

## Quick Guide: How to use the SphereFlash® AI

This quick guide explains how to use the SphereFlash® AI to process plates. It is intended for users who want to work with the general AI model, as well as for partners who wish to train the model for specific plate types or develop a custom model tailored to their own plates. For more details on the various functionalities of the application, please refer to the SphereFlash® AI Instructions for Use (50008740).

Each user may work with different plate types, and these can have different challenges. For this reason, using the correct AI settings is important to improve the accuracy of automatic colony counting. The most important setting when using the application is the **AI score**.

AI score: This is a ratio between specificity and sensitivity. It controls how strict the SphereFlash® AI is when counting colonies.

- A higher AI score makes the AI **stricter**, more specific. It only counts colonies that meet clear conditions, so fewer colonies are counted.
- A lower AI score makes the AI more **flexible**, more sensitive. It accepts more colonies, so more colonies are counted.

High AI score = fewer colonies

Low AI score = more colonies

Different plate types need different AI scores. Setting the correct AI score for a plate type helps minimize or delete:

- False positives (colonies that should not be counted)
- False negatives (colonies that should be counted but are missed)

### Single colony class

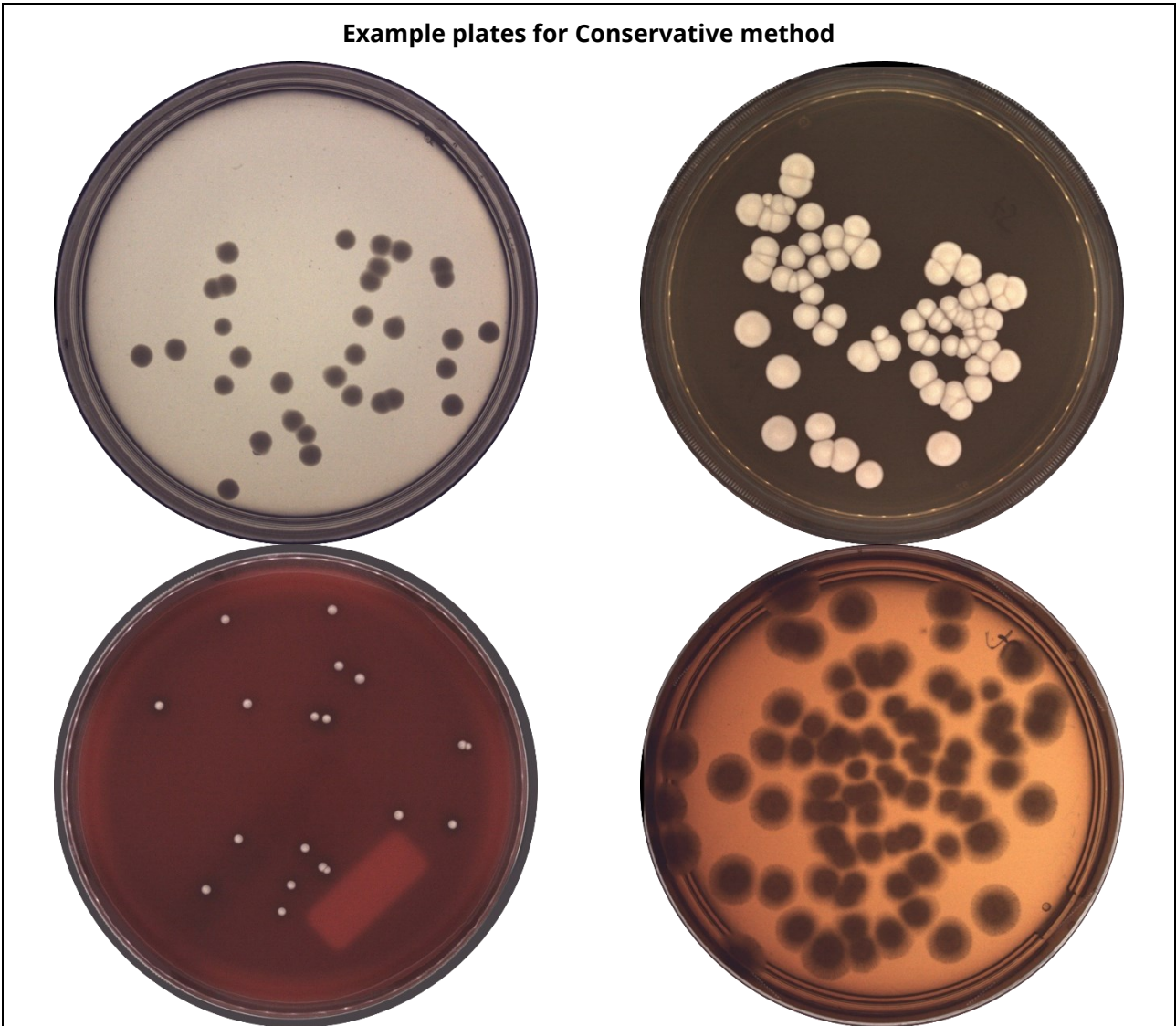
Follow these steps to use the AI if you have only 1 colony class in the plate:

