ProtoCOL

Colony Counting and Zone Sizing System

Quick Start Guide - Pour Plates

Starting ProtoCOL

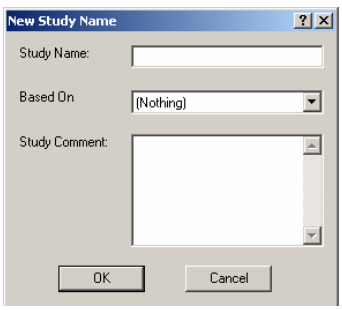
1. Connect ProtoCOL to a monitor keyboard and mouse, and switch on via the button at the back of the unit. This will start the internal PC and initialise the internal camera.
2. Double click the ProtoCOL icon on the desktop. If required enter your specific user name and password, otherwise ProtoCOL will start automatically.
3. When ProtoCOL opens for the first time, the Applications Window will open but will be empty. On subsequent occasions the same will happen but the window will open showing the same information as the last time it was closed.

Data storage in ProtoCOL

Data is stored in **STUDIES** and **BATCHES** with each study being divided into a series of batches. Each batch must contain results from the same type of plate although a study may contain different batches with different types of results. Before taking measurements using ProtoCOL you will need to have a study file open. You can either open an existing file or create a new one. ProtoCOL will automatically save results to an open study file.

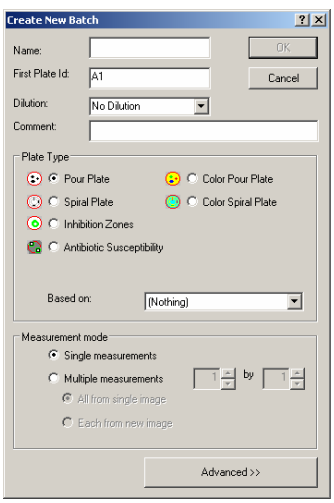
Creating a new study and a new batch

1. Choose NEW from the File menu, or click the "New Study" button in the standard tool bar.
2. Enter a name for the new study.



3. If you are going to use existing batch definitions for your new study, choose the name of the file from the "Based On" drop down menu. Or choose "Nothing" for a brand new study.
4. If required enter a comment in the Study Comment box.
5. Click OK to confirm the settings and close the dialog box.

If you choose to base your new study on an existing one the process of creating a new study is complete. However, if you are creating a completely new study you now need to create at least one new batch to go in it. The "Create New Batch" dialog box will open automatically if you do not base your new study on an existing one. To create a new batch within an existing study choose "New Batch" from the Edit menu.



6. Enter a name for the new batch.
7. Enter an ID for the first plate in the batch.
8. Choose a dilution factor for the plates from the drop down menu.
9. If required enter a comment for the batch.
10. Choose the "Pour Plate" option from the left hand side of the dialog box. If required choose a set of previously saved settings from the appropriate "Based On" drop down menu.
    If you do this the new batch is now created. If this is a brand new batch click "Nothing" from the drop down menu.
11. If you have purchased the ProtoCOL Colour Analysis option and wish to distinguish between colonies of different colours check the "Colour Pour Plate" button.

    To set more advanced options click the Advanced Option button to expand the dialog box.

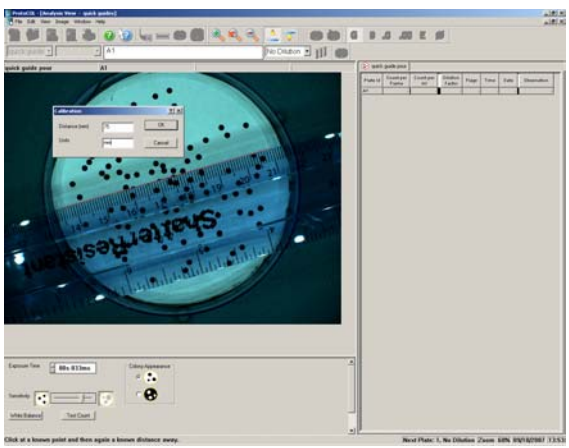
12. The sample volume is assumed to be 1ml, if this is not the case type the sample volume in the "Sample Volume" box.
13. Type the dish size in the "Dish Size" box.
14. In the "Saved Image Options" box check the Automatic option to save the displayed image automatically after taking a measurement.
15. Choose whether mean values for a dilution series should be given by the Arithmetic mean or by an Alternative calculation (refer to the ProtoCOL Systems User Manual page 2-12 for a definition of the alternative mean calculation).
16. For pour plates without colour analysis, set the Feature Size Sensitivity by either typing a number between 0 and 100 or by using the up and down scroll arrows. The Feature Size Sensitivity parameter controls the operation of detecting and counting colonies. The value set here can be changed at a later date.
17. When all of the information is complete click "Hide" to shrink the dialog box and OK to close it and accept the new settings.
    The new batch is now created.

Calibrating ProtoCOL and setting up batches to take measurements

1. Choose the light setting by using the up-light and down-light buttons in the image tool bar. For clear agars with good contrast colonies best results are usually obtained using the lower lamp.

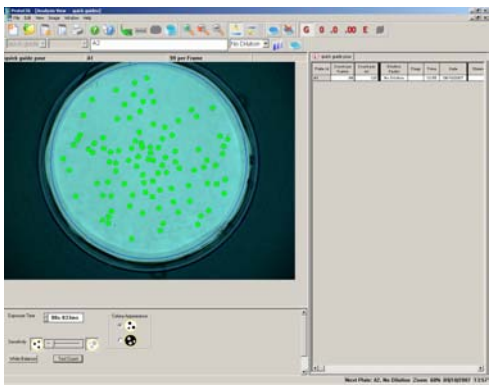
   Before using ProtoCOL to count plates you will need to perform an individual calibration. If you try to use an uncalibrated study you will be reminded that calibration is required.

