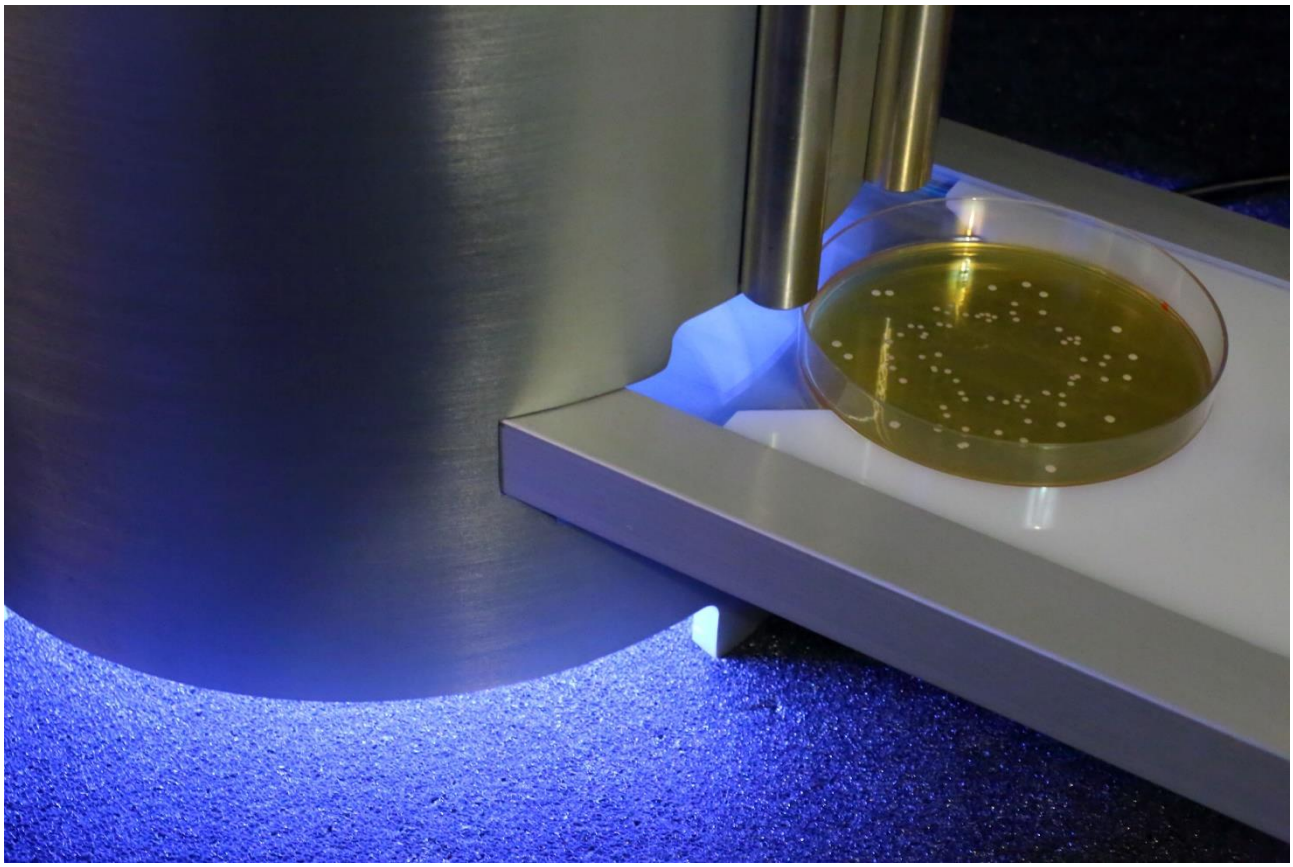
BIORAS
Rapid Analysis Systems for Biology and Life Sciences

Petrilyzer™

Automatic colony counter



Users manual

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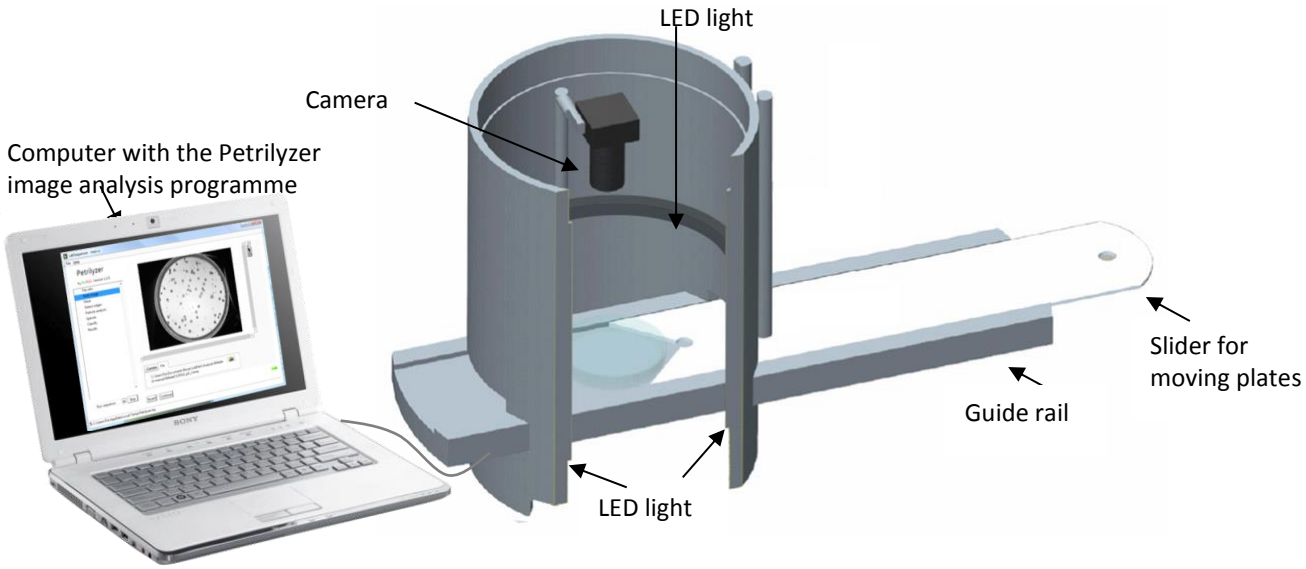
Overview

The Petrilyzer colony counter automatically counts bacterial colonies on agar plates using image analysis. Most plate types can be analysed. The plates are counted at a rate of approx. 3 sec. per plate. The camera inside the light-box is connected to a computer where images are analyzed to identify colony numbers and sizes of colonies on each plate. An image of each plate is stored together with the results from the analysis.