1. Choose a default counting method. There are two options:
  - Option A: Conservative
  - Option B: Standard

#### Option A: Conservative

This is the default method. This is method is the choice for most plate cases.

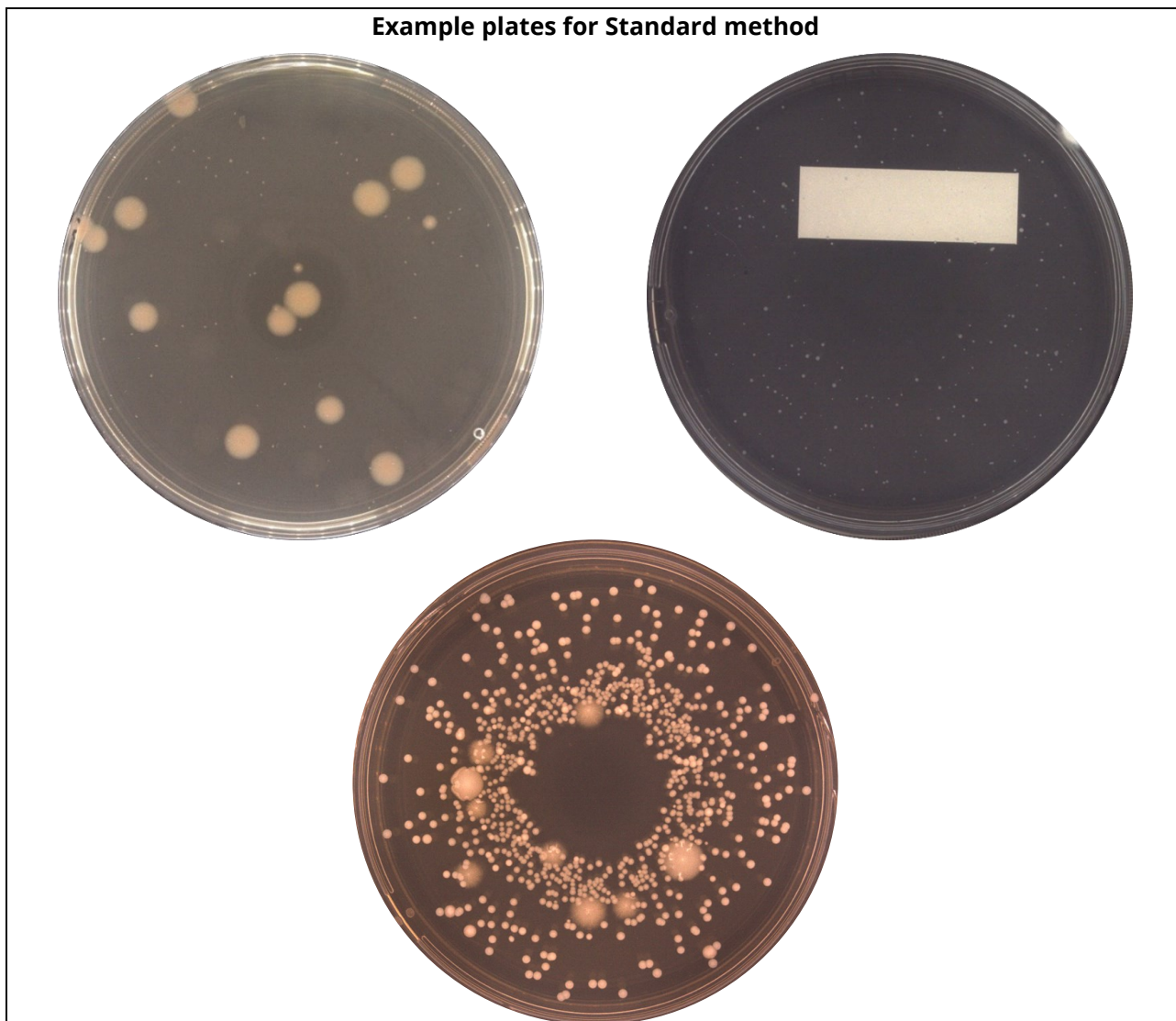
Example plates for Conservative method



### Option B: Standard

Choose this method for specific cases only. This includes plates with lots of colonies (200+), very small colonies, lots of small overlapping colonies (if there are only a few medium or big overlapping colonies, choose Conservative), and rounded colonies (colonies with fuzzy edges, choose Conservative).

Example plates for Standard method



