, Results and Edit, these groups indicate the action they would affect. Below is a list of permissions. If the box is ticked then that represents that user groups have the ability to perform that action and unticked means that action is not permitted.

General

Create Batch: The 'Create Batch' permission allows a user group the permission to create new batches.

If disabled, the user group will no longer be able to create new batches and can only use existing batches previously created in the software.

This is typically disabled when consistent batch settings are required or optimal batch settings have been achieved.

Calibrate XY: The 'Calibrate XY' permission allows a user group to calibrate the measurements performed on the device. This is generally performed when the device is manufactured but, if required again, then this should only be performed by an experienced operator due to its potential to invalidate all device measurements.

If disabled, the user group will not be able to adjust the XY calibration.

Calibrate Colour: The 'Calibrate Colour' permission allows a user group to calibrate the colour balance of a device. This is performed when the device is manufactured and requires a special plate holder to function. This should only be performed by an experienced operator.

If disabled, the user group will not be able to perform colour calibrations.

Calibrate Marker Position: The 'Calibrate Marker Position' permission allows a user group to adjust colour balance marker positions. This is performed when the device is manufactured and should not require adjustment unless you are experiencing technical problems with the device. This should only be performed by an experienced operator.

If disabled, the user group will not be able to calibrate the marker positions.

Edit LIMS Transmission Parameters: The 'Edit LIMS Transmission Parameters' permission allows a user group to adjust the LIMS connection parameters in the ProtoCOL menu.

If disabled, the user group will not be able to access this menu.

Edit Spiral Plate Properties: The 'Edit Spiral Plate Properties' permission can be found in the main ProtoCOL menu, this option allows user to adjust and edit the properties of spiral plates.

If disabled, the user group will not be able to access this menu.

Delete Batch: The 'Delete Batch' permission allows a user group the ability to delete previously created batches from the software; performing this function requires the user to enter an audit reason and the option will also be given to remove the images captured during the batch measurements. This function will remove all measurements obtained and once executed, the data cannot be recovered.

Disabling this setting will prevent user groups from deleting batches.

Edit System Settings: The 'Edit System Settings' permission gives users the permission to adjust system settings. These settings are advanced configuration settings and are generally restricted to administrator level only as they can dramatically change the way the software operates and security authentication method.

Disabling this will prevent a user group from making changes to system settings.

Allow Manual Stats Entry: The 'Allow Manual Stats Entry' permission allows a user group to add manual entries during the set-up of statistical analysis.

If disabled, manual entries will not be allowed.

Allow Manual AST Entry: The 'Allow Manual AST Entry' permission allows a user group to add manual AST entry during an AST batch.

If disabled, manual entries will not be permitted during AST batches.

Edit AutoZone Parameters: The 'Edit AutoZone Parameters' permission allows a user group to adjust automatic measurement parameters of automatic inhibition zone set-up.

Disabling this will prevent changes to the automatic zone classification parameters.

Image

Import Image: The 'import image' permission gives the user group the ability to import either a previously captured image or an external image into the software for analysis.

Disabling this will prevent the user group from importing images into the software.

Select Input Type: The 'Select Input Type' permission gives the user group the ability to change the device used for image capture. The standard input will be the camera within the ProtoCOL device but if using either the ProcScan or an external camera, this can be changed to suit this function.

Disabling this will prevent user groups from changing the default input type.

Classification

Alter Based-On Batch: The 'Alter Based-On Batch' permission gives the user group the ability to adjust batch setting when creating a new batch based on a previous batch.

If disabled, the user group will not be able to adjust the settings of new batches when they are based on existing batches.

Measure

Measure Only: When ticked the 'Measure Only' permission will restrict all options on the measure tab apart from the measure button. This is typically used when consistent batch settings are required or optimal batch settings have already been achieved.

If disabled, all options will be available on the 'measure' tab for that user group.

Results

View Reports: The 'View Reports' permission will allow a user group to view the batch reports and audit reports for batches.

If disabled, the user group will not be able to save or view batch or audit reports in the results tab.

Edit

Edit Results: The 'Edit Results' permission gives user groups the ability to make manual adjustments to plates that have been measured in the edit/review screen.

If disabled, the user group will not be able to make any adjustments to measured plates within the edit/review screen.