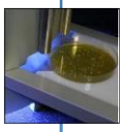


Specifications

- **Materials**: solid aluminium and stainless steel
- **Weight**: 14.4 kg Diameter: 25 cm, height: 34.5 cm
- **Lighting**: Waterproof LED strip with high bright LEDs for 12 Volt DC, lifetime more than 10.000 hours
- **Camera**: USB CMOS industrial machine vision colour camera, 5.0 Megapixel resolution
- **Lens**: Lens 2/3" SHRL 8mm f/1.4
- **Software**: LabView application

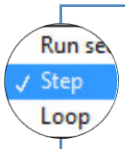
Petrilyzer Quick Start Guide

Start the Petrilyzer program

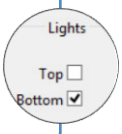


Insert a Petri-dish into the light box

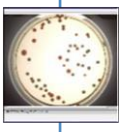
Setup of analysis:



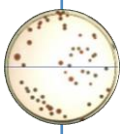
In the menu to the lower left select "Step"



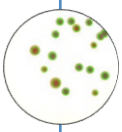
1. Start the setup: Adjust lighting



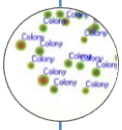
2. Grab image



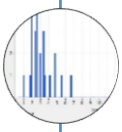
3. Mask to remove area outside of the petri-dish



4. Detect edges



5. Classify according to shape and colour



6. Size of colonies is measured

7. Results for current sample are shown

8. Save data

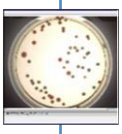
9. Save settings

Routine analysis:

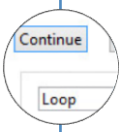
Load settings for the current analysis.

In the menu to the lower left select "Loop"

1. Start the analysis. Push the "Go" button

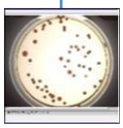


2. Grab image



3. Push the "Continue" button

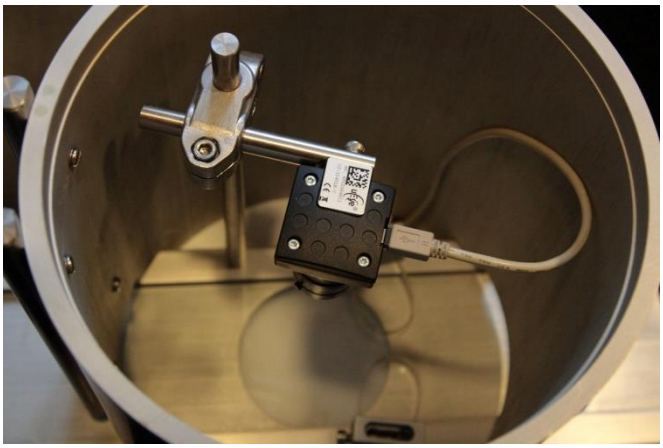
4. Results are shown. Push "Continue"



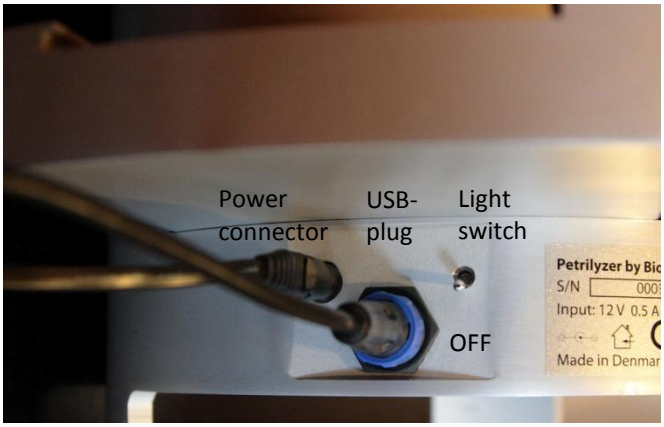
5. Analyze the next sample: Insert a new Petri-dish, Grab image, and run cycle again

Assembly of the Petrilyzer

Open the lid and insert the USB plug into the camera inside the cylinder.



- Plug the other end of the cable into a USB port on your computer.
- Plug the second USB cable, which controls the lights, into a second USB port on your computer
- Plug the power adapter into the slot below the cylinder and into a wall outlet.
- Turn on the light with the light switch.