2. Capture (if using the SpherFlash® instrument) or upload the plate image and then process your image.
3. Use the AI score slider to manually adjust the AI score:
  - Lower AI score = more colonies counted
  - Higher AI score = less colonies counted

If, after AI Score optimization, the plate already shows the **correct final counting result**, the results can be saved.

4. Click the **“Save”** button to store the result.



See example video for AI score slider [HERE](#)

If the plate could not be successfully counted using the AI score alone, do NOT save the results yet.

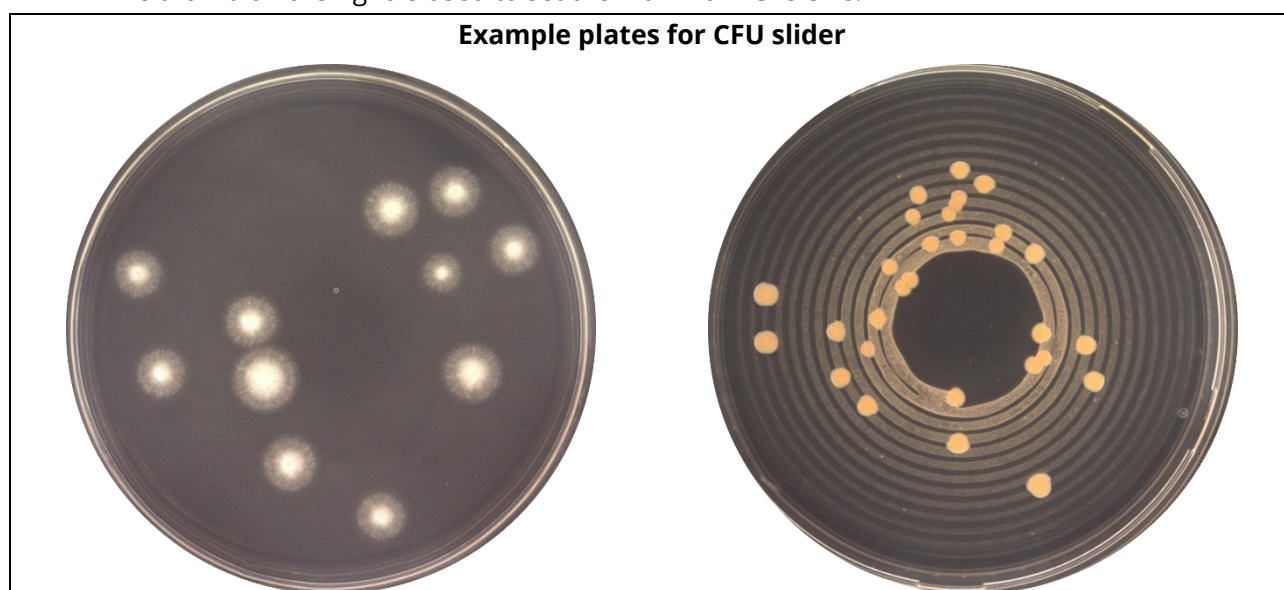
Depending on your specific case, use one of the following:

- Option A: CFU slider
- Option B: Manual addition or deletion of colonies

### Option A: CFU slider

For plates with lots of artifacts that are either bigger or smaller than the colonies, use the CFU size slider. The CFU size slider has two thumbs:

- The thumb on the left is used to set the minimum CFU size
- The thumb on the right is used to set the maximum CFU size.



1. Move the left thumb to the right to reject smaller colonies/artifacts or move the right thumb to the left to reject larger colonies/artifacts.  
If, after CFU Size optimization, the plate already shows the **correct final counting result**, this image can be saved.
2. Click the "Save" button to store the result.

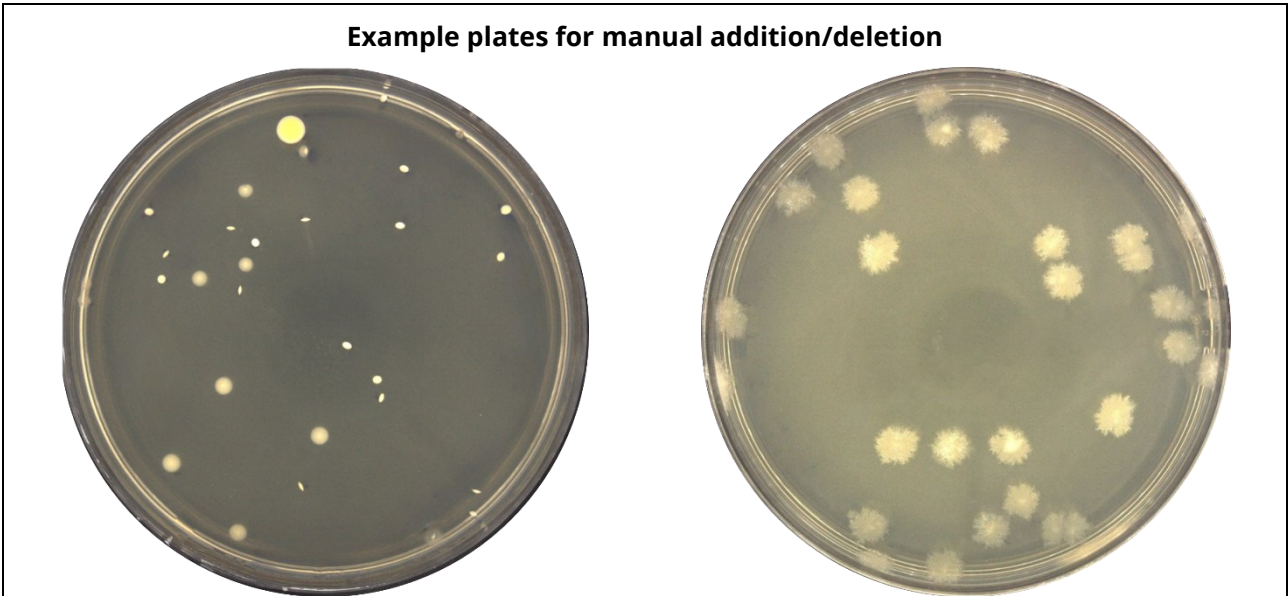


See example video for CFU size slider [HERE](#)

### Option B: Manual addition or deletion of colonies

For other cases, such as overlapping colonies (that cannot be separated by the CFU size) or plates with just a few artifacts especially close to the size of the desired colony to count, colonies can be manually added or artifacts can be manually deleted.

