



SphereFlash[®] Colonies Software

Automatic Colony Counter

Advanced Instructions for Use

Doc. No 50007939 -05



Contents

1. Introduction.....	5
1.1. Intended use of the SphereFlash®	5
1.2. Basic and advanced features.....	5
 2. Methods.....	 6
2.1. Creation, edition, and testing of methods	6
2.2. Filtering and classifying colonies by colors and diameters	8
2.2.1. Counting Modes	8
2.2.2. Filtering by color	9
2.2.3. Filtering by diameter	11
2.2.4. Warning limits for count and concentration	11
2.2.5. Multiple Colony classes	12
2.3. Image Capture settings.....	14
2.3.1. Lighting mode	14
2.3.2. Exposure Time	14
2.3.3. Plate and image diameter	15
2.4. Options settings.....	16
2.4.1. Drawing lines and colors.....	16
2.4.2. Run editable values	16
2.5. Advanced settings	17
2.5.1. Visualizing the effects of each control	17
2.5.2. Filling holes inside colonies	18
2.5.3. Removing dim or narrow artifacts	18
2.5.4. Detecting colonies with smooth or fuzzy edges.....	19
2.5.5. Detecting low-contrasted colonies	19
2.5.6. Changing color detection behavior.....	19
2.5.7. Controlling how overlapped colonies are segregated.....	19
2.6. Importing and exporting methods.....	20

3. Calibration and Verification	22
3.1. Calibration	22
3.2. Verification.....	22
 4. Working with worksheets.....	 24
4.1. Creating and editing a worksheet.....	24
4.2. Using a worksheet to count colonies.....	26
4.3. Importing, exporting, and deleting worksheets	27
4.4. Working with remote file input.....	27
 5. Views Management.....	 28
5.1. Built-in Views	28
5.2. Customized Views	29
5.3. Actions on the views	29
5.4. Audit Trail	30
5.5. Managing results groups.....	31
 6. Entering the data of manually counted plates	 32
6.1. Using IUL's DOT manual colony counter	32
6.1.1. Spread distributions	33
6.1.2. Spiral distributions	33
6.1.3. Using other means for a manual count	33
 7. General administration.....	 35
7.1. Users Management	35
7.1.1. Roles and permissions	35
7.1.2. View existing users.....	36
7.1.3. Add new user.....	37
7.1.4. Other user management operations.....	37
7.2. Environment and System options.....	37
7.2.1. System options	38
7.2.2. Expiration times	38
7.2.3. List separator.....	39
7.2.4. Remote input/output folders.....	39

7.2.5.	Value Limits.....	40
7.2.6.	Barcode reader.....	40
7.2.7.	Date and Time format	40
7.3.	Customizing the logo on the reports.....	40
7.4.	Digital signature of PDF reports and tables.....	41
7.5.	Removing outdated results (purge)	42
7.6.	Managing the database files	43
7.6.1.	Location and backup of the active database files	43
7.6.2.	Location of archived database files resulting from purge operations	43
7.6.3.	Temporarily restoring archived databases	43
7.7.	Interfacing with a LIMS (Laboratory Information Management System)	44
7.7.1.	Remote input file example	45
7.7.2.	Remote output file example	45

8. Regulatory compliance: FDA 21CFR part 11..... 46

1. INTRODUCTION

Thank you for choosing the SphereFlash®. We are confident that this instrument will become an integral part of your laboratory.

Before installing and using the SphereFlash® software, using the SphereFlash® instrument, it is essential that you read the SphereFlash® instrument Instructions for Use carefully. Following the instructions and safety information in that Instructions for Use will ensure safe operation and maintain the system in a safe condition.

1.1. Intended use of the SphereFlash®

The SphereFlash® is an automatic colony counter that meets all the requirements that may be required in a modern microbiology laboratory.

With its SphereFlash® Colonies LITE Software it performs the colony counting simply and reliably thanks to its world-class software and its patented built-in lighting system.

For more demanding users the software can be upgraded to the SphereFlash® Colonies PRO Software to increase its capabilities, e.g. setting user's permission levels, LIMS connectivity, worksheets, barcode reading, reporting, audit trail, or CFR21 Part 11.

With the optional SphereFlash® Halos PRO Software, the SphereFlash® unit can be transformed to measure inhibition halos, accepting various circle approximation criteria to obtain the corresponding diameters of virtual circle areas.

In all versions, the lighting is done through LEDs that are oriented to the internal wall of a moving spherical chamber. The result is an unprecedented uniform and reflections-free illumination in a colony counter device.

The moving sphere closes the chamber and prevents the area in which the Petri dish is located from any external light. It avoids any reflection or interference from external light, creating a luminous atmosphere without reflections from the walls of the Petri dish or the colonies. For the transparent media, the light by transmission is also available in all versions.

1.2. Basic and advanced features

This guide explains the **advanced features of the SphereFlash® Colonies Software**. It is advisable that before addressing the topics in this guide, you become familiar with the basic features, available with the default *SphereFlash® Colonies LITE Software* license, as described in the *Basic Use Guide**.

* See Basic Instructions for Use – SphereFlash Colonies Software, Doc. No 50007937

Please be aware that most of the advanced features described in this guide are only available to the *SphereFlash® Colonies PRO Software* version; to upgrade to the *PRO* version you need to get an *Activation Key* from an IUL representative*.

2. METHODS

A *Method* is a named collection of settings, that is suited to count colonies for a range of plate, colony, and procedural particular conditions.

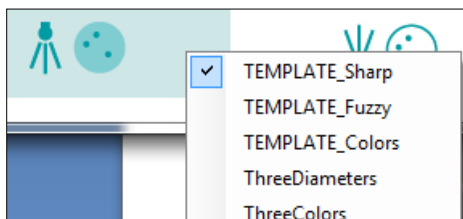
The SphereFlash® application is natively equipped with several general-purpose methods that cover some of the usual plate appearances that can be found in normal usage. These native methods are ready-to-use or can be used as templates to modify some of their settings to create new methods*.

2.1. Creation, edition, and testing of methods

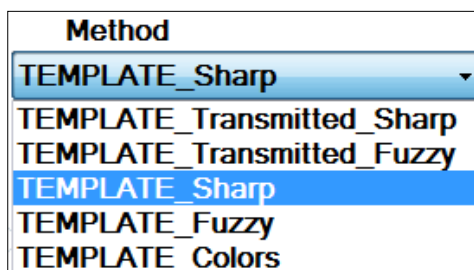
Users with the role *Operator* have not permission to edit a method; instead, *Programmer* and *Administrator* roles must be used (see section 35).

To create a new method, you must start from an already existing method (e.g., 'TEMPLATE_Sharp') and then modify it by edition. When editing and testing have finished and you are satisfied with the results if you want to create a new method, save it with a new name. And if you just want to update the original method with the new settings, save it with the same name.

To edit a method, select it either right-clicking one icon from the bottom bar*:



or selecting the desired method from the method list on the *Colony Count* panel:



and then press the edition button  at the bottom of the *Colony Count* panel.

* See Basic Instructions for Use– SphereFlash Colonies Software, Doc. No 50007937

The **Method Settings** subpanel will be presented. You can distinguish it from the *Colony Count* main panel because the *Method Settings* subpanel has a greenish background:

The box **Method Name** displays the original method that you will edit; as said above, later when saving, you will have the choice to change the method name.


The box **Method Description** allows the user to introduce a text describing the features or the intended use of the method.

Delete Method button allows erasing the selected method. Note that the built-in template methods cannot be deleted.

Hovering the cursor over the small boxes under **View mode**, different overlays are applied to the plate image displayed on the image panel, showing the partial results of each image processing step, corresponding to the settings in the *Advanced* Tabs.

There are several tabs that group the settings according to their functionalities (*Classes*, *Image capture*, *Option settings*, *Advanced 1*, and *Advanced 2*). If any of the tabs become hidden, you can reach it by clicking on the small arrows to the right of the tabs.

Buttons at the bottom of the panel:

 Test the current settings by running a *Colony Count* operation on the current plate in the instrument; then you can see the count results:

Class	Count	CFU/ml	CountVol
Class 1	33	3.92E+002	0.842



Save the method with the modified or edited settings values.



Restore the settings of the original method, discarding the edited values.

Buttons at the top of the panel:



Return to the *Colony Count* run panel.

2.2. Filtering and classifying colonies by colors and diameters

Select the tab *Classes* in the *Method Settings* subpanel:

The screenshot shows the 'Classes' tab in the 'Method Settings' subpanel. The 'Class 1' section contains the following fields and controls:

- Colony Class Id:** A text field containing 'Class 1'.
- Class Mark Color:** A color selection button (red square) and a text field showing 'RGB=(255, 0, 0)'.
- Count Mode:** A dropdown menu currently set to 'Auto'.
- Accept Colors:** A text field containing 'none'.
- Reject Colors:** A text field containing 'none'.
- Min Diameter (mm):** A text field with a spinner, set to '0.30'.
- Max Diameter (mm):** A text field with a spinner, set to '20.00'.
- Counted Limits:** A section with four input fields labeled 'lower', 'upper', 'warning', and 'alarm'.
- CFU/ml limits:** A section with two input fields.

At the bottom of the panel are two large buttons: a plus sign (+) and a minus sign (-).

2.2.1. Counting Modes

The default selection for the *Count Mode* field is **Auto**; with this mode, when the image is processed, the application counts either the bright colonies or the dark colonies, whichever has the higher count. Dark or bright are qualified concerning the background.

The screenshot shows the 'Count Mode' dropdown menu. The menu is open, displaying the following options:


- Auto (selected)
- All
- Dark Only
- Light Only
- Filtered by color

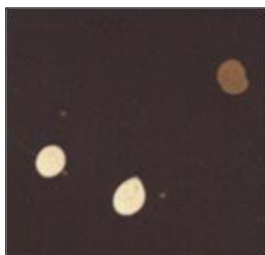
Other Counting Modes available: **All** (forces counting all the detected colonies, regardless of being dark or bright), **Dark Only** (counts only dark colonies), **Bright Only**

(counts only bright colonies), and **Filtered by color** (counts colonies having some specific color).

2.2.2. Filtering by color

To count the colonies corresponding only to a range of colors, before entering the method edition, select on the *Colony Count* panel, as a starting point, one of the methods **TEMPLATE_Colors** (if the colonies have clearly defined edges) or **TEMPLATE_Fuzzy_Colors** (when the colonies have blurry edges).

Click the button  to acquire an image of a plate containing colonies of colors, both those you want to be counted, and also those you want not being counted:




Then enter the method edition using the button .

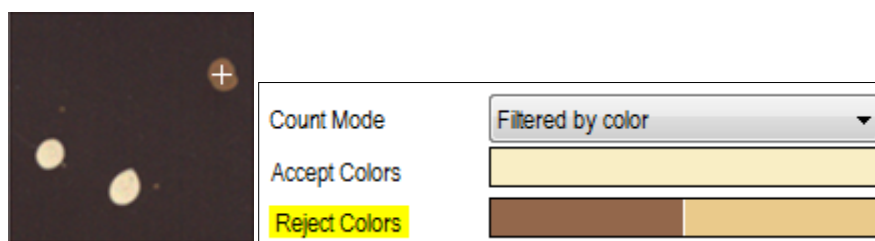
The *Count Mode* will be automatically set to *Filtered by color*, and one box for *Accept Colors* and another for *Reject Colors* will appear, both empty:

Count Mode	Filtered by color ▼
Accept Colors	none
Reject Colors	none

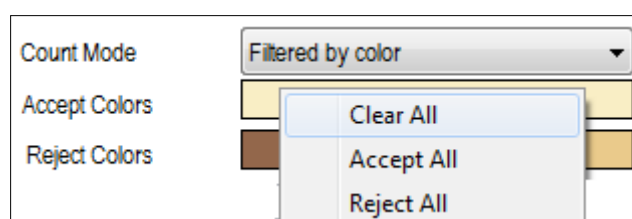
With the label *Accept Colors* selected (yellow background), click on the image of some colonies having the colors you want being accepted, these colors will appear in the *Accept Colors* box:


	Count Mode	Filtered by color ▼
	Accept Colors	
	Reject Colors	none

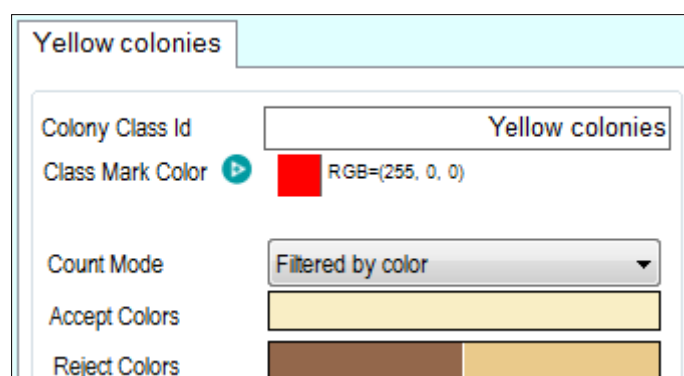
Similarly, click the *Reject Colors* label to select it, and click on the image of some colonies having the colors you want being rejected. Repeat as many times as you need until the result is good enough for you. Now the boxes will show a representation of the colors that will be accepted and rejected.