Petrilyzer startup

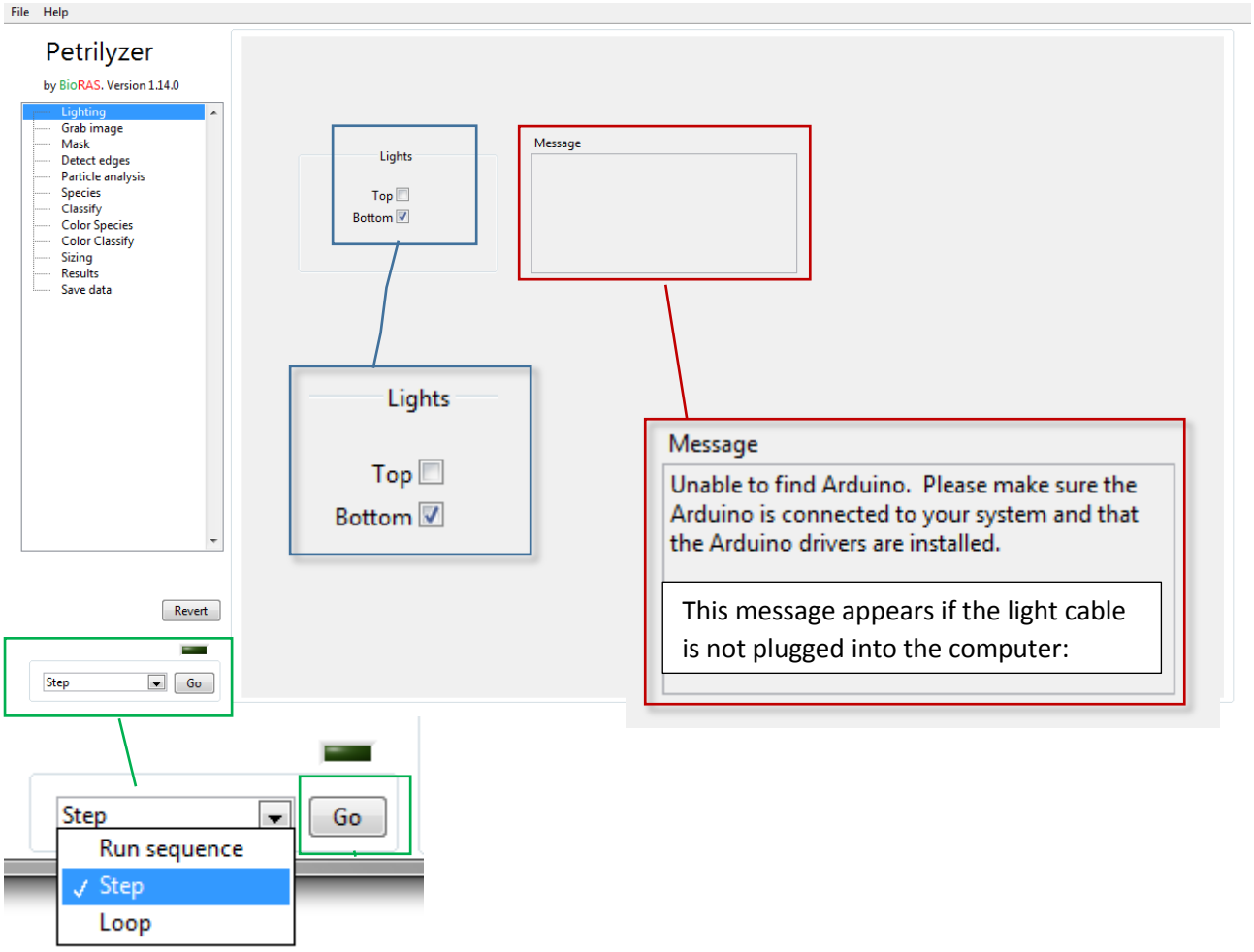
Open the application by double clicking the Petrilyzer icon on the desktop.

Petrilyzer will open in the Lighting dialog window.

Petrilyzer is delivered with standard settings for PCA plates.

Settings should be adjusted for each plate type.

Set the lighting

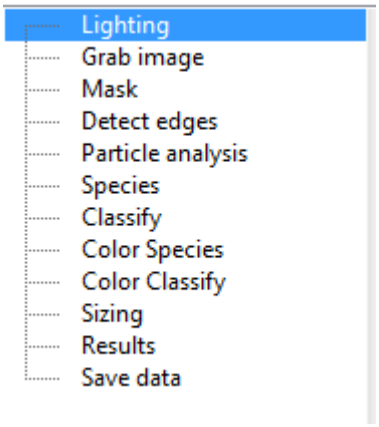


Start the program

Start the program by pressing the "Go" button. The program will start and "Continue" and "Stop" button will appear if "Run Sequence" or "Loop" is selected (see above).

Analysis sequence

Analysis is performed in a sequence of steps. Petrilyzer runs through the sequence automatically during normal operation, or the operator can step through the program sequence step by step (see below).



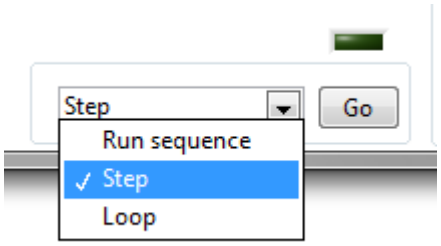
Choose between run sequence, step or loop

In the menu to the lower left choose between three ways of running the program:

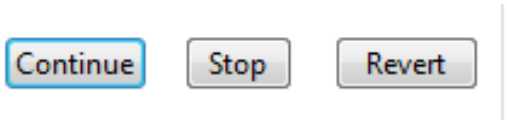
Run sequence: The entire program sequence runs through once. Start the analysis with a click on the "Go" button. The sequence will stop at the "Grab image" to display the image. Make sure the petri dish is positioned correctly and press "Continue". The program then stops at the "Results" step. Click "Continue" again to finish the run and the program is ready for the next sample.

Step: Step through the program step by step by pressing the "Go" button for each step. Step is used to customize and control the application settings.

Loop: **Recommended for routine analysis.** The program behaves as in the "Run Sequence" mode but will start a new analysis automatically (no need to start each run by pressing the “Go” button).



Continue, Stop, Revert Buttons



In "Loop" or "Run Sequence" mode the "Continue" button will appear. In "Step" mode the "Continue" button will not show up, and "Stop" will appear briefly during the execution stage.

When the "Continue" button is pressed the program sequence continues.

Activate ”Continue”:

- Click on the “Continue” button OR
- Press the ”Enter” button on the keyboard

Stop button:

- Stops the sequence
- When the Stop button has been pressed “Continue” will disappear
- Continue by clicking ”Go”

Revert:

- Goes back to the latest saved settings in the current step

Petrilyzer always remembers the last used settings after program closure. Go back to the latest settings by clicking the "Revert" button. This applies only to settings in the current step.

Saving and retrieving settings via the "File" menu is the most secure way to revert to a known protocol in case the settings have been changed, see Save settings.

Tools (Zoom, Arrow, and Others)

The tools vary somewhat in between the sequences

1. Zoom tool

 Enlarge image: Select the magnifying glass, click in the picture with the mouse (“+” magnifying glass);

 Minimize image: Select the magnifying glass, keep the shift key pressed down and click simultaneously in the picture window (“-“ magnifying glass);

2.  The arrow tool is for pointing

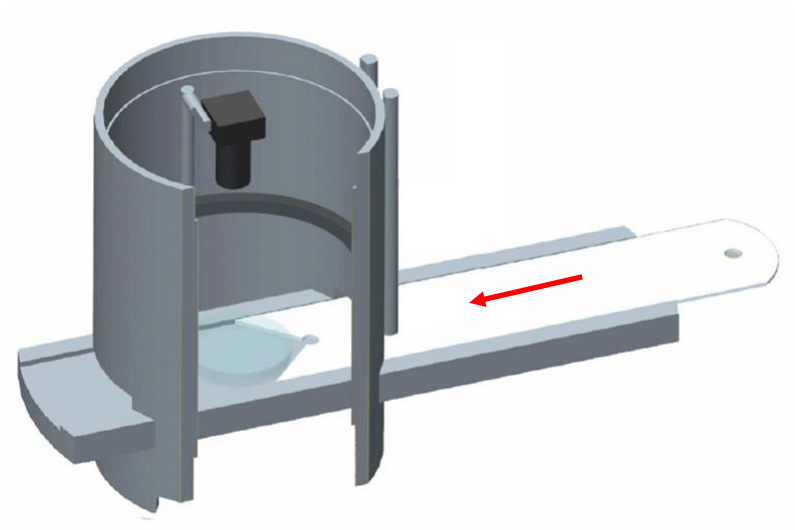
3.  Hand tool for moving the view in the image window;

4.  The line tool is used in the ”Mask” sequence step (masking off the area outside the petri-dish, see below), the “Particle analysis” step (calibrate pixel size. see below).