Allow Delete Plate: The 'Allow Delete Plate' permission allows plates to be deleted in the edit/review screen.

If disabled, the user group will be unable to delete plates in the edit/review tab.

Approve Edit: The 'Approve Edit' permission gives the user group the permission to approve plates in the edit/review screen.

When enabled, the Save Changes button will become a dropdown box with the option to 'Save Approve'. This requires an audit reason and once saved a flag will be added to the batch to show it has been approved.

If disabled, the standard save option will only be visible.

Review Edit: The 'Review Edit' permission gives the user group the permission to review plates in the edit/review screen.

When enabled, the Save Changes button will become a dropdown box with the option to 'Save Reviewed'. This requires an audit reason and once saved a flag will be added to the batch to show it has been reviewed.

If disabled, the standard Save option will only be visible.

Add/Remove Colonies: The 'Add/Remove Colonies' permission allows user groups to add additional or remove excess colonies in the edit/review tab.

If disabled, the user group will not be able to add/remove colonies from measured plates.

Set Zero/TNTC: The 'Set Zero/TNTC' permission allows a user group to set a measured plate to either zero or TNTC in the edit/review tab.

If disabled, the user group will not be able to set plates to zero or TNTC in the edit/review tab.

Exclude Region: The 'Exclude Region' permission allows user groups to set an excluded region in the edit/review screen.

If disabled, the user group will no longer be able to create an excluded region in the edit/review screen, but will still be able to set an excluded region in the colour classification set-up.

Clear Markers: The 'Clear Markers' permission allows the user group to clear all colony markers on the measured plate in the edit/review tab.

If disabled, the user group will not be able to clear the markers.

Adjust Frame: The 'Adjust Frame' permission gives a user group the ability to adjust the size and position of the graticule in the edit/review tab.

If disabled, the user group will not be able to adjust the size or position of the graticule in the edit/review tab.

Set Sensitivity/Small Particle: The 'Set Sensitivity/Small Particle' permission allows the user group to adjust the sensitivity and the reject small particle limit on the edit/review tab of a measured plate.

If disabled, the user group will not be able to adjust these settings.

Comment: The 'Comment' permission allows comments to be added to measured plates within the edit/review tab.

If disabled, the user group will not be able to add comments to plates in the edit/review tab.

Rename Plate: The 'Rename Plate' permission allows the name of a measured plate to be changed in the edit/review tab.

If disabled, the user group will not be able to change the name of a measured plate in the edit/review tab.

Change Dilution: The 'Change Dilution' permission allows a user group to change the dilution of a measured plate.

If disabled, the user group will not be able to change the dilution of a measured plate.

Delete Zone: The 'Delete Zone' permission gives a user the permission to delete a zone in the edit/review tab during an inhibition zone batch. The zone measurement will be recorded as zero and an audit reason will be required when saving the changes.

If disabled, zones cannot be deleted.

Automatic Measurement: The 'Automatic Measurement' permission allows a user group to perform an automatic measurement in the edit/review tab whilst in an inhibition zone batch, this measurement is of a previously measured plate.

If disabled, the user group will not be able to perform the automatic measurement in the edit/review tab.

Reset Plate: The 'Reset Plate' permission allows a user group to reset a plate back to the automatic count value in the edit/review screen, this option is only available once changes have already been made to an automatic count.

If disabled, the user group will no longer be able to reset a plate in the edit/review tab.

LIMS connection properties

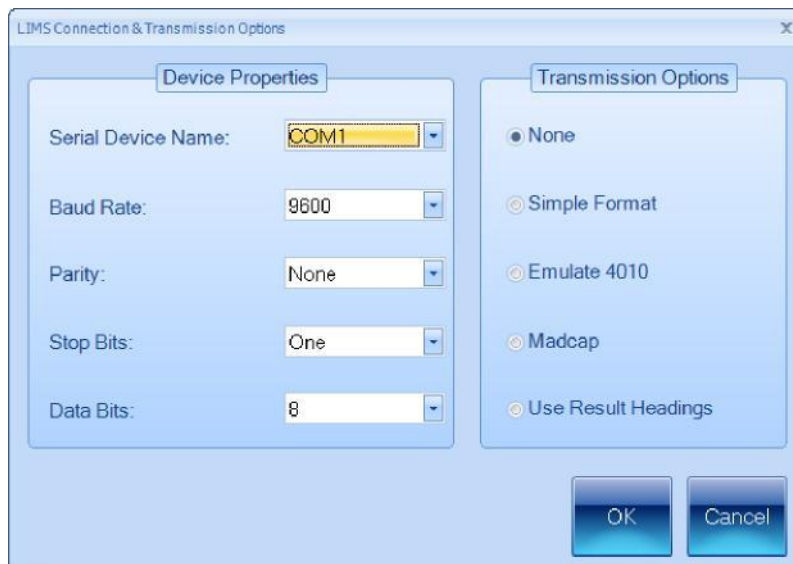
You can connect ProtoCOL 3 to a LIMS system holding the names and dilutions of the plates in a batch – see *Linking to a LIMS*, page 116.