If you want to clear the currently selected colors, right-click on the color boxes and select *Clear All*:



You can go on refining the colors by acquiring images of other plates and adding colors to both boxes until the result is good enough. Then, you can give a name to the *Colony class ID field* to better explain the color criteria used (this name will replace the default tab name *Class 1*). You can also change the outlining *Class Mark Color* to increase the visibility of the selected colonies (click the  button to access the color palette selector):



Finally, the created method should be saved with the button . You will be prompted to introduce a description of the method and to give a new name for it.

2.2.3. Filtering by diameter

In any of the *Count Modes* you can restrict the range of diameters of the individual colonies that will be counted. Colonies with diameters less than the minimum or exceeding the maximum will be excluded from the count.

Min Diameter (mm)	<input type="text" value="0.30"/>
Max Diameter (mm)	<input type="text" value="20.00"/>

2.2.4. Warning limits for count and concentration

When editing a method, you can establish a minimum and maximum count for each *Colony Class*; then when executing a *Colony Count* operation, if the resulting count is outside both limits, a pink background will appear in the *Count* box, and the text <<Lower count warning for Yellow colonies.>> will be automatically appended to the *Comments* field of the results. These limits are required by certain protocols in order to guarantee that the count is statistically significant.

	lower	upper
CountedLimits	<input type="text" value="30"/>	<input type="text" value="300"/>

Class	Count	CFU/ml
✗ Yellow colonies	29	3.44E+002

Similarly, you can define *Warning* and *Alarm* limits for the concentration *CFU/ml*. After executing a *Colony Count* operation, if the resulting concentration exceeds the *Warning* limit but not the *Alarm* limit, the *CFU/ml* box background will become yellow:

	warning	alarm
CFU/ml limits:	<input type="text" value="2.00E2"/>	<input type="text" value="3.00E2"/>

Class	Count	CFU/ml
✗ Yellow colonies	19	2.58E+002

And if the *Alarm* limit is exceeded, the background will turn to pink:

	warning	alarm
CFU/ml limits:	<input type="text" value="2.00E2"/>	<input type="text" value="3.00E2"/>

Class	Count	CFU/ml
✗ Yellow colonies	27	3.21E+002


If you need an opposite behavior (alarm or warning when the concentration is below the limit), fill the *Alarm* limit box with a value less than the *Warning* limit box:

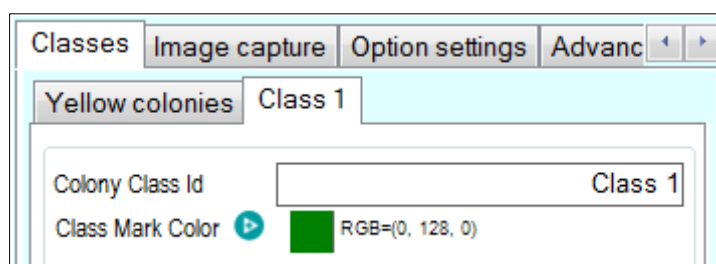
	warning	alarm
CFU/ml limits:	<input type="text" value="3.00E2"/>	<input type="text" value="2.00E2"/>

Class	Count	CFU/ml
✗ Yellow colonies	7	1.93E+002

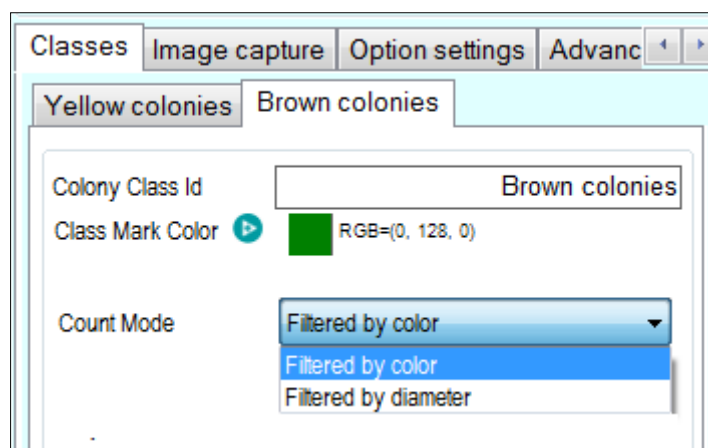
2.2.5. Multiple Colony classes

You can create more colony classes to obtain, from the same plate, differentiated counts for each type of colony, classified by color or diameter range.

After defining the first (default) *Colony Class* as described in sections 2.2.2 and 0, click the button  to create a new class. A new tab with a default name will appear beside that of the first class:



Give a new class a meaningful name in the field *Colony class ID*, choose a *Class Mark Color* if the proposed default color is not appropriated, and select the *Count Mode* according to the desired filtering criteria:



Filtered by color if you want the new class to be defined by a color distinct from that of the previous classes.

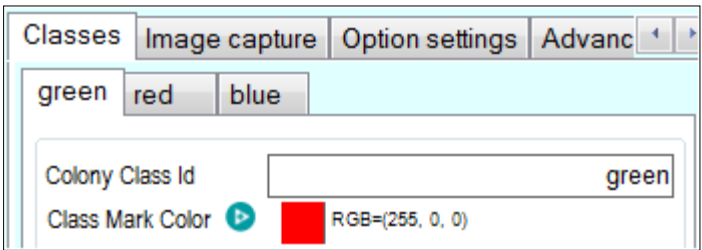
Filtered by diameter if you want the new class to be defined by a diameter range distinct from that of the previous classes but having the same colors as the previous class.


Then proceed for this new class the same way as for the first (default) *Colony Class* as described in sections 2.2.2 and 0.

NOTE: Colonies already selected on the previous classes will not be available for selection in the subsequent classes so that a single colony will only be counted once regardless of the number of classes defined.

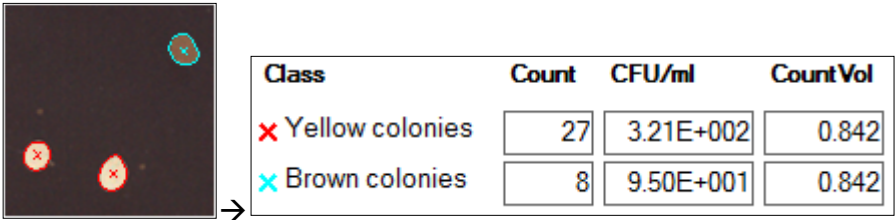
You can go on creating more classes as described above.

A previously defined class can be reviewed, modified, or edited by selecting its corresponding tab:



and the currently selected class can be deleted by clicking the button .

When a *Colony Count* operation is run with a method implementing several colony classes, each colony in the processed image of the plate will be outlined and marked with the color corresponding to its class, and the count results will be detailed for each class:

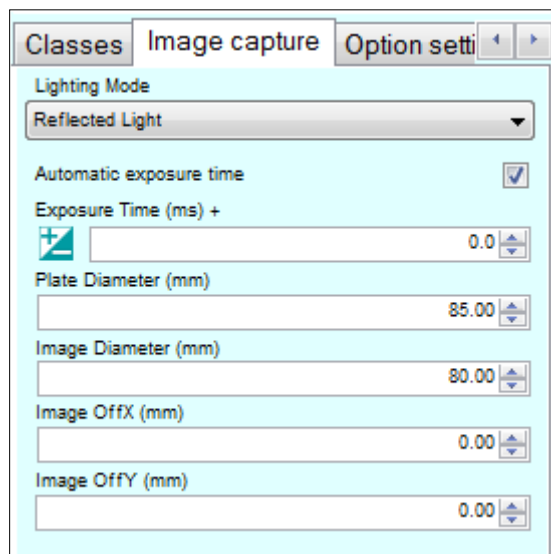


When accepting the result, several rows will appear on the *Data Panel* view for the same plate ID, one row for each *Colony Class*:

Results Group Name	Plate Id	Method	Colony Class	Counted Colonies	Counted Volume	Concentration
Count Results	plate25	TwoColors	Yellow	29	0.842	3.44E+002
Count Results	plate25	TwoColors	Brown	2	0.842	2.38E+001

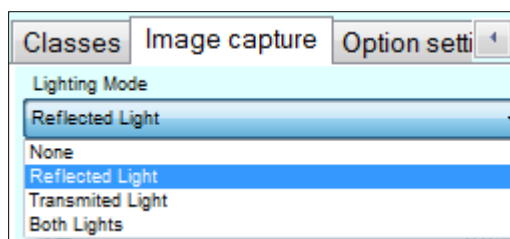
2.3. Image Capture settings

Select the tab **Image capture** to access the settings that control how the plate image is captured. The *Image capture* panel will be displayed:



2.3.1. Lighting mode

Choose which light source will be used to light up the plate: *Reflected Light* (sends diffuse light from above the plate), *Transmitted Light* (sends uniform light from below the plate), *Both Lights* and *None*.

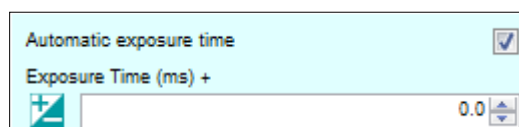


The default mode is *Reflected Light*, which allows to differentiate colonies from artifacts by appearance (color or brightness), and also to separate different colony classes.

Where the colonies are opaque and the substrate is translucent enough, *Transmitted Light* can yield good results if there are no opaque artifacts.



2.3.2. Exposure Time

Exposure Time establishes the global brightness of the image captured from the plate using the selected *Lighting Mode*.



By default, the box *Automatic exposure time* is checked, which means that at every *Colony Count* operation, the best exposure time is automatically determined for the particular plate being processed.

If you want to establish a fixed exposure for all the *Colony Count* operations using the currently edited method, uncheck the *Automatic exposure time* checkbox. Then, the instrument will determine the best exposure for the current plate in the instrument, which will be applied to all the future *Colony Count* operations using this method.

You can manually modify the exposure value in the *Exposure Time (ms)* box with the box arrows or entering a new value and test the results on the current plate by clicking on the run button . To reload the best exposure, click on the  button.

2.3.3. Plate and image diameter

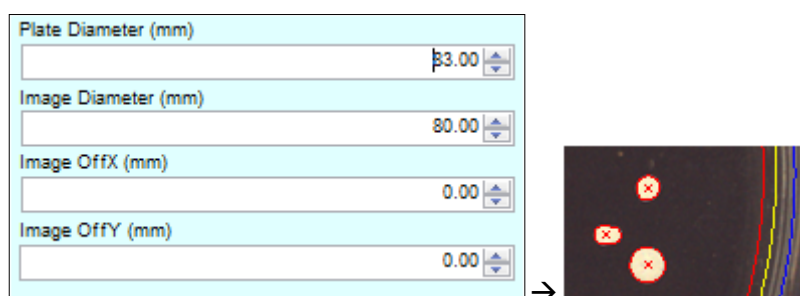


Plate Diameter: This corresponds to the blue circle on the image, and the user should adjust its diameter so that the blue circle perimeter matches the inner edge of the plate cylindrical wall. The application assumes that, in a spread distribution, the *Inoculated Volume* has been uniformly spread up to the limit defined by the blue circle.

Image Diameter: This corresponds to the yellow circle on the image, and the user should adjust its diameter so that the artifacts and reflections typically found close to the walls of the plate will be placed outside the yellow circle.