Slide a petri dish into the Petrilyzer

Slide a sample into the Petrilyzer by means of the slider, as shown below.

The plate is properly positioned when the slider reaches the stopper inside the light box, and the petri-dish is visible in the program's image window (Grab-image program step).



Grab image

In this step images are imported into the program directly form a connected camera or from saved pictures.

Camera is connected: Pictures will be retrieved from the camera, as shown below, when the "Camera" tab is open.

Exposure settings should be adjusted when there is a petri dish in the Petrilyzer.

Set the exposure by moving the "Exposure" slider until the area inside the Petri dish between the colonies appears light gray (the area should not contain all-white sections, which indicate overexposure).OBS. It may take a few seconds for the camera exposure to adjust to the new settings.

The exposure can also be written directly into the “Exposure” field.

Camera is not connected or off-line (file) analysis: The file tab opens. Stored images can be retrieved and analyzed.

Select images from a photo archive by clicking the folder icon and specify the location on the computer for the image to be analyzed. If the image is in an archive with multiple images, Petrilyzer analyzes images one after the other while looking for an index suffix.

NOTE: Petrilyzer creates a duplicate image in the location and with the filename indicated in the "Save data" step (see the section "Save Data").

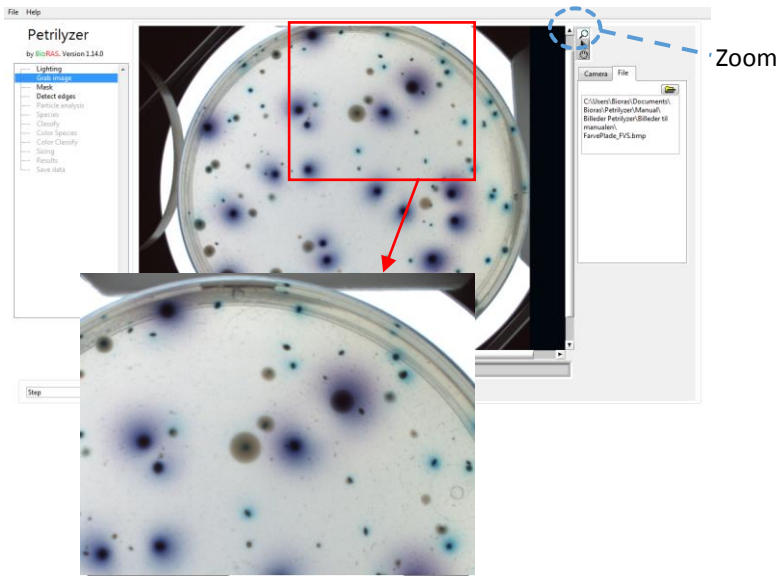
Lens settings

The aperture on the lens should be set to f/4 (half way mark if no numeric values are visible). This gives a good depth of field and sharp images compared to when the lens is wide open.

Check that the camera is placed correctly. Focus can be adjusted on the focus ring on the lens, to get sharp pictures.

Locking screws for aperture (inner) and focus (outer)

It is an advantageous to zoom in on the image using the magnifying glass whilst adjusting the focus, as shown below.