**Example plates for manual addition/deletion**



1. Double-click on the colony class (it will be highlighted in yellow). Two buttons should appear on the top right corner of the Image Panel.
2. Click on the minus (-) button, then start clicking on the center of the artifacts that you want to exclude.
3. Click on the plus (+) button, then start clicking on the center of the colonies you want to include.
4. Once all the colonies have been added or all artifacts have been deleted, click the "Save" button to store the result.



See example video for manual addition/deletion [HERE](#)

## Two different colony classes

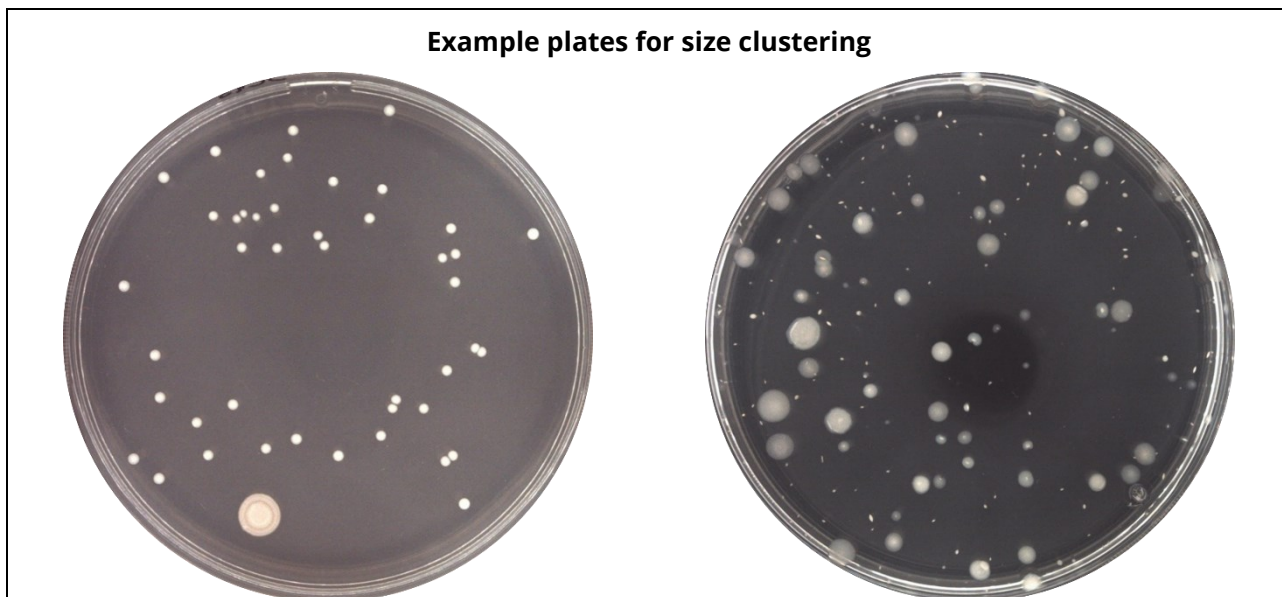
For plates with different colony classes in a single plate, there are two clustering methods that can be created:

- Option A: Differentiation by CFU size
- Option B: Differentiation by colony colors

### Option A: Differentiation by CFU size

For plates with different colony classes of different CFU sizes in a single plate, a CFU size clustering method can be created.

**Example plates for size clustering**



Follow these steps to create the method:

1. Click on the 'Method settings' button.
2. Choose the Method name: 'Conservative' or 'Standard' and fill in the rest of the name manually.
3. In the 'Count Mode', choose 'Filtered by size'.
4. Add the size range for the first colony class by defining the Min. and Max. CFU size.
5. Click on the 'Plus' button to add another colony class.
6. Repeat steps 3 and 4 until all colony classes have been defined.
7. Click the 'Run' button to test the method with a plate of choice.
8. Adjust the parameters as required.
9. Click the 'Save' button to save the method.
10. Use this method to count several similar plates to train the model.