To configure ProtoCOL 3 to work with a LIMS system:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Choose **LIMS Connection Properties** from the **Settings** submenu to display the **LIMS Connection & Transmission Options** dialog box:



3. Consult the documentation for the LIMS system you are using and select the appropriate settings in the dialog box.
4. Press **OK** to confirm the settings and close the dialog box.

Database location

If you are using several ProtoCOL 3 instruments connected across a network, they can share a single ProtoCOL 3 database. In order to do this, you will need to specify the location of the shared database in each of the ProtoCOL 3 installations. Alternatively, you may have multiple databases on the local database server on your PC, in which case, you can use the following procedure to specify which one to use.

Configuring ProtoCOL 3

To specify the location of the ProtoCOL 3 database:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:

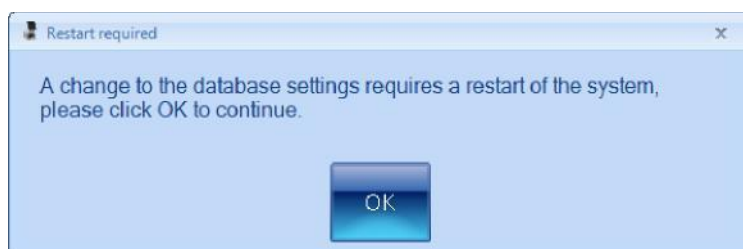


2. Choose **Edit Database location** from the **Settings** submenu to display the **Create ProtoCOL 3 Database Connection** dialog box:



3. Enter the names of the ProtoCOL 3 **Database** and **Server**.
4. Press **Connect**.

A dialog box will be displayed warning you that ProtoCOL 3 will restart:



5. Press **OK**.

ProtoCOL 3 will close down and restart automatically and be connected to the new database location.

Calibration

ProtoCOL 3 is fully calibrated in the factory and in normal circumstances will require no further calibration after delivery. However, in exceptional circumstances you may need to carry out some recalibration. For details, see:

- Color balance*, on the facing page
- Marker positions*, page 194
- Camera measurements*, page 196.