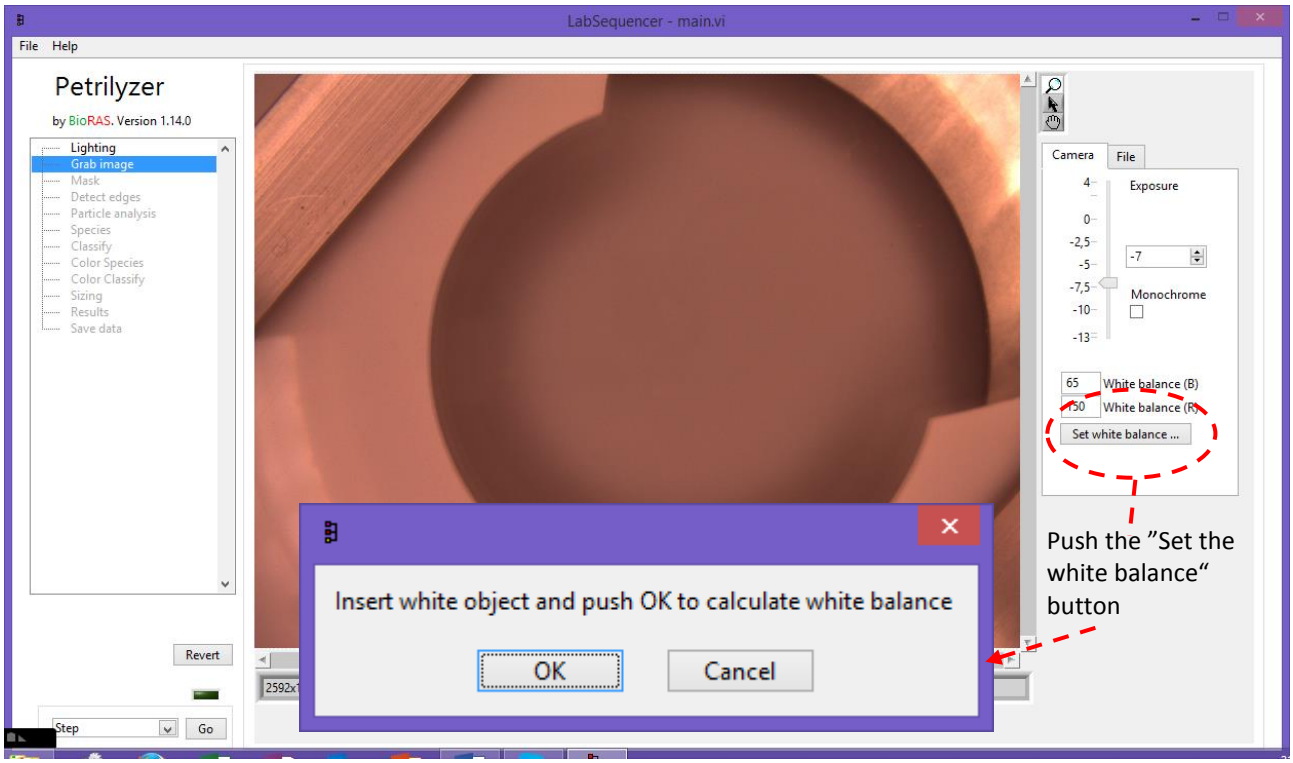


Setting the White Balance

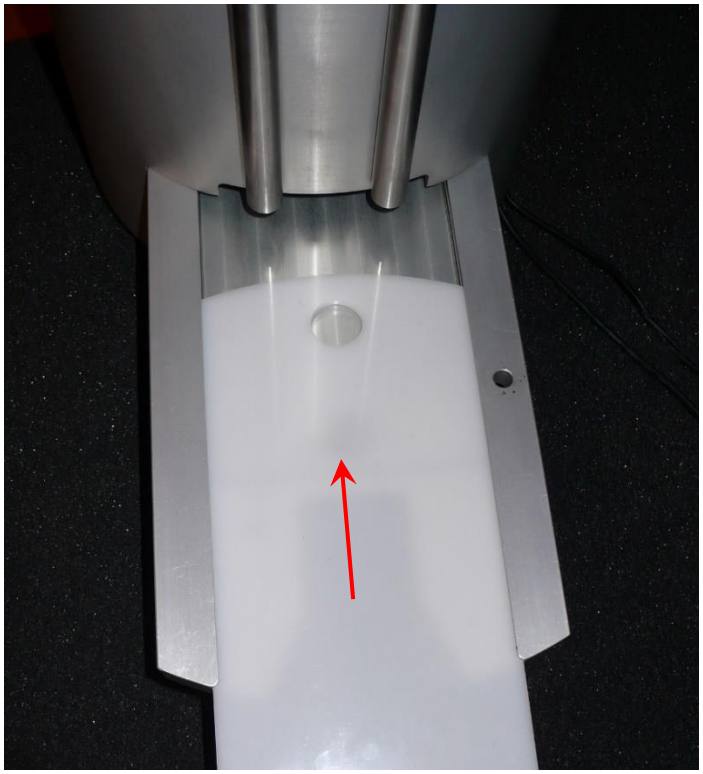
In order to obtain accurate colour display in images with coloured colonies, the white balance should be set. Setting the white balance is very easy, and needs only to be done during initial start-up of the Petrilyzer.

In the example below white objects are reddish. Adjusting the white balance in the “Grab image” step, will render accurate colours.

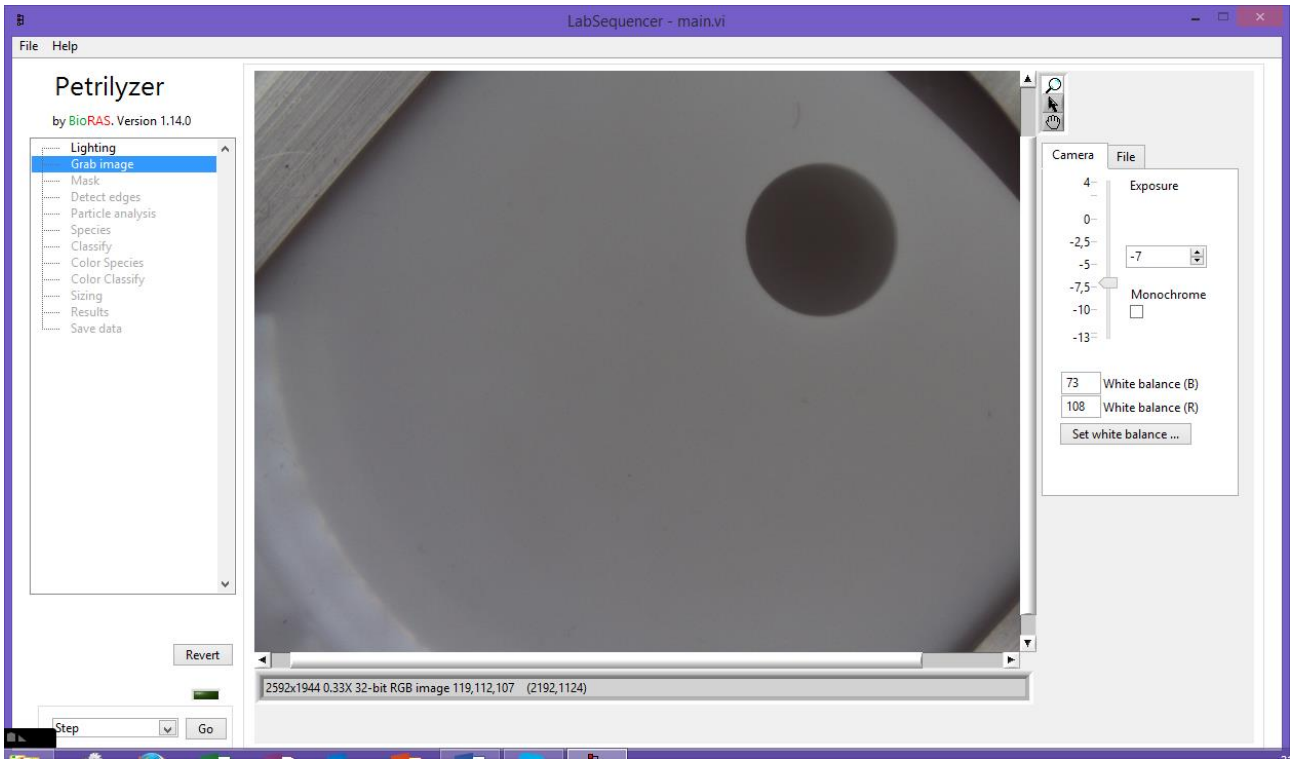
Push the “Set white balance” button, a dialogue will appear asking you to insert a white object.



Use the slider as a white object by inserting it the opposite way as shown below, and push OK.