There is a red circle automatically placed slightly inside the yellow circle. No image processing will be done outside the yellow circle, and only colonies whose center lies inside the red circle will be counted. The area of the red circle in relation to that of the blue circle determines the actual *Counted Volume*.

NOTE: For spiral distributions, the circles of the rings pattern and their associated section volumes determine the counting area and the *Counted Volume*. The blue and red circles do not affect the spiral distributions count unless they are inside the outer ring circle of the spiral pattern.

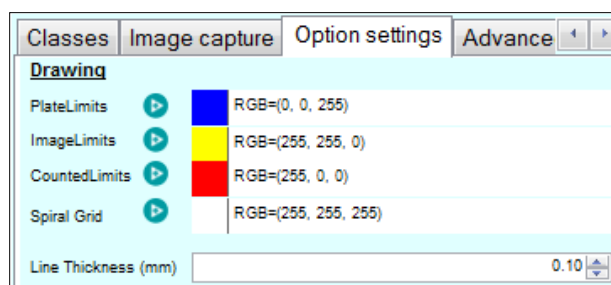
Image OffX / OffY: The user can manually displace the center of the circles by introducing some vertical and horizontal offset values.


NOTE: Automatic calibration procedure and the auto-centering device of the instrument will ensure that the prescribed center of the circles matches the actual center of the plate so that for normal plates there is no need to introduce any offset.

2.4. Options settings

Select the tab *Option settings* to access the line *Drawing* settings and *Run Editable Values* options.

2.4.1. Drawing lines and colors

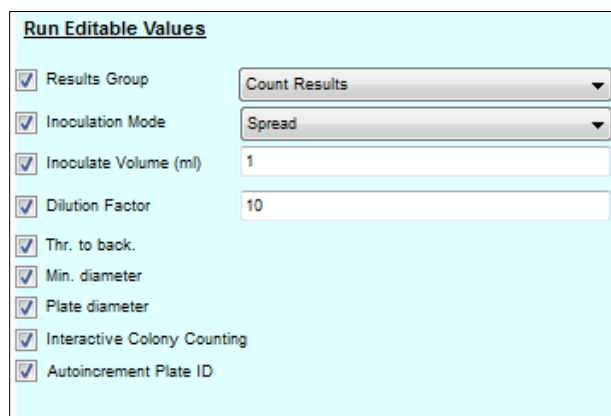


You can change the default color used to draw any of the three circles (*PlateLimits*, *ImageLimits* and *CountedLimits*), as well as that of the *Spiral Grid*, by clicking on the  button to access a color palette selector.

You can specify a common *Line Thickness* for all these circles and spiral grid, which will also apply to the colony outlines and center marks.

NOTE: The color of the colony outlines and center marks is defined for each *Colony Class* (see section 2.2.2).

2.4.2. Run editable values



The *Run Editable Values* are those that can be introduced or modified by the user during the execution of a *Colony Count* operation, given that the method allows for it. The permission to modify by the user one of these values is granted by checking the respective checkbox at the left of the label of the value.

If the checkbox is unchecked, the default value assigned by the method is always applied and the user cannot modify it. If the checkbox is checked, the method default value will be presented but the user may change it.

The method default values for **Results Group**, **Inoculation Mode** and **Inoculate Volume** can be assigned in this same tab and dialog. Default values for **Thr. to back.**, **Min. diameter**, and **Plate diameter** are the settings assigned in the method edition tab where each of them is defined (see 2.5.5, 0, and 2.3.3).

Interactive Colony Counting checkbox allows the user to interactively draw regions on the plate to be excluded or included for counting, as well as manually add or remove individual colonies*.

Autoincrement Plate ID checkbox enables to automatically create a new plate ID after each *Colony Count* operation has been executed and its result stored. The subsequent plate IDs are created by adding or incrementing a number to the first plate ID name entered by the user.

2.5. Advanced settings

The tabs *Advanced 1* and *Advanced 2* contain several settings that control the way clusters of colonies are discriminated from the background, how they are differentiated by color, and how the clusters are decomposed into individual colonies.

The default values of these settings in the *TEMPLATE_** built-in methods have been optimized to successfully process a representative set of plate cases, but if you feel that the results are not good enough for a particular case, you can try to modify some of these *Advanced* settings in order to try improving the counting performance.

2.5.1. Visualizing the effects of each control

By sequentially hovering the cursor over each slider in the tab *Advanced 1*, you can visualize the progression of each partial processing, determining the effect on the result of each setting value, from locating primary contours until recognizing segregated and differentiated colony clusters.

On the displayed processed image, when the cursor is not placed over any slider, the colony clusters that are not contrasted enough or that do not match the color selection of the current *Colony Class*, are not outlined. The colonies whose cluster is outlined but are not matching the diameter range of their *Colony Class* or are identified just as protrusions of neighbor colonies in the cluster, aren't marked with a cross.

When you let the cursor resting on a setting control, a tip will appear summarizing the intended use or effect of that setting.

* See Basic Instructions for Use – SphereFlash Colonies Software, Doc. No 50007937

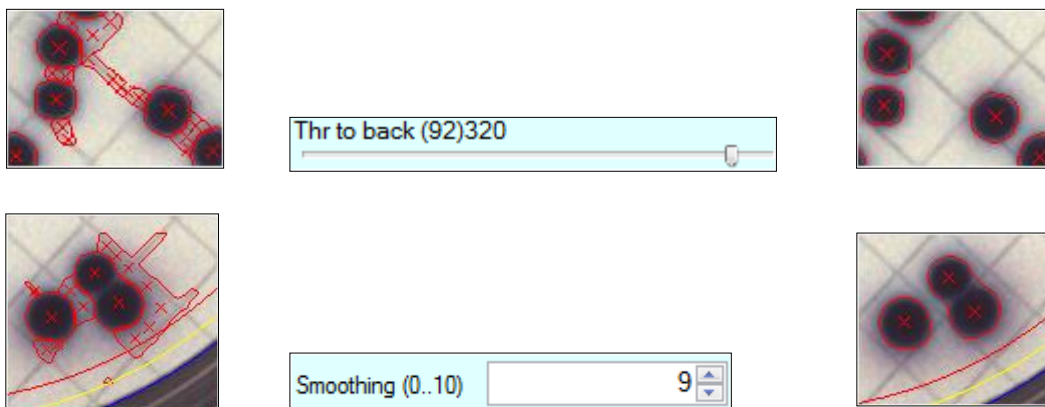
2.5.2. Filling holes inside colonies

In order to avoid counting as colonies holes and small stains overlapped inside a big colony, you can try to check the box *Fill holes*. If the stain or hole has a color similar to that of the background, you can also try to check the box *Backless*:

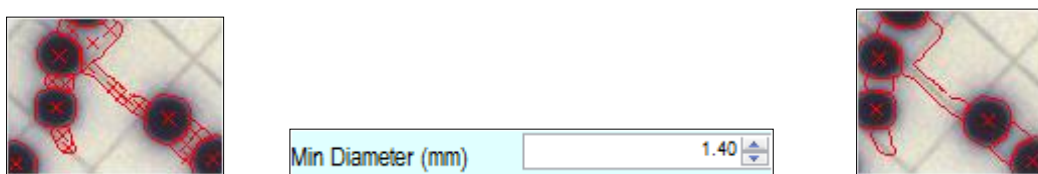


2.5.3. Removing dim or narrow artifacts

When there are dim or narrow artifacts on the background, such as grid lines of a filter, you can avoid them being wrongly counted as colonies by means of increasing the *Threshold to background* setting, or raising the degree of *Smoothing*:

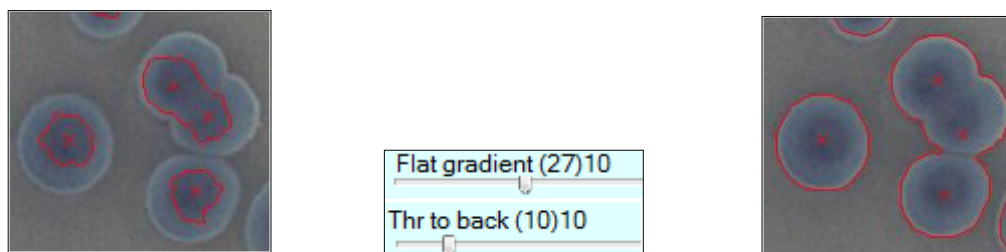


If the actual colonies are big enough, an alternative is to raise the *Min Diameter* setting in the *Classes* tab:



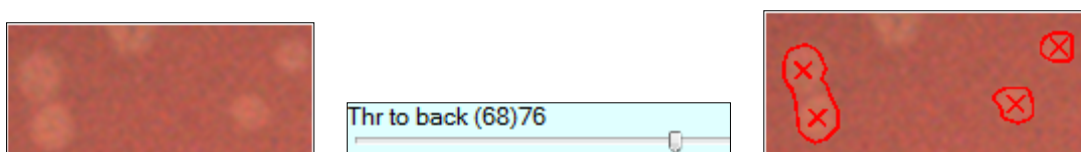
2.5.4. Detecting colonies with smooth or fuzzy edges

In order to detect colonies having smooth or fuzzy edges, it is recommended to use the build-in methods *TEMPLATE_fuzzy* or *TEMPLATE_transmitted_fuzzy*; but if the result with these methods is not good enough, you can improve it by trying some of the settings of the group *Segmentation* in the tab *Advanced 1* (particularly *Flat gradient* and *Ctr. gradient*), and possibly lowering *Threshold to background*:



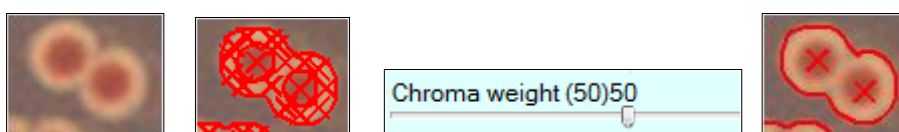
2.5.5. Detecting low-contrasted colonies

When the colonies are faint and low-contrasted against the background, lowering the setting *Threshold to background* can help to properly detect and count them. In some circumstances, checking the box *Backless* can also help, as well as changing the *Count Mode* to *All* instead of *Auto* (see section 2.2.1).



2.5.6. Changing color detection behavior

When the colonies show an internal structure with different colors, sometimes a weird result is obtained with the default settings. You can improve the results by changing the setting *Chroma Weight*:

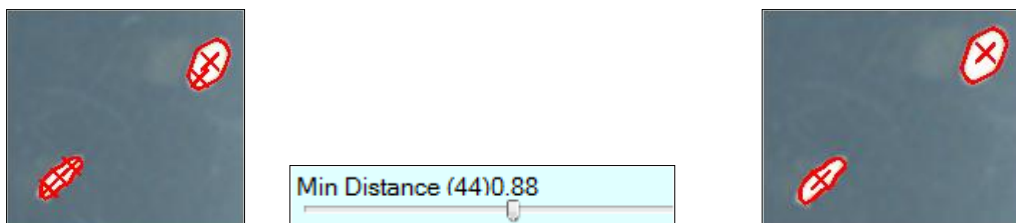


You can also try to modify the settings *Color Threshold* and *Backless*.

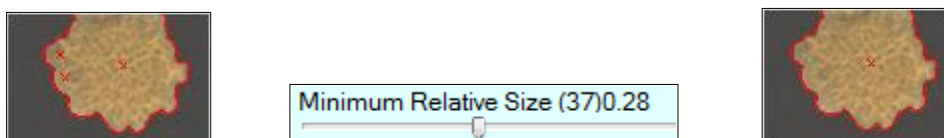
2.5.7. Controlling how overlapped colonies are segregated

The settings in the tab *Advanced 2* control how the clusters of overlapped colonies are decomposed into the colonies they contain.

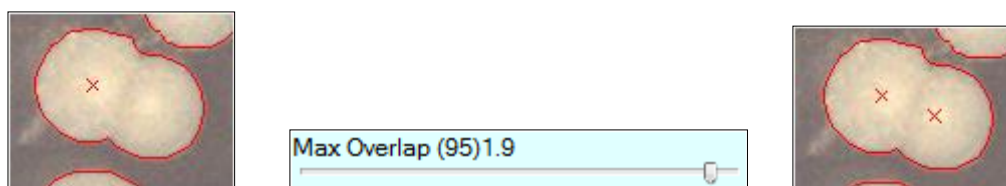
When there are **elongated colonies**, they could mistakenly be interpreted as a cluster of overlapped colonies, resulting in a count of several colonies where there is only one. By rising the setting *Min Distance*, you can avoid counting spurious colonies that are too close to other colonies:



In the case of **colonies with irregular or bulged edges**, sometimes their lobes could wrongly be counted as colonies. By adjusting the setting *Minimum Relative Size* you can avoid that the small bulges be recognized as independent colonies.