Color balance

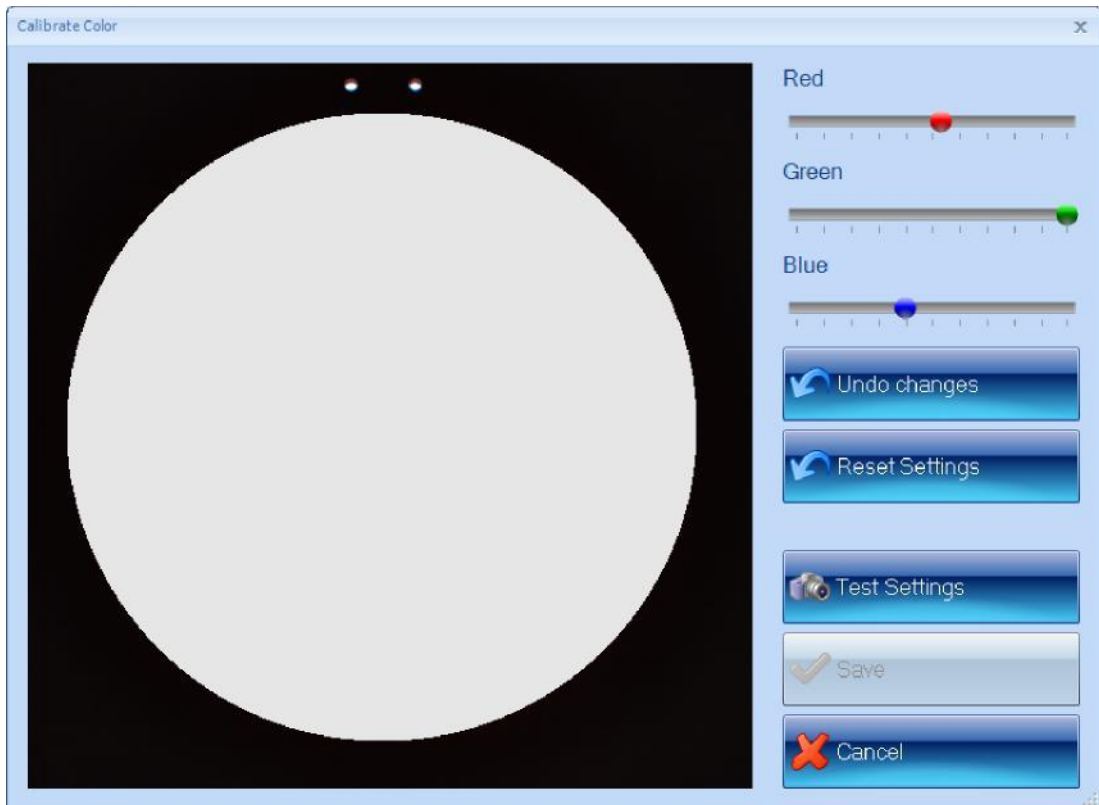
ProtoCOL 3 is fully calibrated in the factory and in normal circumstances will require no further calibration after delivery. However, in exceptional circumstances you may need to recalibrate the color balance.

To recalibrate the color balance:

1. Insert a neutral test sheet into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
2. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



3. Choose **Calibrate Color Balance** from the **Settings** ▾ **Calibration** submenu to display the **Calibrate Color** dialog box:



4. Compare the color of the test sheet image on the screen with the actual color of the test sheet.
5. If the image has a color cast compared with the test sheet, adjust the color sliders

accordingly. For example, if the image is too yellow, increase the **Blue** setting, or decrease the **Red** and **Green** settings.

The image will not reflect the new settings yet – see the next step. 6.

Press



to check the effect of the changes.

7. Repeat Steps 4–6 until the color balance of the image matches the color balance of the test card.
8. Press



to save the new color balance settings and close the dialog box. If required, during the above procedure:

To undo all the changes you have made since you opened the dialog box:

Press



To reset the settings to the values set in the factory:

Press



Marker positions

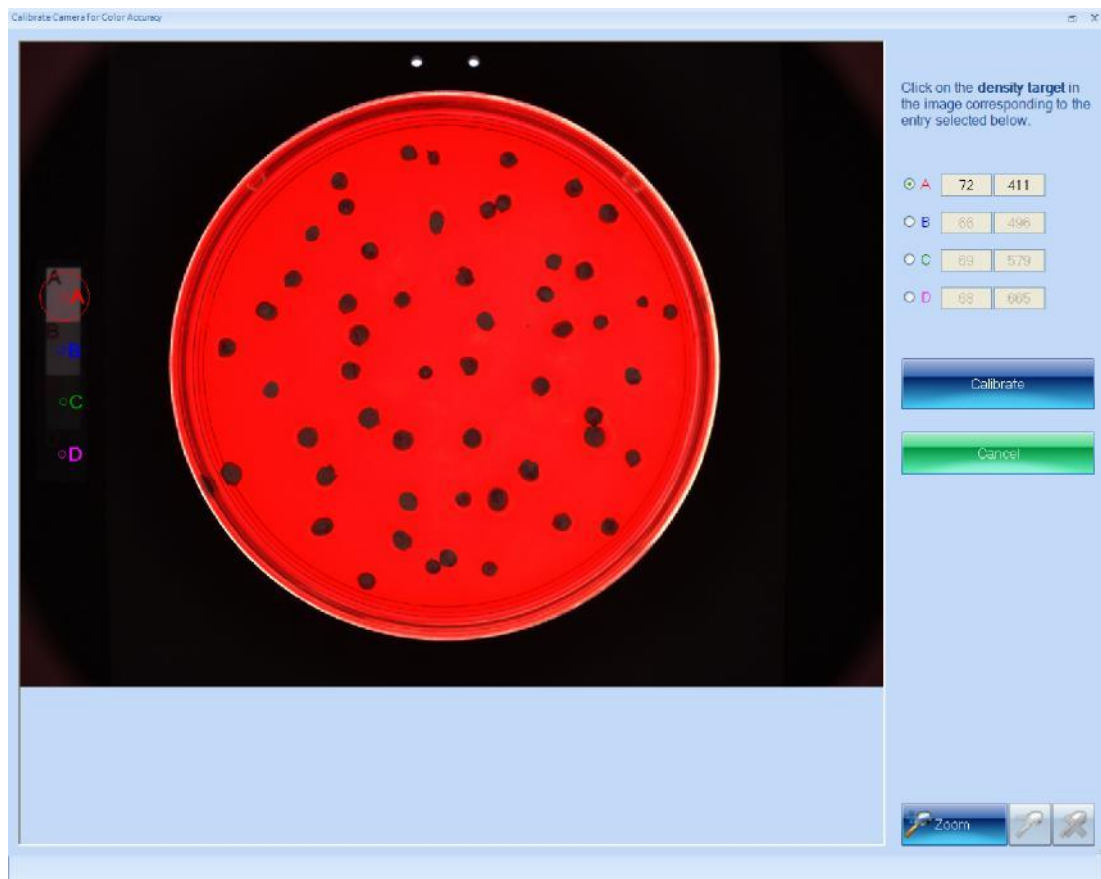
Each time you capture an image in ProtoCOL 3, the instrument carries out an automatic recalibration from a grayscale on the instrument stage. In order to do this, it must have an accurate record of the positions of the gray patches. ProtoCOL 3 is fully calibrated in the factory and in normal circumstances will require no further calibration after delivery. However, in exceptional circumstances you may need to recalibrate the marker positions over the gray patches.

To recalibrate the marker positions:

1. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



2. Choose **Calibrate Marker Positions** from the **Settings** > **Calibration** submenu to display the **Calibrate Camera for Color Accuracy** screen:



The **A B C** and **D** markers on the left-hand side of the screen should lie at the center of each of the gray patches, with **A** on the top patch and **D** on the bottom patch.

3. If any of the markers are out of position, press the radio button at the top right-hand corner of the screen with the corresponding letter to select it. A circle will appear around the marker showing it is selected – for example, marker **A** is selected in the picture.
4. Drag the marker until it is at the center of its gray patch.
5. Repeat Steps 3 and 4 for any other markers that are out of position.

6. Press



to save the new marker positions and return to the main ProtoCOL 3 screen.

Camera measurements

ProtoCOL 3 is fully calibrated in the factory and in normal circumstances will require no further calibration after delivery. However, in exceptional circumstances you may need to recalibrate the camera measurements, which enable the instrument to measure distances on the image accurately.

To recalibrate the camera measurements:

1. Insert an object of known size into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
2. Press the ProtoCOL 3 button at the top left-hand corner of the window to display the ProtoCOL 3 menu:



3. Choose **Calibrate Camera Measurements** from the **Settings** ▾ **Calibration** submenu to display the **Calibrate Camera for Measurements** dialog box.

This dialog box is also displayed when you calibrate an imported image, and is used in exactly the same way – see *Calibrating the image*, page 13, for detailed instructions.

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Appendix 1:

Ames module application notes:

Ames Module

The Ames module has two levels of functionality. The *Simple Ames* module allows the user to measure plates with the same name and find the difference in colony numbers to assess the level of mutagenicity. The *Ames Study Manager* is a more in-depth report focused module that allows the user to input all the relevant experimental information before calculating the ratio of revertant colonies on test sample and solvent control plates.

The general procedure for counting colonies using the Simple Ames module is:

1. Open a *Simple Ames* batch – see section, *Opening and selecting batches*.
2. Load the plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
3. Capture an image – see *Capturing an image*, page 10.
4. Adjust the measurement frame(s) if required – see *Adjusting frame settings in Measurement mode*, page 141.
5. Classify the colonies – see *Classification – colony counting batches* page 30.
6. Name and accept the batch to begin measuring your plates.
7. To compare colony numbers on plates, enter or select the same plate identifier as you did for the first measurement – see *Entering plate identifiers and dilutions*, on page 148.
8. This will calculate the difference between the first and second measurements, i.e. the increase / decrease in colonies present.