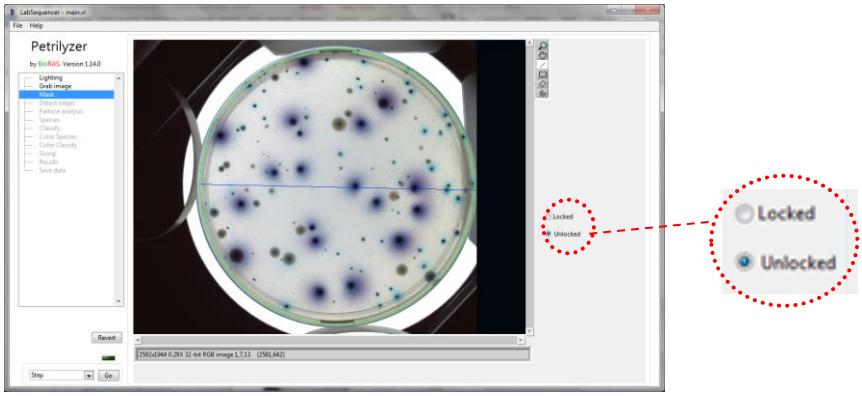
The white balance has been set, and colours are accurate:



Mask

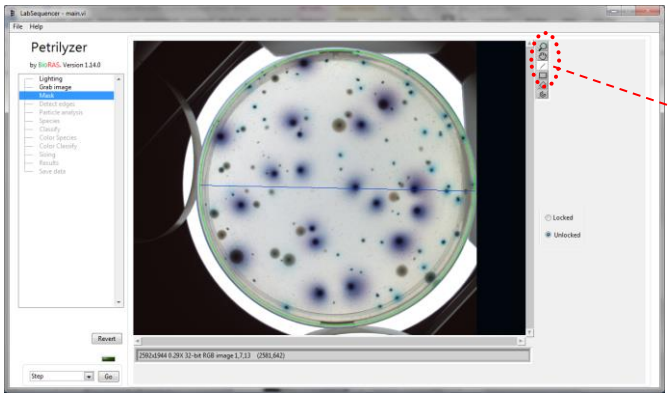
In the mask phase, the area outside of the petri-dish is defined, to remove unwanted disturbance. The mask is usually a circle but it can also take on other shapes.

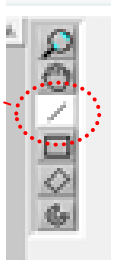
- The Mask is locked when ”Locked” is activated. Mask is, by default set on ”locked”
- To adjust the mask ”Unlocked” should be activated, whereby the line tool will appear.



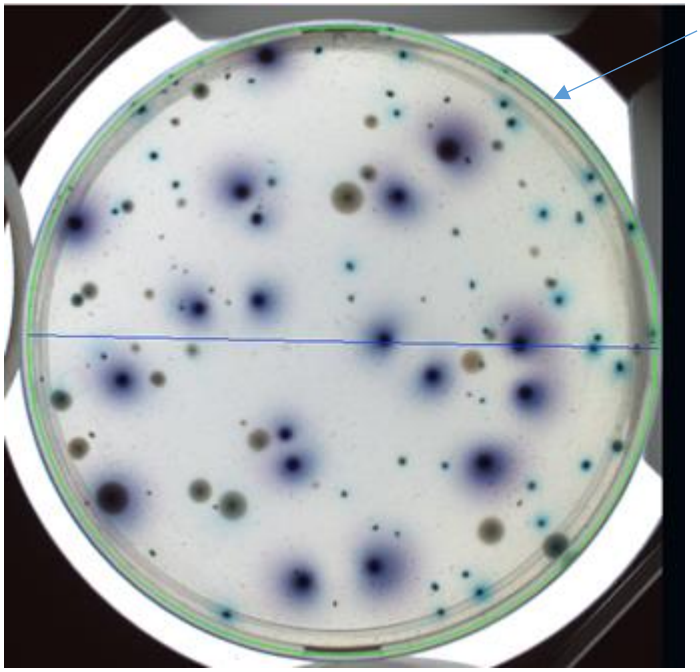
Create a circular mask around the plate:

- The line tool is used to draw a circle with the line as the diameter. The line tool can also be used to adjust an existing mask.
- Select the line tool



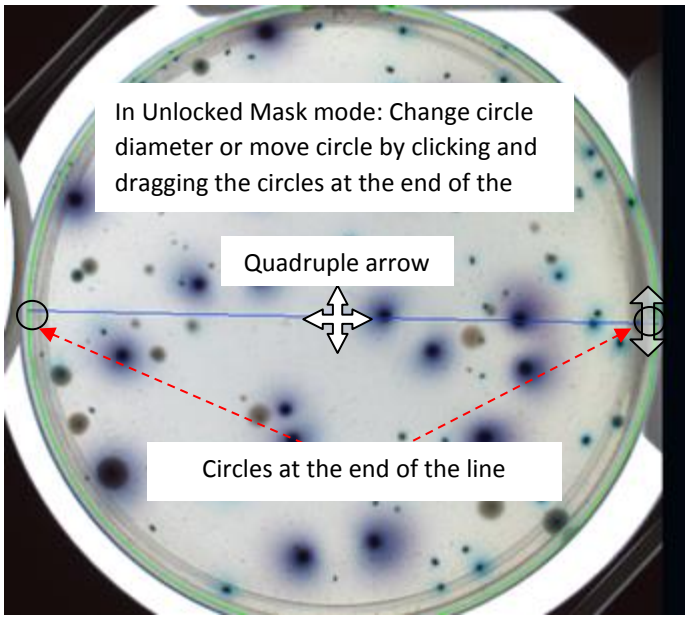


- Place the mouse on the edge of the petri-dish. Draw a line across the dish. Left-click on the mouse at the start of the line, release the button at the end of it. To get the line completely horizontal, press the shift key simultaneously.
- A circle will appear



Adjustment of the mask:

- Move the whole circle by placing the mouse over the line. A quadruple arrow will appear. Click on the line and drag the mouse to move the entire circle
- The circle's radius can be changed by placing the mouse over the end of the line. A double arrow and small circles at each end of the line will appear. Click on one of the ends and drag the mouse to change the circle's radius as well as the line direction.
- Lock the Mask after adjustment.