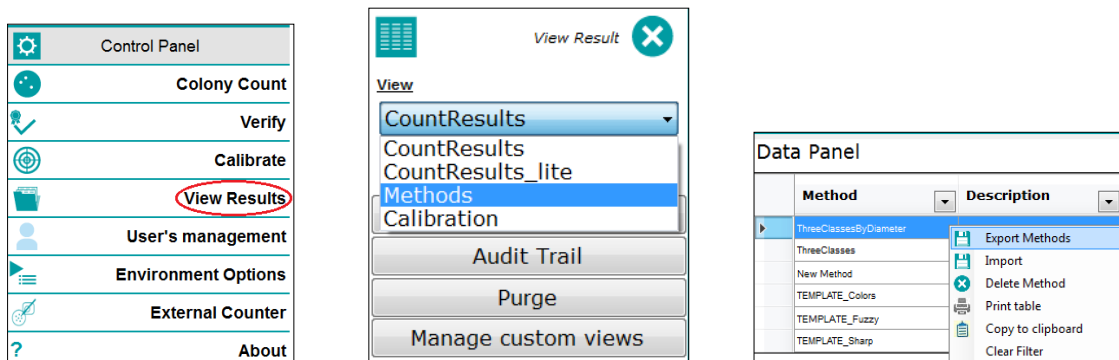
Sometimes, **two round colonies that overlap** may be counted as a single elongated colony; in this case, *Max Overlap* setting can be useful to achieve counting both colonies instead of only one.



2.6. Importing and exporting methods

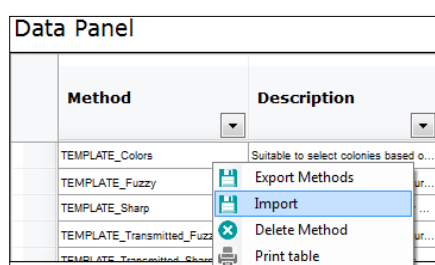
If you have several SphereFlash® instruments working in different computers, you may want that some methods developed on one of these workplaces could be used in the other ones, without the annoyance of manually copying all the settings of the method from one workplace to the other.

The *Method Export* feature allows you to create on the source workplace an XML file containing the settings of the methods that you want to copy. To export a method, go to *View Results*, select from the drop-down list the *View 'Methods'*, and you will get in the *Data Panel* a list of all the available methods. Select all the methods you want to export, and right-click on the selection to access the context menu; from it select *Export Methods*:



A dialog will be opened, where you can give a name for the exported methods XML file, and choose the destination folder.

Now copy the generated file onto a folder on the PC of the new workplace where you want to copy the methods. Proceed similarly as above, but this time select *Import Method*:



A dialog will be opened to select the method file to be imported. Then, if they already exist a method with the same name as the imported one, you will be prompted to change the name of the imported method.

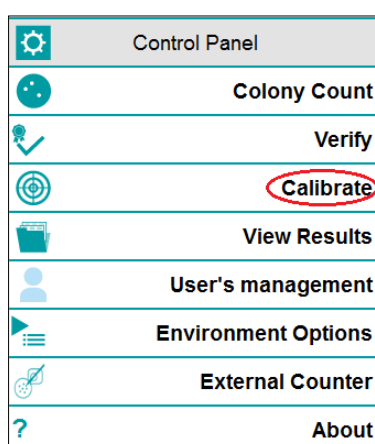
3. CALIBRATION AND VERIFICATION

3.1. Calibration

The *Calibration* procedure automatically determines, for the particular instrument in use, the required dimensional centering and scaling, and the proper lighting adjustments to achieve precise and repeatable results.

The first time a SphereFlash® instrument is used, the software application will request that a calibration procedure be performed. Calibration will also be requested after some prescribed time since the last calibration, as well as when some changes have been done to the *Environment* settings. Users with the role of *Administrator* can define the calibration lapse time, and whether the calibration is compulsory or not when requested (see 7.2.2).

To perform a calibration, select *Calibrate* in the top-right control panel and insert the calibration disk ref.900010081 on the platform:



Push the Run button on the bottom right: . The instrument will proceed to perform several image captures, and finally, an image of the calibration disk will appear on the Image Panel.

You will be prompted to accept the calibration using the Accept button: . Now the application is ready to count colonies on your Petri Dishes.


3.2. Verification

To assess whether the instrument is correctly counting the colonies, there is a verification disk ref. 90004113 which simulates colonies of different sizes; one side of the test plate contains dark colonies over a bright background, and the other side contains bright colonies over dark background.

To run a *Verification* operation, insert the verification disk on the platform and select *Verification* in the top-right control panel:



The *Verification* panel will be displayed; choose among *Black Colonies on White Background* or *White Colonies on Black Background* according to the side of the disk



which is presented on the top, and click on the *Run* button . The instrument will acquire an image of the disk, display the count results in separate diameter classes, and if the count of a class is outside the expected range, the corresponding *Count* box will be highlighted with pink background:

The Verification panel displays the following information:

- Imaging Device**
 - Last Verification Date: 2017/03/21 13:03
 - Due Verification Date: 2017/04/20 13:03
- Verification Data**
 - ☒ Black Colonies on White Background
 - ☐ White Colonies on Black Background
- Admissible Results (Test Plate)**
 - 1 mm: 169 - 189
 - 2 mm: 153 - 163
 - 3 mm: 120 - 126
- Results Table**

Class	Count	CFU/ml	CountVol
1 mm Count	179	2.13E+003	0.842
2 mm Count	158	1.88E+003	0.842
3 mm Count	118	1.46E+003	0.842

The '3 mm Count' row is highlighted with a pink background. Below the table are buttons for Run, Confirm, and Cancel. An arrow points from the panel to a circular image of the verification disk showing colonies.

Now you can accept or reject the verification result using the respective buttons  or .

Users with the role of *Administrator* can define the verification lapse time, and whether a message requesting the verification is shown after the time expiration (see 7.2.2).

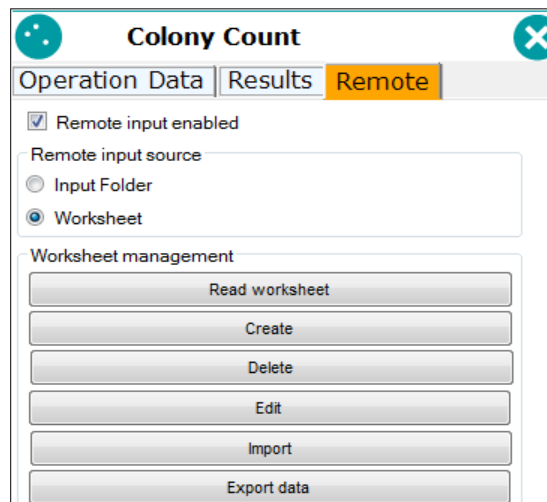
4. WORKING WITH WORKSHEETS

To avoid errors of the operator when selecting the method or typing-in the *Run Data and Input Values* (*Distribution*, *Inoculated volume*, *Dilution*, *Plate diam*, *ThrToBack*, *AbsMinDiam*) required to process each particular plate, a user with the role *Programmer* can create a table in advance, where for each *Plate ID*, the method to be used, and the optional input values can be defined.

Later when the operator will process a particular plate, by just introducing its *Plate ID* (typing in it, or better using a barcode reader), if this *ID* exists in the active worksheet, all the required parameters to process it will be automatically loaded.

4.1. Creating and editing a worksheet

In the *Colony Count* panel, select the tab *Remote*, and then check the box *Remote Input Enabled* and the radio button *Worksheet*. The tab *Remote* will be highlighted in orange to indicate that the *Remote* mode is active:



To create a new worksheet, click *Create* and you will be prompted to enter a name for the new worksheet. In order to edit an existing worksheet, first click *Read Worksheet*, select the desired one from the list, and then click *Edit*. Then, the worksheet editing dialog will appear:

Plate_Id	Method_Name	Inoculation_Mode	Inoculated_Volume	Dilution_Facto
PD192643	TEMPLATE_Sharp	Spread	1	10
PD192644	TEMPLATE_Sharp	Spread	1	100
PD192645	TEMPLATE_Sharp	Spread	1	100
PD192646	TEMPLATE_Fuzzy	E Mode 50ul	1	10
PD192647	TEMPLATE_Fuzzy	C Mode 100ul	1	10
PD192648	TEMPLATE_Tra...	Spread	1	10

For each plate you want to include in the worksheet, type-in (or barcode read) its *Plate ID* in the top-left box, and select the *Method* you want to be applied to count this plate. Optionally, if you want that some of the *Input Values* applied to this plate to be distinct from the default values in the method, check the box *Add input values* and select or type-in below the particular value you want to apply to this plate.

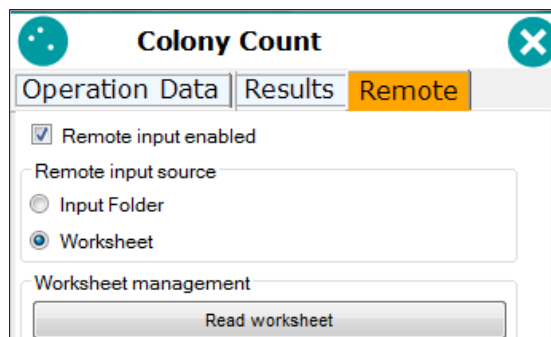
NOTE: The Input values modified for a plate will also apply to the next plate unless you re-load the method or use a new method for the new plate.

Press *Add* to include the edited plate in the worksheet list on the right. You can re-edit an existing plate in the list by selecting its row, changing the values, and then pressing *Overwrite*. And you can remove a plate from the list by selecting its row and pressing *Remove*.

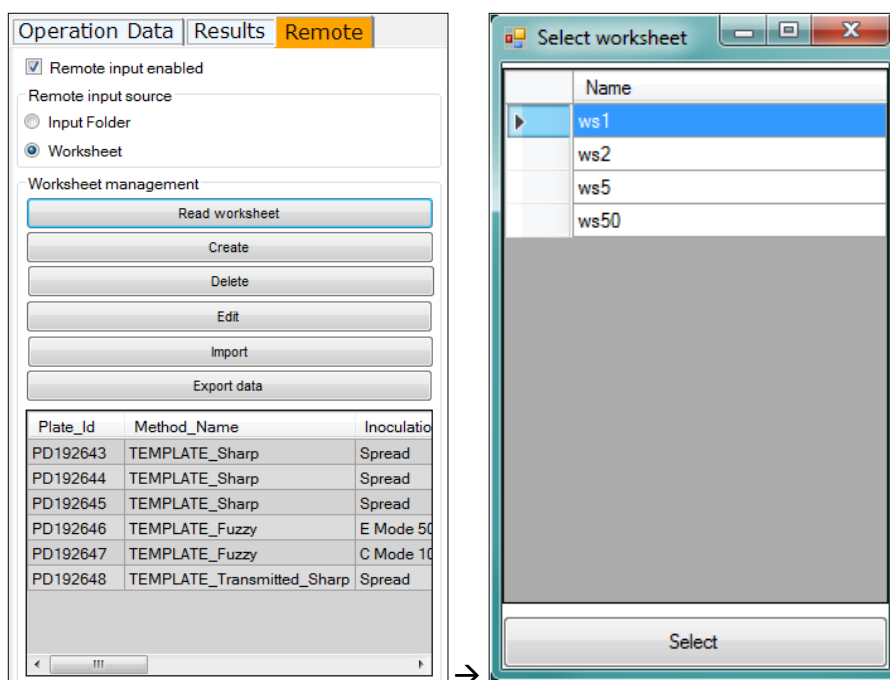
Finally, press *Save* to store the worksheet and its contents in the database.