2. To calibrate ProtoCOL insert an object of known size or with known graduations on it within the camera's field of view.
3. Choose "Calibration" from the edit menu or use the calibration button on the tool bar.
4. Move the pointer to the starting point on the object and click the left mouse button. As you move the pointer along the object a line will be drawn behind it to the final position. When you reach the end at which you decide to take the calibration measurement click the left mouse button again.
5. The calibration dialog box will be displayed. Enter the distance between the two points and the units used for the measurement.
6. Press OK to confirm the calibration and close the dialog box.



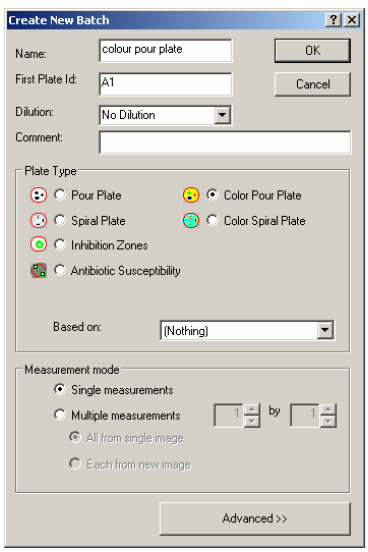
Optimising camera settings

1. Place a typical plate for the batch under the camera. ProtoCOL will automatically capture an image of the plate and display it in the image pane.
2. Adjust the camera gain / sensitivity and exposure time using the slider and spin arrows as appropriate.
   The camera gain slider should be set to the left to give a low value.
3. Set the colour correction of the camera by placing a piece of white paper under the camera and clicking on "White Balance". This is now complete and should not have to be done very often.
4. To move or re-shape the frame i.e. the area of the plate which ProtoCOL counts, move the pointer so that it is within the image of the plate and click the left mouse button. Eight points will appear on the image and the pointer will change to a four way arrow. Light blue "Drag Handles" will appear on the screen allowing you to move and re-shape the frame over the image.
5. Use the controls in the "Controls Pane" to select the appearance of the plate, either light colonies on a dark agar or dark colonies on a light agar.
6. Adjust the sensitivity by moving the slider. As these changes are made you can see in real time the difference that this will have on the count made by ProtoCOL. Adjusting these settings carefully allows accurate distinction to be made between colonies and debris.
7. Click "Test Count".
8. Colonies that have been counted are highlighted in green. Carefully adjust the camera settings again so that only colonies that require counting are highlighted.
9. Click the "Count Colonies" icon.
10. Results are shown in the top right of the screen along with information on time and date that the plate was read, dilution factor, batch name and any observation. The study name is displayed on the top left of the screen.

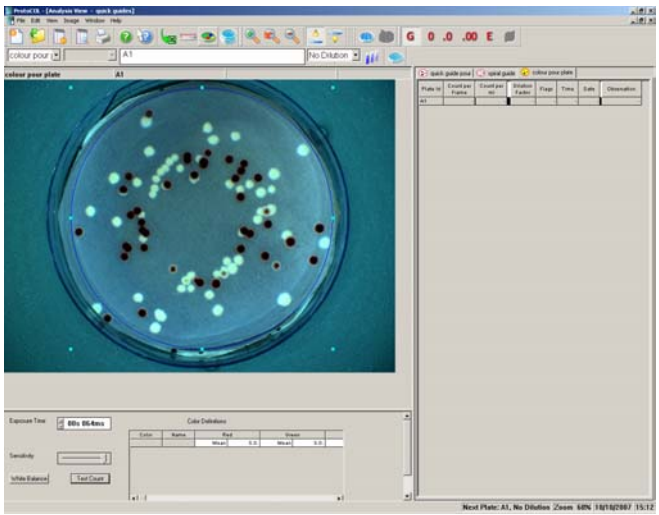


Colour analysis

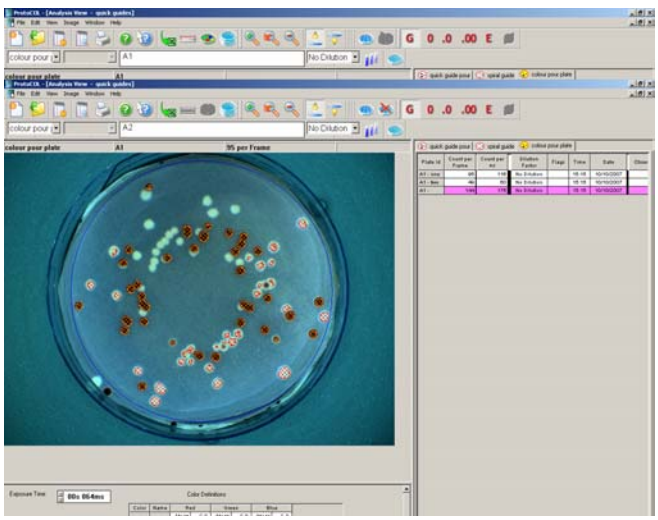
1. If you purchased the Colour Analysis option and wish to distinguish between colonies of differing colours you can do this by checking the "Colour Pour Plate" button in the the "New Batch" dialog box.



2. After calibrating the plate in the normal way click the "Colour Definition" icon.
3. Click on the agar to define the colour in a maximum of 8 regions around the plate. The size of the defining regions can be adjusted using the up and down spin arrows.
4. Click "Next Organism".
5. Define the colour of the required organisms in the same way clicking on "Next Organism" if more than one type of colony needs counting.
6. When all the required colony types have been defined click "Finish".
7. Name the colonies next to the relevant colours in the dialog box.
8. Click "OK".



9. Adjust the camera as for a non colour defined plate.
10. Count as previously described.



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