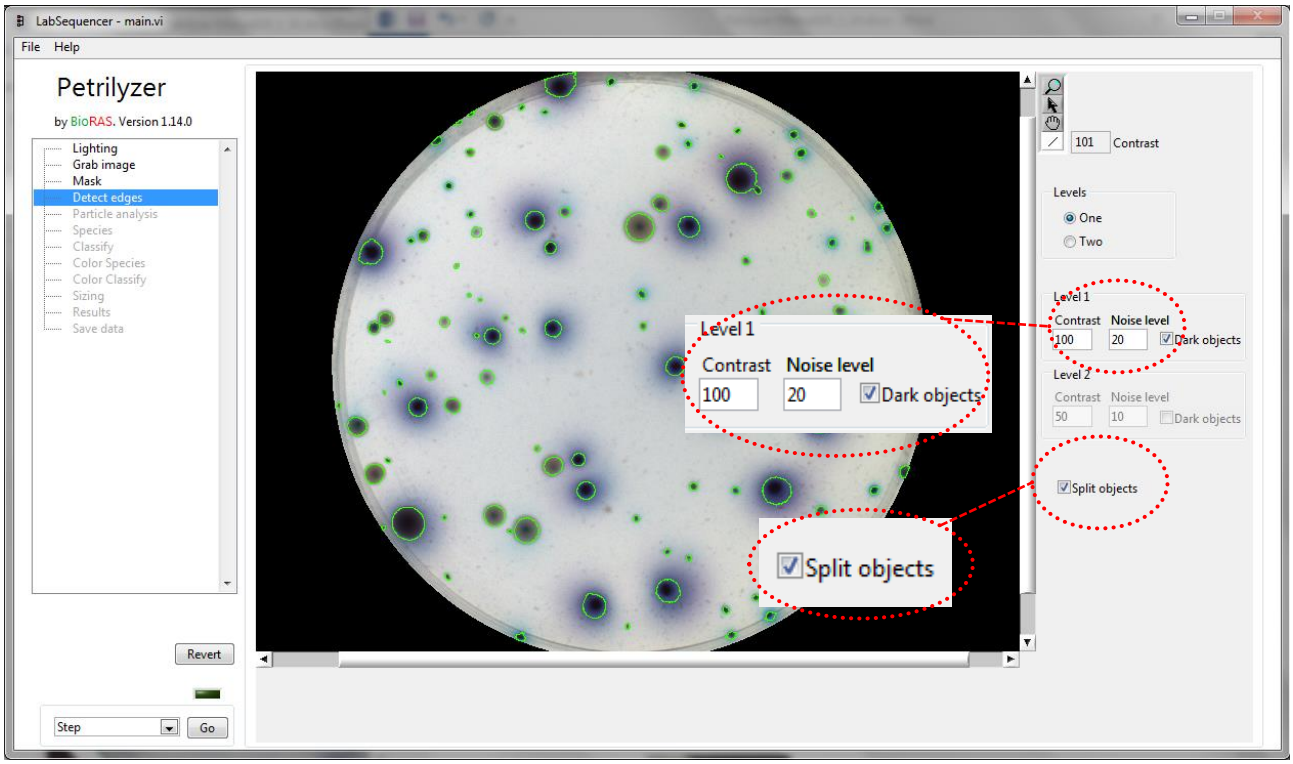


Detect edges

In this step, the programme finds the colonies or particles that are to be counted. Detected colonies are marked with a green ring around each colony.

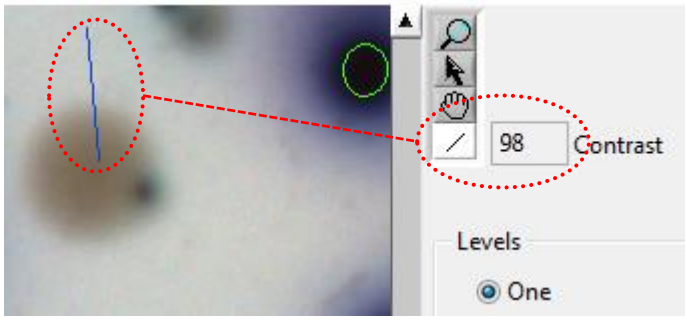
The following settings are available:

- Contrast
- Noise level
- ”Dark Objects”: should be checked if the colonies are dark against a light background
- For light colonies against a dark background, uncheck ”Dark objects”
- Levels: select 1 if the colonies have roughly the same contrast, select 2 if there are two different categories of colonies on the same plate; i.e. both dark and light colonies



Contrast settings

Contrast affects the ability of the program to find colonies or contours in the image. It is typically set once and for all for a specific type of plate. Contrast can be measured using the line tool. Draw a line over the edge of a colony, and the contrast will be shown in the window next to the line tool. For the best contrast estimate, measurements should be done on several colonies, and the lower average value should be used for contrast settings.

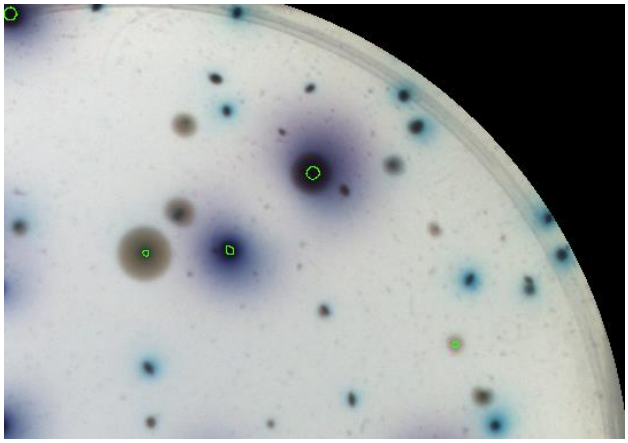


The pictures below shows result of increasing contrast.

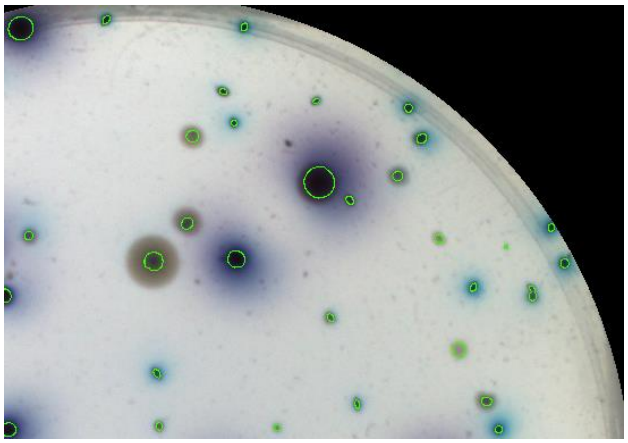
If Contrast is too low, small colonies will not be detected. If it is too high, colonies next to each other may merge. It is therefore important to find the best value for any given type of samples.