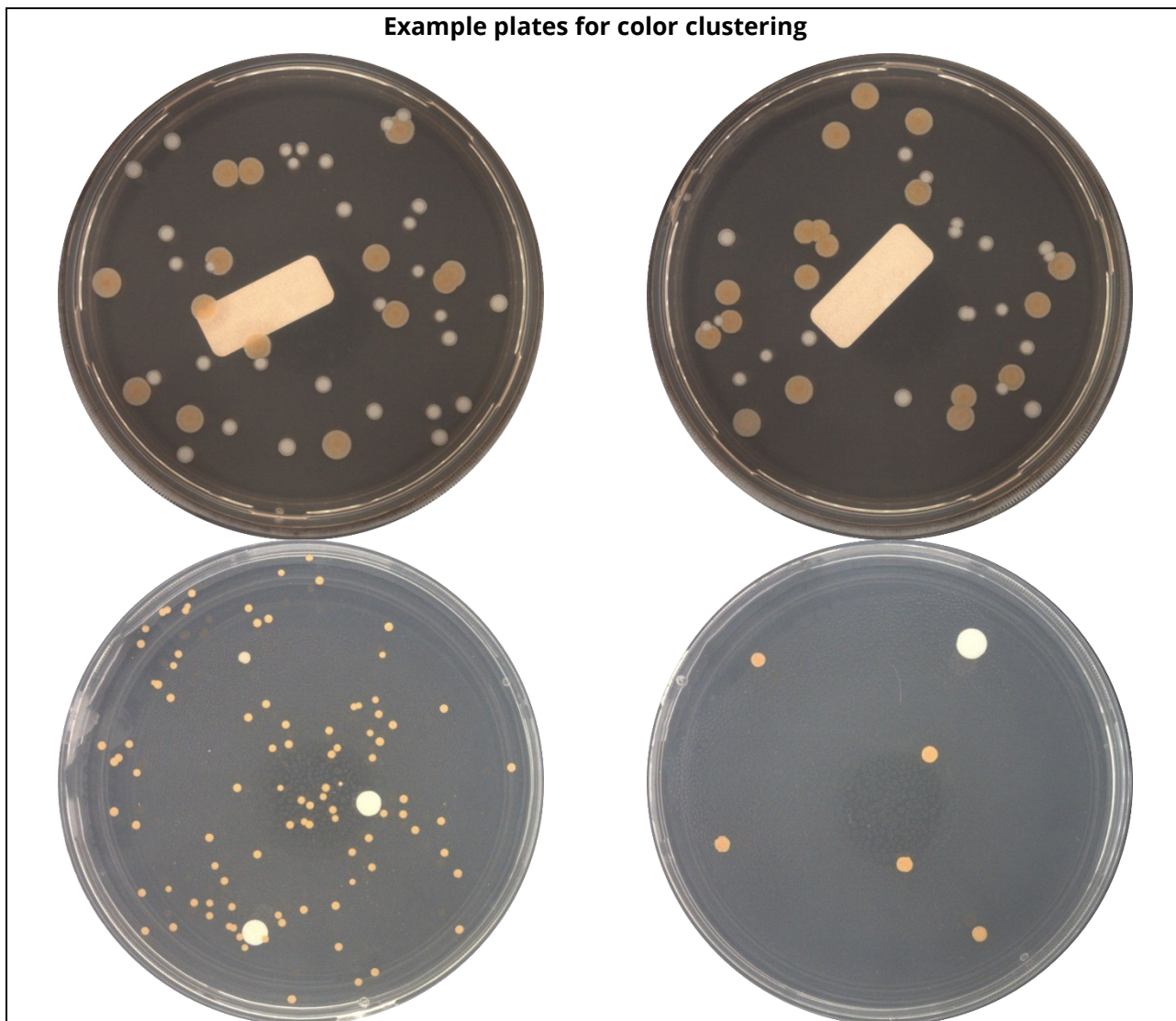
See example video for size clustering method creation [HERE](#)

### **Option B: Differentiation by colony colors**

For plates where colony classes are defined by colony colors, a method based on color classification is recommended. Use a color classification method for the following cases:

- Case 1: Plates with colonies of different colors and ALL colonies must be classified into a colony class i.e. there are no colonies to be rejected.
- Case 2: Plates with colonies of different colors and only ONE colony class must be counted and others rejected.
- Case 3: Plates with several colonies of different colors with some colonies classified into different colony classes and others rejected.