4.2. Using a worksheet to count colonies

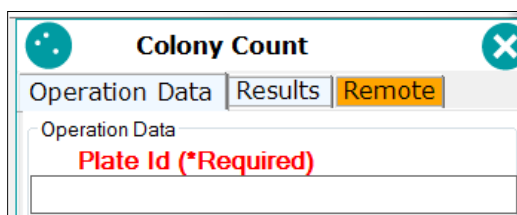
In the *Colony Count* panel, select the tab *Remote*, and then check the box *Remote Input Enabled* and the radio button *Worksheet*. The tab *Remote* will be highlighted in orange to indicate that the *Remote* mode is active:





Then press *Read worksheet*, select the worksheet you want to use, and the list of plates included in this worksheet will be displayed at the bottom:



Select the tab *Operation Data*, and type-in (or barcode read) a *Plate ID* in the top-left box:



If this *ID* exists in the worksheet, the *Run* button will turn green  and you can proceed to run the colony count with the method and values specified in the worksheet for this plate.

If the *ID* doesn't exist in the worksheet, the *Run* button will keep gray  and you will be prevented to run a count operation.

4.3. Importing, exporting, and deleting worksheets

You can create a worksheet in an external application and then import it into the SphereFlash® application for its use. There are three formats supported: Excel, CSV, and XML. Importation is done by clicking on the *Import* button.

In order to externally create a worksheet to be imported, you may find it helpful to create a sample worksheet with the SphereFlash® application, export it in the desired format, and use the generated file as a template to create your external worksheet with the expected structure and field names. Exportation is done by clicking on the *Export data* button.

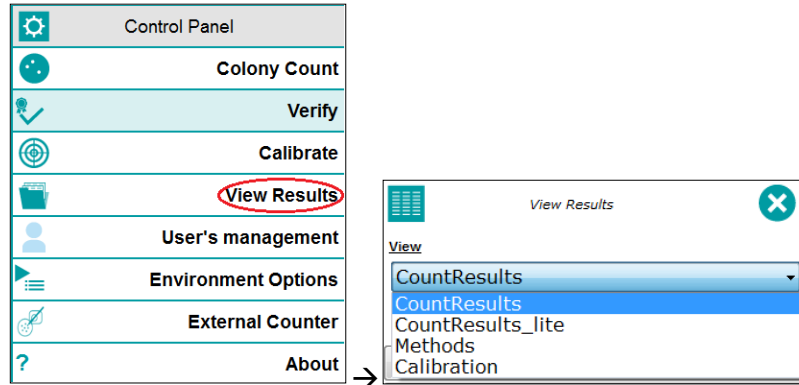
To delete an existing worksheet, push the button *Delete* and select from the list the worksheet to be deleted.

4.4. Working with remote file input

If you select the *Input Folder* radio button instead of the *Worksheet* button, a similar behavior as that of the worksheet will be achieved, but the information concerning which *Method* and *Run & Input values* apply to each *Plate ID* will be automatically obtained at each *Colony Count* operation, from an XML file located in a remote folder, rather than from a worksheet. See sections 7.2.4 and 7.7.

5. VIEWS MANAGEMENT

You can display in the bottom *Data Panel* several types of views of colony count results, methods, and calibrations. To select the view to display, go to the *View Results* panel and select from the drop-down list the desired view:



5.1. Built-in Views

CountResults: Displays all the available information fields of each result. For a result with multiple *Colony Classes*, one row for each class is displayed

CountResultsLite: Displays a summarized selection of the most relevant fields of each result.

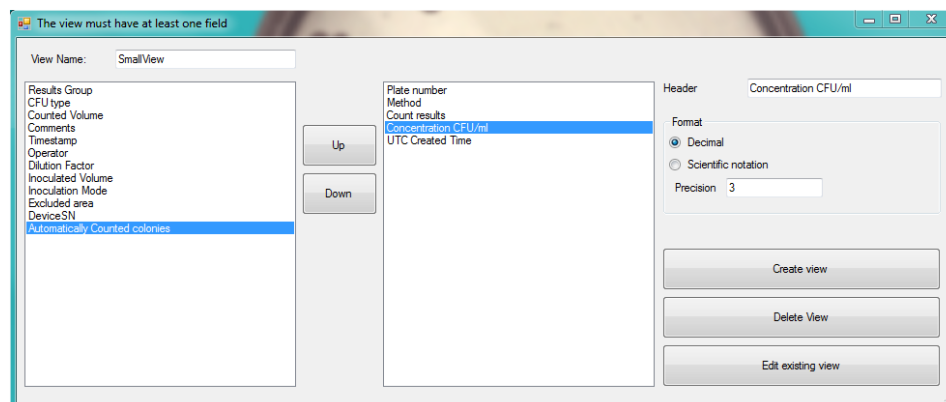
Methods: Displays a list of all the available methods. You can select a method and delete it if your user has a role with enough permissions.

Calibration: Displays the list of all calibration operations performed.

5.2. Customized Views

The users with a role that has permission for it, can create a customized view of the results, by selecting which columns will be shown, in which order, change the heading name of the columns, and define the numeric format to display for numeric, non-integer fields.

Press the *Manage custom views* and a view tailoring dialog will appear:



You must give a name to the view in the upper-left box. In the left panel, there are the available column names, select those you want in your view by double-clicking on each of them, after clicking, the column name will be moved to the right panel.

One in the right panel, you can re-order the columns with the *Up* and *Down* buttons. You can change the header name for the selected column in the top-right box. You can define a numerical format for the fields that admit it (scientific or decimal, with the desired number of decimal places). And you can remove a column from the right panel by double-clicking on it, the column will be moved back to the left panel with its original name.

When you are done, click on the *Create View* button to save the view. Then, this created view will be available in the Views drop-down list.

To modify an existing custom view, press *Edit existing view* and select the name of the view to edit. You can delete an existing view when editing it, by pressing the button *Delete View*.

5.3. Actions on the views

When a view is displayed in the *Data Panel*, you can sort the rows in ascending or descending order by clicking on the header of the column that contains the desired sorting values. A filter can be placed on each column to only select the rows that meet the combined criteria of all the filters. And a manual selection of individual results or a range of them can be established by clicking on the desired rows while holding the *Shift* or *Ctrl* keys as is usual in Windows programs*.

* See Basic Instructions for Use – SphereFlash Colonies Software, Doc. No 50007937

Once a selection has been established, by left-clicking on it you can export the selected results to a variety of formats, print them as a table or as a report, and copy them to the clipboard. To export to a PDF file of the selected results, you can print them using a third-party virtual PDF printer*.

5.4. Audit Trail

The *Audit Trail* is a historical record of all the changes and actions that any user has done when using the application. The *Audit Trail* is mandatory under some regulatory schemes, such as the FDA CFR21P11.

To access the *Audit Trail*, go to the *View Results* and click on the button *Audit Trail*. A grid panel with multiple tabs is displayed:

Event	MethodName	ColonyClassName	TimeStamp	UTCTimeStamp	ModifiedBy	ChangedProperty	PreviousValue	NewValue	ReasonForChange
New CountSettings	New Method Depre...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New CountSettings	VERIFICATION_Bla...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New CountSettings	VERIFICATION_W...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	New Method Depre...	Count Group 1	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	VERIFICATION_Bla...	1 mm Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	VERIFICATION_Bla...	2 mm Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	VERIFICATION_Bla...	3 mm Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	VERIFICATION_W...	1 mm Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	VERIFICATION_W...	2 mm Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
New ColonyClass	VERIFICATION_W...	3 mm Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			
Discarded Method	New Method Depre...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	Discarded record			
Discarded Method	VERIFICATION_Bla...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	Discarded record			
Discarded Method	VERIFICATION_W...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	Discarded record			
New CountSettings	VERIFICATION_Col...		2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			null
New ColonyClass	VERIFICATION_Col...	Red Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			null
New ColonyClass	VERIFICATION_Col...	Green Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			null
New ColonyClass	VERIFICATION_Col...	Blue Count	2017/03/16 13:23	2017/03/16 12:23	User Administrator	New record			null

Each record in all the grid panels includes the name of the user who performed the action and the timestamp when the action occurred. By clicking on the header of a column, the records can be ordered according to the values of this column. The records can be filtered by the time of occurrence, by introducing the start and end dates on the top boxes *Time from* and *Time to*.

In a very similar way that with the results views, you can manually select a set or range of rows and export or print them, by left-clicking on the selection.

There are the following tabs available:

Users: Records all the user management actions, such as creation, deletion, role assignment, password change, etc.

Methods: Records all the events related to the creation, edition, modification, and deletion of methods.

Environment: Records the changes done in the settings under the *Environment* and *System options* panels.

Sessions: Records each session login and logout data.

Versions: Records each update done to the application.

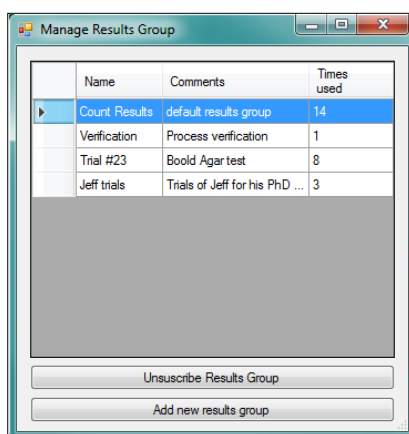
Results Groups: Records the creation and deletion of results groups.

Failed Logins: Records the logins that failed because the password was incorrect or the user name was wrong.

Calibration Results: Shows the results of each calibration operation that has been done.

5.5. Managing results groups

Result Group is a label or tag that can be applied to the result, allowing subsequent grouping or filtering of the results in the views based on this label. To access the management of the groups, click on *Manage Results Groups* button in the *View Results* panel, and a dialog will open where the existing groups are listed and there are buttons to create a new group or remove an existing one:



The native built-in groups are the *Count Results* group, which is the default for colony count operations, and the *Verification* group, which is automatically assigned to the results of the *Verification* operations.

When creating or editing a method, you can define the *Results Group* that will be assigned to the results obtained with this method, and whether the operator is allowed to change this assigned group (see 2.4.2).

NOTE: A *Result Group* cannot be deleted if there are in the database some results that have this group assigned. Prior to deletion, you must purge the old results (see 7.5) in a fashion that no remaining results have this group assigned.

NOTE: Methods using a deleted results group, will be automatically re-assigned to the first *Results Group* of the list, the default *Count Results* group.

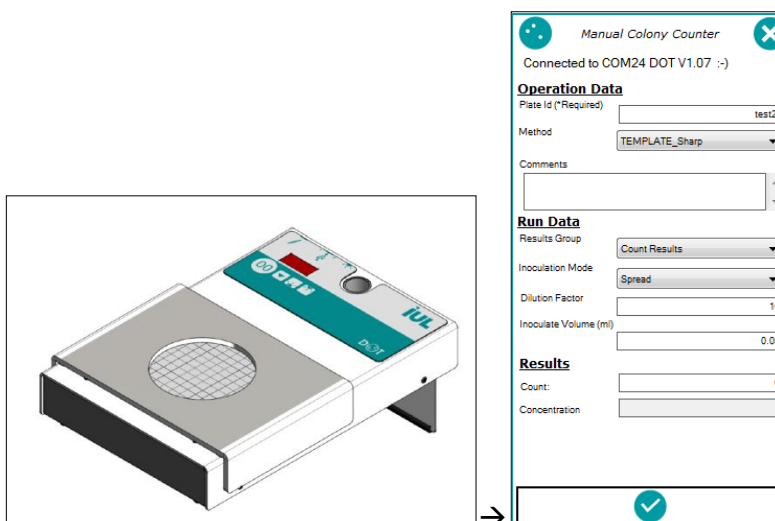
6. ENTERING THE DATA OF MANUALLY COUNTED PLATES

Sometimes the size, shape, or typology of the plate or their colonies prevent the use of the SphereFlash® instrument for automatically count the colonies. In such a case, you can still introduce the manual count results and run data values into the SphereFlash® database, where they will be handled the very same way as the results automatically obtained using the SphereFlash® instrument.

6.1. Using IUL's DOT manual colony counter

You can use the DOT manual colony counter by IUL ref. 90008700/9000871, and automatically send the final resulting colony count to the SphereFlash® application via DOT USB socket #1 (VCP – serial emulation) *.