The general procedure for the Ames Study Manager module is:

1. Open an *Ames Study Manager* batch – see section, *Opening and selecting batches*.
2. Load the plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
3. Capture an image – see *Capturing an image*, page 10.
4. Adjust the measurement frame(s) if required – see *Adjusting frame settings in Measurement mode*, page 141.
5. Classify the colonies – see *Classification – colony counting batches* page 30.
6. Name and accept the batch to begin measuring your plates.
7. Measure all the plate required for the study.
8. In the Results tab click on *Create Report*, select *Ames Study* and *Run Ames Test*.
9. Select the plates you wish to include in the study from the list and press OK.
10. To *Create a New Assay*, select the number of replicas used and whether or not metabolic activation was included (including metabolic activation will generate a double report format with each report side-by-side in the spreadsheet).
11. The Report Headings window allows you to input additional information about the study but can also be left blank.
12. The *Ames wizard* table will then open allowing you to input details such as the bacterial strain used, positive and negative controls, doses and replica numbers.

13. Once the table has been completed, it can be saved as a *Template* so that studies can be based on it in the future. Ames Templates are usually saved in the ProtoCOL 3 database export folder but this can be changed if desired.
14. Press Finish to generate the Ames Report, which will open in Excel of Open Office. This report is locked for compliance purposes.
15. To base an Ames experiment on an existing template that was previously saved, follow steps 1-9 and then click on *base on saved assay template* in the opening window.
16. A pop-up window will open allowing you to choose a template. Ames Templates are usually saved in the ProtoCOL 3 database export folder but this can be changed if desired.
17. Select the template you wish to use and press *Open*. If the number of plates in the template does not match the number of plates selected, a pop-up warning will appear. In this case, the template can still be used but some of the information might need to be filled-in manually to ensure it is correct.

Appendix 2:

Antibiotic Susceptibility application notes

AST Batch

The AST module can be used to measure inhibition zones and compare the resulting data to the most up-to-date EUCAST and CLSI guidelines. Breakpoint values can now also be entered manually, allowing researchers to effectively study incidence of antibiotic resistance.

The general procedure for measuring zones using the AST module is:

1. Open an *Antibiotic Susceptibility* batch – see section, *Opening and selecting batches*.
2. Load the plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
3. Capture an image – see *Capturing an image*, page 10.
4. Classify the zones – see *Classification – zone measurement batches* page 79.
5. In the classification window, select *Allow Breakpoints and Expert rules* and select whether EUCAST, CLSI or Manual breakpoint values are being used. Manual breakpoints can be added to EUCAST and CLSI batches but Manual batches are designed for manual breakpoints only.
6. *Test measure* the plate to enable the *Accept New Batch* button.
7. When *Measure Plate* is selected, the AST wizard will open. Select the breakpoint organism / group you are testing and press next.
8. Input the required information into the table (EUCAST/CLSI doses and breakpoint values will be set but manual entries can be made here by selecting *Add Manual Data*.
9. When entering manual data, make sure to *Apply Changes* in order for the information to be saved in the database. These entries can be deleted at a later stage.
10. In the next window, select whether or not *Expert Rules* should be applied. If yes, then select the most suitable breakpoint organism. Press *Finish*.
11. The results tab will now display the AST results for that plate. If *Apply settings to all plates in batch* was selected in the first window (step 8) then steps 9-11 will be skipped and the same settings will apply to every plate in the batch.

Appendix 3:

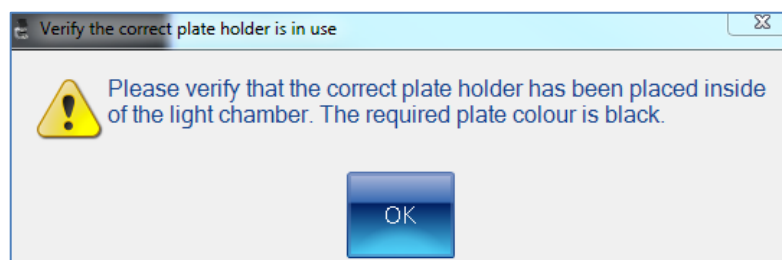
Chromogenic module information:

Chromogenic Batch

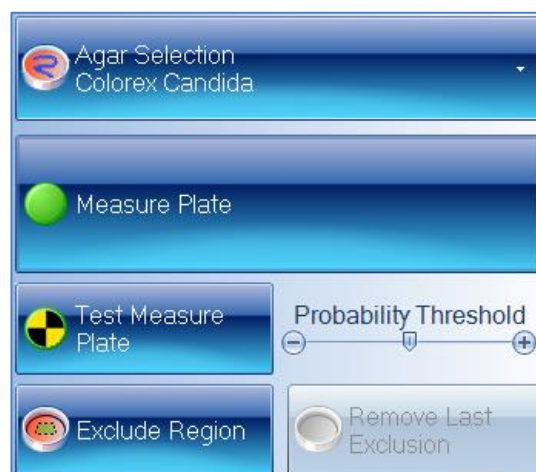
The Chromogenic Media module can be used to identify microorganisms using colour recognition software. Colonies on chromogenic agar are analysed and compared against colours from a comprehensive library of reference images to provide a detected / not detected result.

The general procedure for the Chromogenic Media module is:

1. Open a *Chromogenic Media* batch – see section, *Opening and selecting batches*.
2. Load the plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9. The black plate holder must be used for Chromogenic Media batches for colour reference purposes (a warning window will pop up as a reminder before accepting the batch).



3. Capture an image – see *Capturing an image*, page 10.
4. Adjust the measurement frame(s) in the Classification tab if required – see *Adjusting frame settings in Measurement mode*, page 141.
5. Assign a name for the batch and the first plate (or configure plate names using the Configuration tab).
6. *Accept New Batch* button to begin taking measurements.
7. Select a supplier and agar type from the drop down menu. This can be changed from plate to plate allowing a variety of agar types to be analysed.



8. *Test Measure* plate will provide a preview of the result but will not record the result.
9. Press *Measure* to analyse the plate and save the result in the database.

10. The results table at the bottom of the screen will now display the Chromogenic results for that plate.

Plate Name	Agar Name	User	Flags	Created	Comments /...
1	Colorex Candida	shauna		11/09/2018 10:17:44	
	Microorganism...	Colour		Detected	
	C. albicans	Green		Detected	
	C. glabrata	Purple		Detected	
	C. krusei	Pale Pink		Not Detected	
	C. tropicalis	Blue		Detected	

11. The *Exclude Region* function can be used to exclude a part of the plate from the analysis. This will be flagged in the results report and audit report. Pressing the *Remove Last Exclusion* button will remove the exclusion, allowing the entire plate to be analysed.
12. The results tab allows results headings to be customised. The results report can be opened in Excel or Open Office. The report can also be opened in PDF format (max 10 headings).

Create Report

- Plate Name
- Agar Name
- Microorganism Name
- Colour
- Detected
- User
- Flags
- Created
- Comments / Notes

OPKA Module

The OPKA software module allows users to analyse OPK assay plates in a variety of formats from single frame to 12 x 12 grids. The general procedure for counting colonies using the OPKA module is:

9. Open an *OPKA* batch – see section, *Opening and selecting batches*.
10. Load the plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
11. Capture an image – see *Capturing an image*, page 10.
12. Adjust the measurement frame(s). OPKA plate frames will usually be rectangular with a grid of rectangular sub-frames. See classifying frame settings for OPKA batches, page 33 - 38.
13. Classify the colonies – see *Classification – colony counting batches* page 30.
14. Name and accept the batch to begin measuring your plates.
15. Colony count results will be given for each sector of the OPKA plate.

SBA Module

The SBA software module allows users to analyse serum bactericidal assay plates in a variety of formats from single frame to 12 x 12 grids. The general procedure for counting colonies using the SBA module is:

1. Open an SBA batch – see section, *Opening and selecting batches*.
2. Load the plate into ProtoCOL 3 – see *Loading plates into the instrument*, page 9.
3. Capture an image – see *Capturing an image*, page 10.
4. Adjust the measurement frame(s). SBA plate frames will usually be rectangular with a grid of rectangular sub-frames. See classifying frame settings for SBA batches, page 33 - 38.
5. Classify the colonies – see *Classification – colony counting batches* page 30.
6. Name and accept the batch to begin measuring your plates.
7. Colony count results will be given for each sector of the SBA plate.