Example plates for color clustering



**Case 1:** Plates with colonies of different colors and must be classified into different colony classes and there are no colonies to be rejected.

Follow these steps to create the method for case 1:

1. Click on the 'Method settings' button.
2. Choose the Method name: 'Conservative' or 'Standard' and fill in the rest of the name manually. See example:
3. In the 'Count Mode', choose 'Filtered by color'.
4. Under 'Accept colors', click on the plus button, then click on the colony that you want to count. The color of that colony should now appear in the 'Accept colors' field.  
**NOTE:** Do NOT use the 'Reject colors' option for the other colonies that you want to count in other colony classes.
5. Click on the 'Plus' button to add another colony class.
6. Repeat steps 3 and 4 until all colony classes have been defined.
7. Click the 'Run' button to test the method with a plate of choice.
8. Adjust the parameters as required.

9. Click the 'Save' button to save the method.
10. Use this method to count several similar plates to train the model.



See example video for color clustering case 1 method creation [HERE](#)

**Case 2:** Plates with colonies of different colors and only ONE colony class must be counted and others rejected.

Follow these steps to create the method for case 2:

1. Click on the 'Method settings' button.
2. Choose the Method name: 'Conservative' or 'Standard' and fill in the rest of the name manually.
3. In the 'Count Mode', choose 'Filtered by color'.
4. Under 'Accept colors', click on the plus button, then click on the colony that you want to count. The color of that colony should now appear in the 'Accept colors' field.
5. Under 'Reject colors', click on the plus button, then click on the colony that you want to reject. The color of that colony should now appear in the 'Reject colors' field.
6. Repeat step 5, until all the colonies that are to be rejected have been added.

**NOTE:** There is NO need to select all the colonies of the same color. You only need to click on one colony of the same color to reject.

7. Click the 'Run' button, to test the method with a plate of choice.
8. Adjust the parameters as required.
9. Click the 'Save' button to save the method.
10. Use this method, to count several plates to train the model.



See example video for color clustering case 2 method creation [HERE](#)

**Case 3:** Plates with several colonies of different colors with some colonies classified into different colony classes and others rejected.

Follow these steps to create the method for case 3:

1. Click on the 'Method settings' button.
2. Choose the Method name: 'Conservative' or 'Standard' and fill in the rest of the name manually.
3. In the 'Count Mode', choose 'Filtered by color'.
4. Under 'Accept colors', click on the plus button, then click on the colony that you want to count. The color of that colony should now appear on the 'Accept colors' field.
5. Under 'Reject colors', click on the plus button, then click on the colony that you want to reject. The color of that colony should now appear on the 'Reject colors' field.

**NOTE:** Do NOT reject a colony if you want it to be counted in another colony class. Only reject colonies that you do NOT want to be counted at all.

6. Repeat steps 4 and 5 until all colony classes have been defined.
7. Click the 'Run' button to test the method with a plate of choice.

8. Adjust the parameters as required.
9. Click the 'Save' button to save the method.
10. Use this method to count several plates to train the model.



See example video for color clustering case 3 method creation [HERE](#)

## Model training for specific plates

The SphereFlash® AI software includes a **pre-trained model** developed and validated by IUL. This is the default model delivered with the system.

Model training is performed exclusively by our Data Science team.

If users wish to retrain or optimize the model for their **specific plate types and counting criteria**, they must **follow the procedure described in this guide**. Custom AI training requires a **sufficient number of representative positive samples** to ensure robust and reliable performance.

For training purposes, **annotated result** is required. An annotated result shows the desired final result for the user — where the colony count shown in the processed image reflects all the desired colonies and excludes all artifacts. This can be achieved by first adjusting the AI score and then if needed, performing manual adjustments through the CFU size filter, manual addition or deletion, or in some cases creation of a new method.

Only plates that reflect the **true final counting criteria** should be saved and used for training.