Select the '*Manual Counter*' panel:



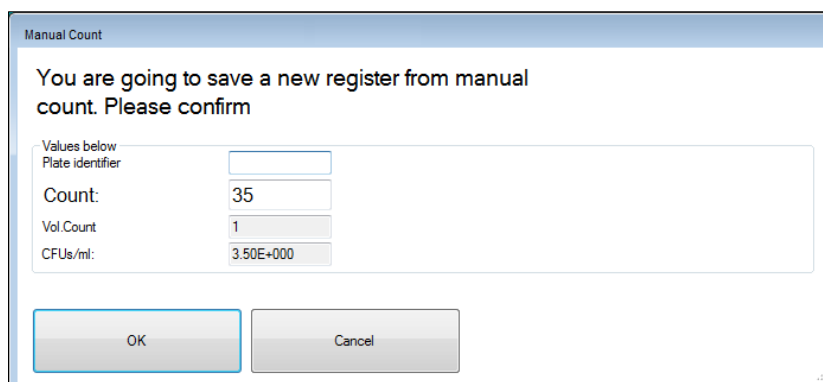
On the panel, you can see on the top whether a DOT instrument has been found connected to the SphereFlash® application.

You can select a *Method* and/or type-in *Run Data* values, with the only purpose of allowing the SphereFlash® application to compute the concentration based on the transmitted manual count and the distribution, volume, and dilution of the *Method* or introduced *Run Data*.

* See Basic Instructions for Use – SphereFlash Colonies Software, Doc. No 50007937

6.1.1. Spread distributions

After finishing the manual count and pressing the '00' button on the DOT instrument, the following dialog will be displayed:

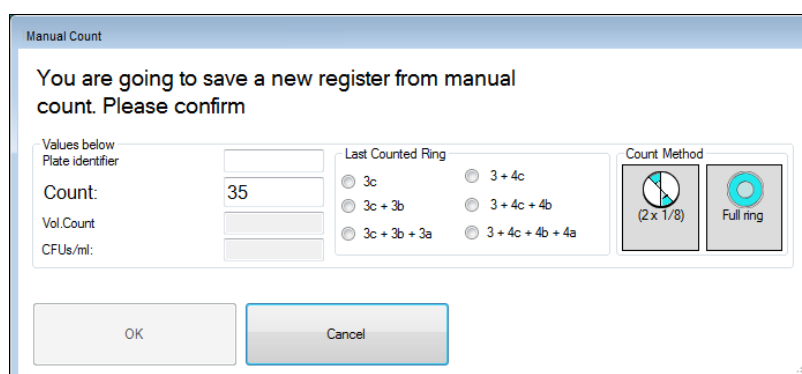


The dialog box titled "Manual Count" contains the text: "You are going to save a new register from manual count. Please confirm". Below this text are four input fields: "Plate identifier" (empty), "Count:" (35), "Vol.Count" (1), and "CFUs/ml:" (3.50E+000). At the bottom are "OK" and "Cancel" buttons.

The received count appears and the concentration has been already computed, assuming that you counted ALL the colonies of the plate; then just type-in or barcode scan the *Plate ID*, and press **OK** to save the result into the SphereFlash® database.

6.1.2. Spiral distributions

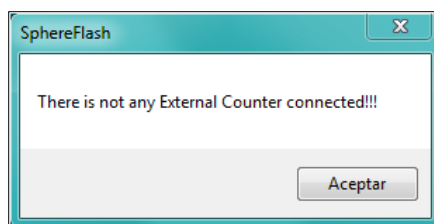
In the case of spiral distributions, proceed similarly as above, but now the displayed dialog will ask you to enter the particular data of how the spiral plate was counted: whether you have counted full rings or only two opposite 1/8 sectors, and at which ring zone you finished the count (the zone 3c being the outermost ring):



The dialog box titled "Manual Count" contains the text: "You are going to save a new register from manual count. Please confirm". Below this text are four input fields: "Plate identifier" (empty), "Count:" (35), "Vol.Count" (empty), and "CFUs/ml:" (empty). To the right of these fields is a "Last Counted Ring" section with six radio button options: 3c, 3c + 3b, 3c + 3b + 3a, 3 + 4c, 3 + 4c + 4b, and 3 + 4c + 4b + 4a. To the right of this is a "Count Method" section with two icons: a pie chart labeled "(2 x 1/8)" and a full ring labeled "Full ring". At the bottom are "OK" and "Cancel" buttons.

6.1.3. Using other means for a manual count

When selecting the *Manual Colony Counter* panel, the application first tries to detect whether a DOT instrument is connected to the computer; after a while, if no external counter has been detected a warning message is displayed:




Still, you can directly introduce a manual count by filling in the *Count* box of the panel:

 A screenshot of the 'Manual Colony Counter' panel. The panel has a title bar with a close button. Below the title bar, there is a message: 'There are any external counter connected!!! :-('. The panel is divided into three main sections:

- Operation Data:** Contains a 'Plate Id (*Required)' text box, a 'Method' dropdown menu (currently showing 'TEMPLATE_Sharp'), and a 'Comments' text area.
- Run Data:** Contains a 'Results Group' dropdown menu (showing 'Count Results'), an 'Inoculation Mode' dropdown menu (showing 'Spread'), a 'Dilution Factor' text box (showing '10'), and an 'Inoculate Volume (ml)' text box (showing '1').
- Results:** Contains a 'Count' text box (highlighted with a red circle and containing the value '25') and a 'Concentration' text box.

 At the bottom of the panel, there is a large button with a green checkmark icon.

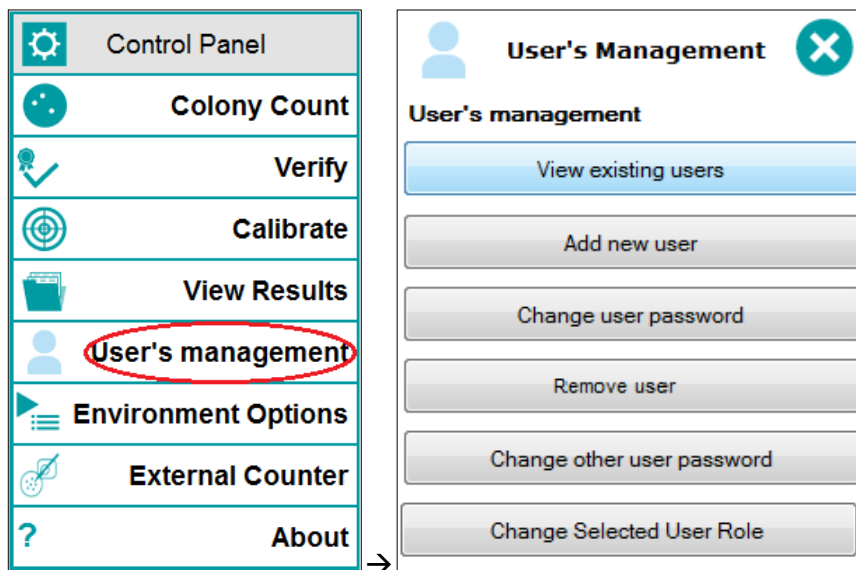
and by clicking on the *Run* button  , you can go on similarly as described in the above sections 0 and 6.1.2.

7. GENERAL ADMINISTRATION

NOTE: In order to manage the features of this section your user needs a role level of *administrator*. Given that some of these features could have implications on the compliance of some regulatory frameworks, you should be authorized by your company's management to act as administrator of the SphereFlash® software.

7.1. Users Management

In the control panel, select the option '*User's management*'; then the corresponding panel will be shown with several options:



7.1.1. Roles and permissions

Every user has a role assigned, and this role determines which features of the application are permitted to the user.

There are three different roles:

Administrator: This role has exclusive access to:

- Manage users (view, create, remove, assign role and password)
- Change environment and general settings
- Purge outdated results from the database
- Create, edit and delete custom views
- View, sort, filter, select, export, and print Audit Trail tables and records

Besides, the *Administrator* has full access to all the features allowed to the *Programmer* and *Operator* roles.

Programmer: This role has privileged access to:

- Create, edit, delete, and import methods

- Defining a method in which *Run Data* and *Input values* can be modified during a colony count, and whether interactive count modification is allowed.
- Save into a method the *Run Data* and *Input values* modified during a colony count operation
- Create, edit and delete worksheets
- Re-process the image of a previously stored result of a colony count operation

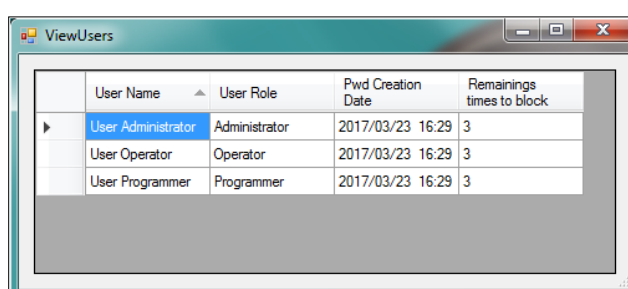
Additionally, the *Programmer* has full access to all the features allowed to the *Operator* role.

Operator: This role has regular full access to all the remaining features not listed above, particularly:

- Run a calibration and a verification operation
- Run a colony count operation, and if the method allows for it, change the *Run Data* and *Input values*, and interactively modify the count results
- Select and use an existing worksheet or *Remote Input*
- *Import and export* a worksheet
- Create and delete *Results Groups*
- Use *External Manual Count*
- Select which type of results *View* is displayed
- Sort, filter, select, export, and print results from the currently displayed *View*
- Export a method
- Change the own password of the user

7.1.2. View existing users

A table is displayed showing a list of all registered users, their name and permission role when their password was changed last time, and how many remaining failed logins are permitted until the user being blocked:



	User Name	User Role	Pwd Creation Date	Remainings times to block
▶	User Administrator	Administrator	2017/03/23 16:29	3
	User Operator	Operator	2017/03/23 16:29	3
	User Programmer	Programmer	2017/03/23 16:29	3

A user is blocked after 3 consecutive failed logins (wrong password). To unblock the user, either wait for a while or ask the administrator to solve the issue (either changing the user's password or removing the user and adding it again).

NOTE: The record of all failed logins can be seen in the *Audit Trail* (see section 5.4)

7.1.3. Add new user

The administrator can create a new user by assigning a name and a role, and entering a password for them:

NOTE: The first time a new user accesses the application, they should change the password assigned by the administrator to a new one.

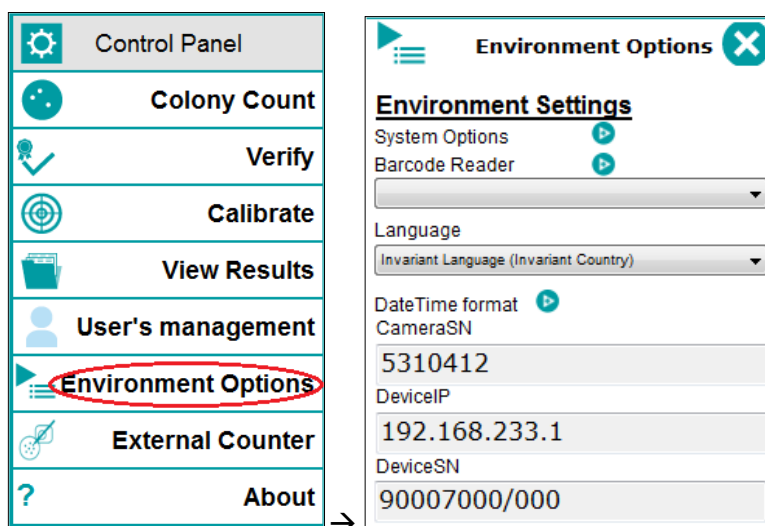
7.1.4. Other user management operations

The administrator can also change the password of other users, remove existing users, and change the role of an existing user.

Additionally, any user, regardless of its role, can change their own password.