Plate Name	User	Flags	Created	Comments /...
1	shauna		11/09/2018 10:30:36	
Sector 1				
Colony Na...				Coun...
A				76
Sector 2				
Colony Na...				Coun...
A				62
Sector 3				
Colony Na...				Coun...
A				60

Appendix 5. Review/Approve feature

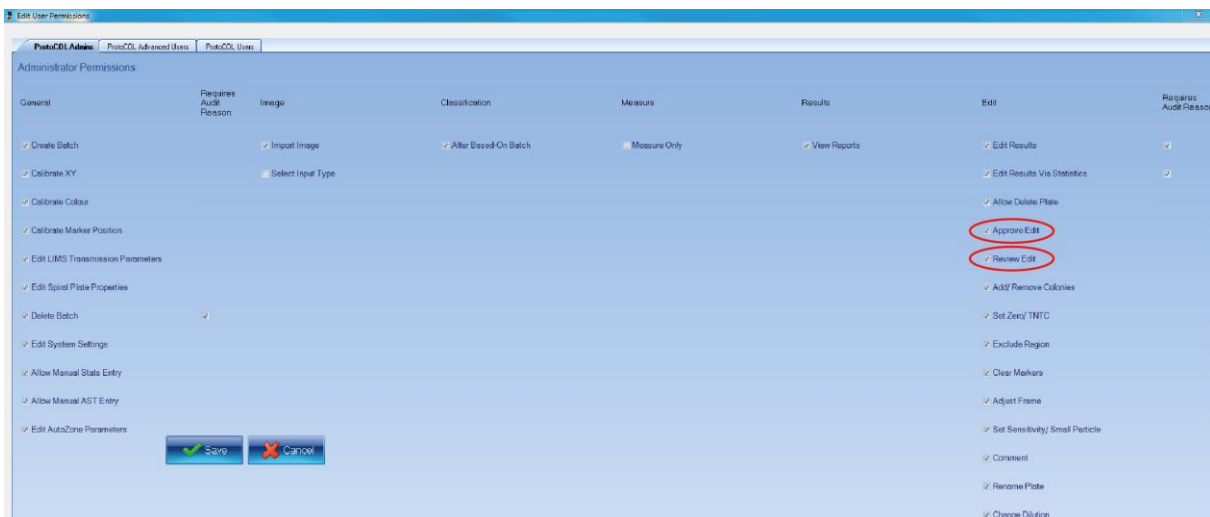
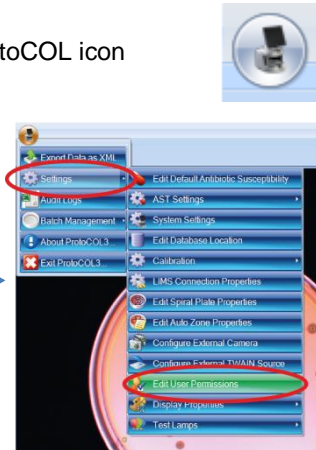
The review/approve feature gives the user/s the ability to mark plates as reviewed and/or approved after they have been manually checked in the edit/review section.

To enable this feature the appropriate permissions needs to be assigned the different user permission tiers on the system.

As this feature is disabled by default it needs to be enabled by clicking the ProtoCOL icon on the top left of the screen. (Administrator level required)

Then press 'Settings' followed by 'Edit User Permissions'

The 'Review Edit' & 'Approve Edit' tick boxes should be selected for any user level required to perform that function. An example of this would be that 'Approve Edit' is ticked for advance users, this will mean that all user of advance level accounts will be able to approve plates from any batch generated on the ProtoCOL software. Once the permission has been ticked for the group, click 'save' and then restart the software for the changes to take effect.




Any user with the appropriate permission will now see a dropdown box on the edit/review screen with either 'Save Reviewed' or 'Saved Approved'.

When saving as reviewed or approved this will save the plate and add Flag to the plate results indicating that the plate has been checked following an automatic count.



If any changes to the plate were made by the reviewer or approver then an audit reason is required.

To maintain correct workflow there is an option in the system setting that can be unticked to restrict the batch report from being printed until all plates in the batch have been approved.

This can be turned on by clicking the ProtoCOL menu  and navigating to settings then system settings and then the option 'Allow Unapproved Batch Report' can be unticked to make it function. Click save changes to apply.