7.2. Environment and System options

In the *Control panel*, select *Environment options*, and the corresponding panel will open:

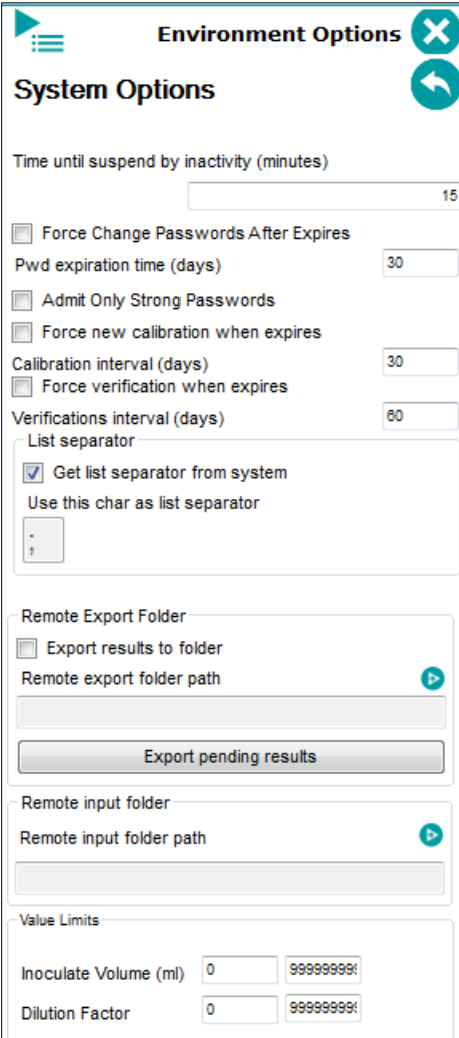



At the lower part of the panel, there is information about the currently connected SphereFlash® instrument.


You can choose the language of the user interface among those that have been shipped along with the installation package.

By clicking on the respective button  you can access to change the *System Options*, define the *Barcode Reader* settings, and select the display format of time and date.

7.2.1. System options



Environment Options 

System Options 

Time until suspend by inactivity (minutes)

☐ Force Change Passwords After Expires

Pwd expiration time (days)

☐ Admit Only Strong Passwords

☐ Force new calibration when expires

Calibration interval (days)

☐ Force verification when expires

Verifications interval (days)


List separator

☒ Get list separator from system

Use this char as list separator


Remote Export Folder

☐ Export results to folder

Remote export folder path 

Export pending results

Remote input folder

Remote input folder path 

Value Limits

Inoculate Volume (ml)

Dilution Factor

7.2.2. Expiration times

Time until suspend by inactivity: After this time elapsed since the last interaction between the user and the application, the application will freeze and a re-login will be requested to continue.

Password expiration time: After this time elapsed since the last password change of the current user, the application will warn the user to change its password.

Force Change Password After Expires: If this box is checked, when the change password message is displayed, the user cannot go on using the application until they change their password.

Admit only strong passwords: If this box is checked, only strong passwords will be allowed.

Calibration interval: After this time elapsed since the last calibration, the application will request the user to perform a calibration.

Force new calibration when expires: If this box is checked, when the calibration requested message is displayed, the user cannot go on using the application until they perform a new calibration.

Verification interval: After this time elapsed since the last verification, the application will request the user to perform a verification.

Force new verification when expires: If this box is checked, when the verification requested message is displayed, the user cannot go on using the application until they perform a new verification.


7.2.3. List separator

For exporting and exporting files in CSV format, you can define the list separator symbol, or let the application use the symbol that is defined in the Operating System regional settings.


7.2.4. Remote input/output folders


In order to interface with LIMs (see section 7.7), you need to define the path to the folders where the input and output XML files will be located.

Export results to folder: check this box if you want that the result of each *Colony Count* operation to be exported to a folder.

Remote export folder path: Click on the  symbol to access the dialog to choose the destination folder of the individual result files. The chosen path will appear in the box.

Export pending results: If the *Export results to folder* box is checked, but there is no destination path assigned or it is unreachable, the results to be exported are marked as pending. After solving the issue, the administrator can press this button to bulk transfer all the pending result files to the remote folder.

Remote input path: Click on the  symbol to access the dialog to choose the destination folder of the input plate-method association files (where the method and input values are defined for each plate ID). The chosen path will appear in the box. The activation of the input from the remote file by means of a button in the *Remote* tab of the *Colony Count* panel (see section 4.4).


Remote export folder path: Click on the  symbol to access the dialog to choose the origin folder of the input plate-method association files. The chosen path will appear in the box.

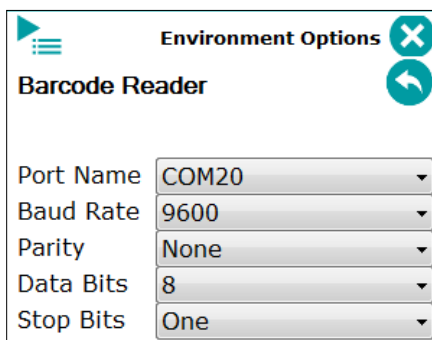
7.2.5. Value Limits

You can specify here the minim and maximum values that an operator is allowed to introduce in the *Inoculated Volume* and *Dilution Factor* boxes when performing a *Colony Count* operation, to minimize the risk of entering by error illogical values leading to wrong results. This is a safety measure required by some regulatory frameworks.

7.2.6. Barcode reader

You can use a serial (RS-232), barcode reader, to scan the plate ID from a label with a barcode.


Click on the  symbol to access the configuration of the barcode reader serial interface, and fill in or select the proper values according to the reader manufacturer indications. You can find the port name where the reader is connected to the computer using the Windows *Device Manager* dialog.

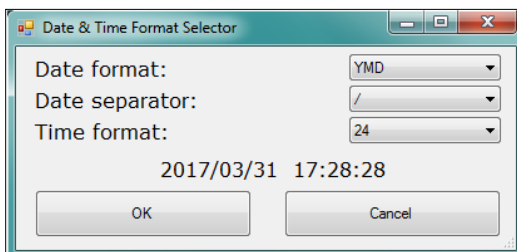


The dialog box is titled "Environment Options" with a subtitle "Barcode Reader". It contains five configuration fields, each with a dropdown menu:

- Port Name: COM20
- Baud Rate: 9600
- Parity: None
- Data Bits: 8
- Stop Bits: One

7.2.7. Date and Time format

Click on the  symbol to configure how the dates and times are formatted (order of year, month and day, separator, and 24 or 12 am/pm time format):



The dialog box is titled "Date & Time Format Selector". It contains three configuration fields, each with a dropdown menu:

- Date format: YMD
- Date separator: /
- Time format: 24

Below the fields, the current date and time are displayed: 2017/03/31 17:28:28. At the bottom are "OK" and "Cancel" buttons.

7.3. Customizing the logo on the reports

The printed reports and tables appear with the logo of IUL. If you want that your logo appears instead, replace it in a .jpg image file with the same path and filename at:

```
%Program Files%\IUL\SphereFlash\logo\logo.jpg
```

7.4. Digital signature of PDF reports and tables


You can apply a digital signature to the PDF reports and tables using third-party applications properly linked to the SphereFlash® application.

Configuring the digital signature feature:

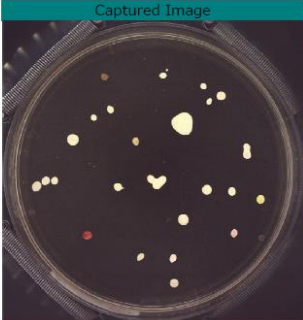
- a) Install in the computer a virtual PDF printer such as *PDFCreator*
- b) Configure the virtual printer to automatically open the document after the PDF file has been created
- c) Install in the computer *Adobe Acrobat Reader DC*®
- d) Associate the file type PDF to be automatically opened by *Adobe Acrobat Reader DC*®

Applying the digital signature to a PDF report or table:


- e) Select or filter the desired rows from the current view
- f) Left-click on them and select *Print Report* or *Print Table*
- g) On the print dialog, select the virtual PDF printer (*such as PDFCreator*)
- h) When asked for, select the destination folder and give a name to the unsigned PDF file to be created
- i) When the file has been automatically opened by *Adobe Acrobat Reader DC*®, in this application go to *Tools > Certificates > Digital Signature*.
- j) You will be prompted to define an area on the document where the digital signature will be printed.
- k) You will be prompted to use an existing certificate or to create a new one
- l) Select the destination folder and give a name to the digitally signed PDF file to be created.



SphereFlash
Automated Colony Count



Captured Image



Processed Image

Plate ID:	plate45
Method:	ThreeColors
Date/Time:	2017/04/04 14:55:31
Operator:	User Administrator
Results Group:	Count Results

Inoculation Mode:	Spread
Inoculated Volume(mL):	1
Dilution Factor:	10
Excluded Area:	15.79 %

ColonyClass	Mark Color	Count	Counted Volume(mL)	Result CFU/mL
white	+	26	0.842	3.09E+02
yellow	+	3	0.842	3.56E+01
brown	+	2	0.842	2.38E+01

Input values (Method related)

Threshold to background: 80; Plate diameter: 85.0; Minimum diameter: 0.5

Comments

John Smith

Digitally signed by John Smith
Date: 2017.04.05 10:59:02 +02'00'

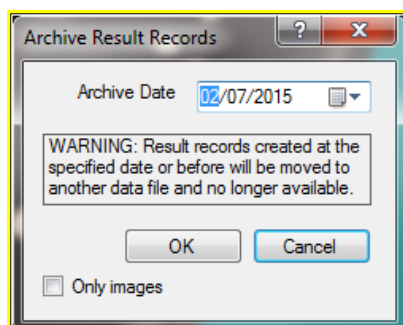
7.5. Removing outdated results (purge)

When there are in the database old results and their images that you no longer need to review, using a purge operation you can remove them from the database, moving them into a historic archive file. This way the database will not grow indefinitely, compromising the performance of the application.

Your user role must be that of *Administrator* to being allowed to perform this purge operation.

Before purge, you have to make sure that you have exported or printed all the old results from which you need to keep their information in external support (Excel, prints, or PDF reports...).

To purge the results, click on the *Purge* button in the *View Results* panel, and a dialog will appear where you can define the limit date where the results older than this will be removed. There is also a checkbox allowing to purge only the images but keeping the results (images take a lot of disk space).



You will be warned that **the purge process can take a long time**, in extreme cases it can last up to one hour. It's advisable that you launch the purge at the end of the working day, leaving the purge operation being run overnight. Please be patient and don't try to shut down the application using the Windows *Task manager* or resetting the computer.

NOTE: There are regulatory frameworks that require keeping the information for a prescribed minimum period of time; it is the responsibility of the administrator user to take the proper measures to make sure that these regulations are not broken when purging the database.

7.6. Managing the database files

7.6.1. Location and backup of the active database files

It is the responsibility of the client's Systems Administrator to perform a reliable regular backup copy of the active database files where the settings and results created by the application are stored.


The path where these active database files are stored is:

```
%ProgramData%\IUL\SphereFlash
```

All the files inside this folder are to be backed up regularly, as prescribed by the client's policies, and by the regulatory frameworks that must be fulfilled.

7.6.2. Location of archived database files resulting from purge operations

The archived databases resulting from purge operations are stored in the same path above, inside a folder whose name starts with '@' followed by the date and time corresponding to the purge limit date of the archive:

 @2016-10-14_636269827042756418

7.6.3. Temporarily restoring archived databases

If the results contained in an archived database must be reviewed or exported/printed, the Systems Administrator can proceed this way:

1. Close the application
2. Backup the active database files (section 7.6.1)
3. Locate the desired archive folder (section 7.6.2)
4. Copy the contents of the archive folder to the active database path (section 7.6.1)
5. Open the application, and perform the review, export, or print off the desired archived results

6. Close the application and restore the active database files previously backed up in step 2.

7.7. Interfacing with a LIMS (Laboratory Information Management System)

The SphereFlash® application can interface in a corporate network with a LIMS to automatically and in real-time:

- Send to the LIMS the Colony Count results after processing each plate (*Remote Out* to a folder).
- Get from the LIMs which method and which Run / Input values must be applied to process each plate, similarly, as working from worksheets (*Remote In* from a folder).

To implement these features, the following subjects must be addressed:

- a) Two folders have to be designated as the *Remote In* and the *Remote Out* folders; they may be located either in the computer containing the application or in some server reachable through the network.
- b) The systems administrator must grant the proper access permissions to both folders by the *SphereFlash®* application as well as the LIMS system.
- c) The systems administrator must ensure the rules of privacy and restricted access to the data in these folders, as mandated by the company policies and any regulatory framework that would be applicable.
- d) In the *System Options* panel (section 7.2.4) define the paths to the respective remote folders.
- e) In the *System Options* panel (section 7.2.4) check the *Export results to folder* checkbox.
- f) In the *Remote* tab of the *Colony Count* panel, check the *Remote input enabled* checkbox, and select the button *Input folder* (section 4.4).
- g) Each plate must have a unique identifier (*Plate ID*), it's advisable to use a barcode to avoid operator errors.
- h) The LIMS must create in advance, for each *Plate ID*, an XML file containing the settings (method and *Run / Input Values*) required to process the plate, and place this file into the *Remote In* folder.
- i) The LIMS must collect from the *Remote Out* folder each XML file that will appear after each *Colony Count* operation, parse it and transfer the count results to its database.
- j) The LIMS should delete from both *Remote In* and *Remote Out* folders the files corresponding to the plates already processed.

7.7.1. Remote input file example

File name:

RI05.xml

(where *RI05* is the plate ID to be processed with the settings in the file)

File contents:

```
<?xml version="1.0" standalone="yes"?>
<plate>
  <plate_id>RI05</plate_id>
  <method_name>TEMPLATE_Sharp</method_name>
  <inoculation_mode>Spread</inoculation_mode>
  <inoculated_volume>1</inoculated_volume>
  <dilution_factor>10</dilution_factor>
  <result_group>Count Results</result_group>
  <th_to_background>79</th_to_background>
  <abs_min_diameter>0.30</abs_min_diameter>
  <plate_diameter>80.0</plate_diameter>
</plate>
```

NOTE: Only nodes <plate_id> and <method_name> are compulsory.

7.7.2. Remote output file example

File name:

37254674.xml

(where *37254674* is an incremental unique number, not the Plate ID)

File contents:

```
<?xml version="1.0" standalone="yes"?>
<DocumentElement>
  <ColonyClass>
    <InputValues>80 ;85.0 ;0.3</InputValues>
    <Id>5</Id>
    <CountFromAutomaticProcess>27</CountFromAutomaticProcess>
    <UTCCreatedTime>2017-03-29T14:01:14.225+02:00</UTCCreatedTime>
    <DeviceSN>90007000/000</DeviceSN>
    <ExcludedFraction>0</ExcludedFraction>
    <InoculationMode>D Mode 50ul</InoculationMode>
    <InoculatedVolume>0.05</InoculatedVolume>
    <DilutionFactor>10</DilutionFactor>
    <Operator>User Administrator</Operator>
    <CreatedTime>2017-03-29T16:01:14.225+02:00</CreatedTime>
    <Comments />
    <CFUml>5400</CFUml>
    <CountedVolume>0.0500000007450581</CountedVolume>
    <Count>27</Count>
    <ColonyName>Class 1</ColonyName>
    <MethodName>TEMPLATE_Sharp</MethodName>
    <PlateId>plate28</PlateId>
    <ResultsGroupName>Count Results</ResultsGroupName>
  </ColonyClass>
</DocumentElement>
```

8. REGULATORY COMPLIANCE: FDA 21CFR PART 11

NOTE: The availability of the features described in this section requires the *Colonies PRO* license.

The SphereFlash® application software is designed to enable the customer to comply with FDA regulations related to 21 CFR part 11, allowing the use of electronic records instead of paper records.

Summary of the key elements of compliance:

- The original electronic records are encrypted and protected to ensure authenticity, integrity, confidentiality, and ready retrieval throughout the retention period (§11.10 a, c).
- The software has the ability to generate accurate and complete copies of records both in human-readable and electronic form (§11.10 b).
- The access to the software, its use, and operation, is limited to authorized individuals (§11.10 d, g).
- The software automatically generates an Audit Trail that records all the user actions that create, modify or delete electronic records. This Audit Trail is time-stamped and encrypted, and cannot be altered by the users (§11.10 e).
- The correct and appropriate sequence of operations, steps, and events is enforced by the software and cannot be altered by the user (§11.10 f)
- Data input from the user is checked or limited to valid and appropriate values (§11.10 h)

Features of SphereFlash® software related to FDA 21CFR part 11 compliance:

#	section of FDA 21CFR part 11	Topic	Requirement	SphereFlash® software implementation	Customer action is required to comply	Comments
1	11.10 (a)	Validation	The system must be validated for the intended use		X	The customer is responsible for the validation of the system in which the SphereFlash® is integrated, according to the particular use intended by the customer.
				X		The SphereFlash® software provides standard template methods to be used for customer validation.
2	11.10 (a)	Changes on records	It must be possible to discern invalid or altered records	X		<p>The results of all the operations and the settings of methods are recorded in an encrypted database, which prevents any modification of its contents neither from the application nor from outside it.</p> <p>If an operator re-processes the same plate or the image of a previously processed plate, both the old and new results records are kept, being distinguishable by their respective timestamps.</p> <p>All the operator actions, changes, and entries to the operation results, system settings, and operation methods, that are relevant for the validity of the results of the operations, are automatically recorded in an Audit Trail which includes the date, local, and UTC time, user, and change that has been performed.</p> <p>If the date/time of the computer has been manipulated, so that there are stored records with a timestamp apparently in the future, a warning comment is automatically added to each new colony count result record.</p>
					X	The customer must ensure that, on the computer where the SphereFlash® software is installed, the date and hour cannot be modified by unauthorized individuals.

#	section of FDA 21CFR part 11	Topic	Requirement	SphereFlash® software implementation	Customer action is required to comply	Comments
3	11.10 (b)	Report, printout, electronic record, FDA review	The system must be able to produce accurate and complete copies of the records on paper as well as in electronic form, allowing for their review by FDA.	X		Records data can be exported to Excel, CSV, and text files. The results data of each operation can be automatically exported in real-time to an XML file when <i>Remote Out</i> mode is active. Records data can be printed to paper or PDF files in table and report formats. Generated PDF reports and tables can be automatically linked to a third-party application, to be digitally signed to ensure its authenticity, integrity, and authorship. When exporting or printing reports, the exact data contents of the records in the database are automatically transferred to the file or printout; there is no possibility to modify the data.
					X	After exporting to a file outside the application, it is the responsibility of the customer to ensure the integrity of its contents.
4	11.10 (c)	Protection of records, retention period	The records must be readily available throughout their retention period	X		The results of all the operations and the settings of methods are kept in an encrypted database, which prevents any modification of its contents neither from the application nor from outside of it. All the records of the active database are directly available for review, export, and print until the authorized user performs a purge of the outdated records into an archive file. Even in the archive file, the outdated records are still safeguarded under encryption, and they can be retrieved for their consultation if needed.
					X	It is the responsibility of the customer to perform periodic back-ups of the database files

#	section of FDA 21CFR part 11	Topic	Requirement	SphereFlash® software implementation	Customer action is required to comply	Comments
5	11.10 (d)	Login, access authorization	The system must limit the access to the system to only authorized individuals	X		<p>The SphereFlash® software is provided with a login feature that limits access only to authorized individuals using a username and a password.</p> <p>Each user is assigned a role that defines the subset of features for which they have permission.</p> <p>The users with administrator roles have the responsibility to create or delete users, and to assign them the proper role.</p> <p>The system can be configured by the administrator to request the users a periodical change of their password.</p> <p>After several failed login attempts, a user becomes blocked and the intervention of the administrator is required to unblock them.</p> <p>All the changes to the users and their roles, as well as the login events, successful or not, are recorded in the Audit Trail.</p> <p>If the operator leaves the computer unattended for more than a scheduled time, the application will be blocked, requiring a new login.</p>
					X	The customer has the responsibility to designate and empower a staff member to act as administrator of the SphereFlash® software application
6	11.10 (e)	Audit trail, timestamps	The system must automatically generate a secure, time-stamped audit trail, that logs the date and time of user entries and actions which create, modify or delete electronic records	X		All the operator actions, changes, and entries to the operation results, system settings, and operation methods, that are relevant for the validity of the results of the operations, are automatically recorded in an Audit Trail which includes the date, local and UTC time, user, and change that has been performed.
					X	The customer must ensure that, on the computer where the SphereFlash® software is installed, the date and hour cannot be modified by unauthorized individuals.

#	section of FDA 21CFR part 11	Topic	Requirement	SphereFlash® software implementation	Customer action is required to comply	Comments
7	11.10 (e)	Overwriting data	When modifying electronic records, the previously recorded information must still be available in the system, not being overwritten by the modification	X		<p>All the operation results data, after being recorded, are kept in the database with no possibility of further modification. If an operator re-processes the same plate, or the image of a previously processed plate, both the old and new results records are kept, being distinguishable by their respective timestamps.</p> <p>All the modifications of the settings of methods or the general system settings are recorded in the Audit trail, where the user, date and time, and changed values can be seen.</p>
8	11.10 (e)	Audit Trail, retention period	The Audit Trail of an electronic recording must be retrievable throughout the retention period of the record	X		<p>The Audit Trail entries are kept in the same encrypted database as the results records, which prevents any modification of its contents neither from the application nor from outside of it.</p> <p>All the Audit Trail entries of the active database are directly available for review until the authorized user performs a purge of the outdated records into an archive file.</p> <p>Even in the archive file, the outdated records are still safeguarded under encryption, and they can be retrieved for their consultation if needed.</p>
					X	It is the responsibility of the customer to perform periodic back-ups of the database files
9	11.10 (e)	Audit Trail, FDA review	The Audit Trail must be available for review and copying by the FDA	X		<p>Audit Trail PDF reports can be generated and automatically linked to a third-party application, to be digitally signed to ensure its authenticity, integrity, and authorship.</p> <p>When exporting or printing those reports, the exact Audit Trail contents in the database are automatically transferred to the file or printout; there is no possibility to modify the data.</p>
					X	After exporting the Audit Trail to a file outside the application, it is the responsibility of the customer to ensure the integrity of its contents.

#	section of FDA 21CFR part 11	Topic	Requirement	SphereFlash® software implementation	Customer action is required to comply	Comments
10	11.10 (f)	Sequence of steps	The system must use operational checks to enforce the permitted sequence of steps and events as appropriate	X		<p>The sequence and setting values required for each operation type are enforced by the method definition, which doesn't allow the operator to modify any of the steps or settings except those data that purposely have been decided that will be entered by the operator.</p> <p>Additionally, the <i>Remote In</i> and <i>Worksheet</i> features will enforce the application of the method and setting values required for each particular plate ID.</p> <p>Only users having assigned <i>Programmer</i> or <i>Administrator</i> role are able to define method settings, and to associate in a worksheet each plate ID with the method and settings required for its processing.</p>
					X	The customer has the responsibility to designate and empower suitable staff members to act as <i>Programmers</i> .
11	11.10 (g)	User permissions	The system must ensure that only authorized individuals can use the system, access the functions, modify a record or perform other operations	X		<p>The SphereFlash® software is provided with a login feature that limits access only to authorized individuals using a username and a password.</p> <p>Each user is assigned a role which defines the subset of features for which they have permission.</p> <p>The users with administrator role have the responsibility to create or delete users, and to assign them the proper role.</p> <p>The system can be configured by the administrator to request the users a periodical change of their password.</p> <p>After several failed login attempts, a user becomes blocked and the intervention of the administrator is required to unblock them.</p> <p>All the changes to the users and their roles, as well as the login events, successful or not, are recorded in the Audit Trail.</p> <p>If the operator leaves the computer unattended for more than a scheduled time, the application will be blocked, requiring a new login.</p>

#	section of FDA 21CFR part 11	Topic	Requirement	SphereFlash® software implementation	Customer action is required to comply	Comments
					X	The customer has the responsibility to designate and empower a staff member to act as administrator of the SphereFlash® software application.
12	11.10 (h)	Validity check of input data	The system must determine, as appropriate, the validity of the source of data input or operational instruction	X		<p>The <i>Activation Key</i> for the <i>Colonies PRO</i> license is linked to the serial number of the SphereFlash® instrument of the client. No plate images acquired with devices other than this particular instrument will be admitted to be processed.</p> <p>The values that the operator is allowed to introduce manually, are either limited to a selection list that contains only the possible meaningful values or are numerical values that can be restricted to lie within a minimum and maximum values, defined in the general settings of the application.</p> <p>Additionally, the <i>Remote In</i> and <i>Worksheet</i> features will enforce the application of the method and setting values required for each particular plate ID.</p>

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