For the image shown here, the contrast was set to 100.

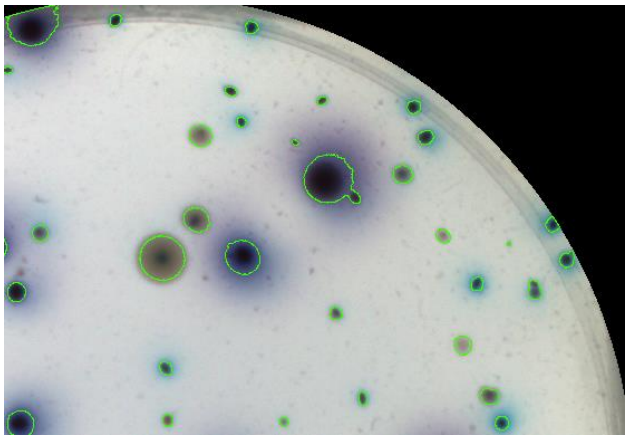
Contrast 25:



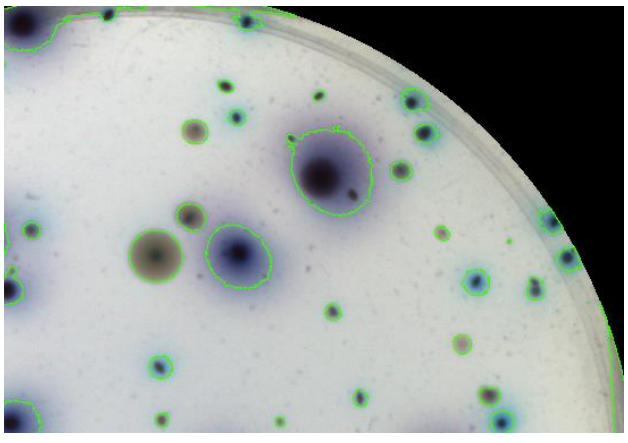
Contrast 50 (default):



Contrast 100:



Contrast 150:

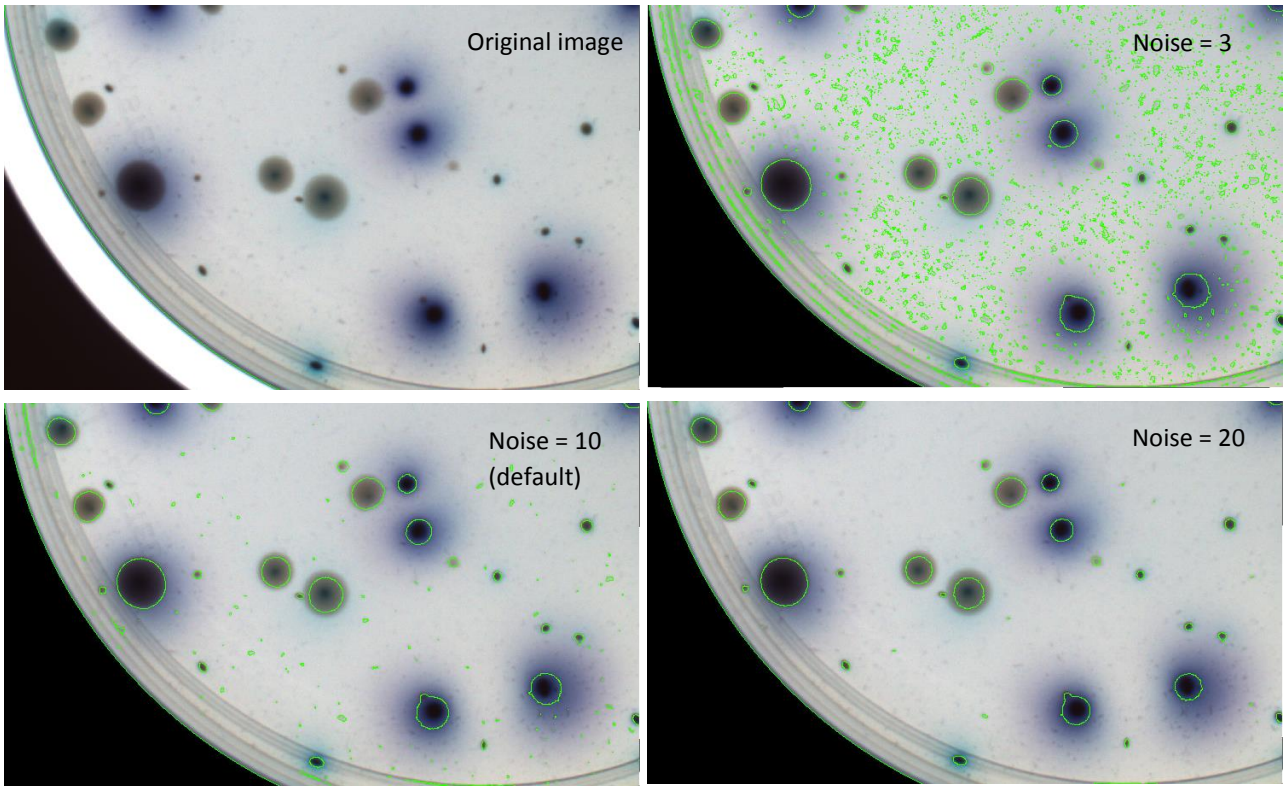


Noise settings

The ”Noise” parameter removes background noise and weak particles.

If background noise is low, ”Noise” should be set to a low value. If noise reduction is not required, it should be set to 1.

If ”Noise” is set at too high a value, weak colonies may disappear along with the noise. The default value is 10. For the image shown here noise level 20 was suitable.

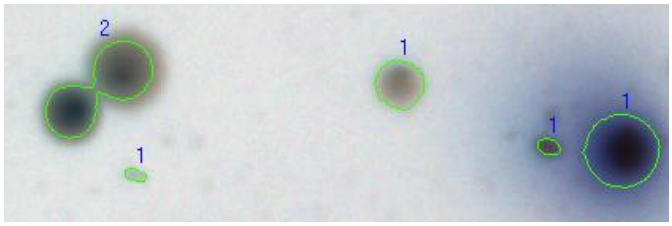


Split objects for counting adjoining colonies

Split objects will separate joint colonies. Split objects is checked by default, see above.

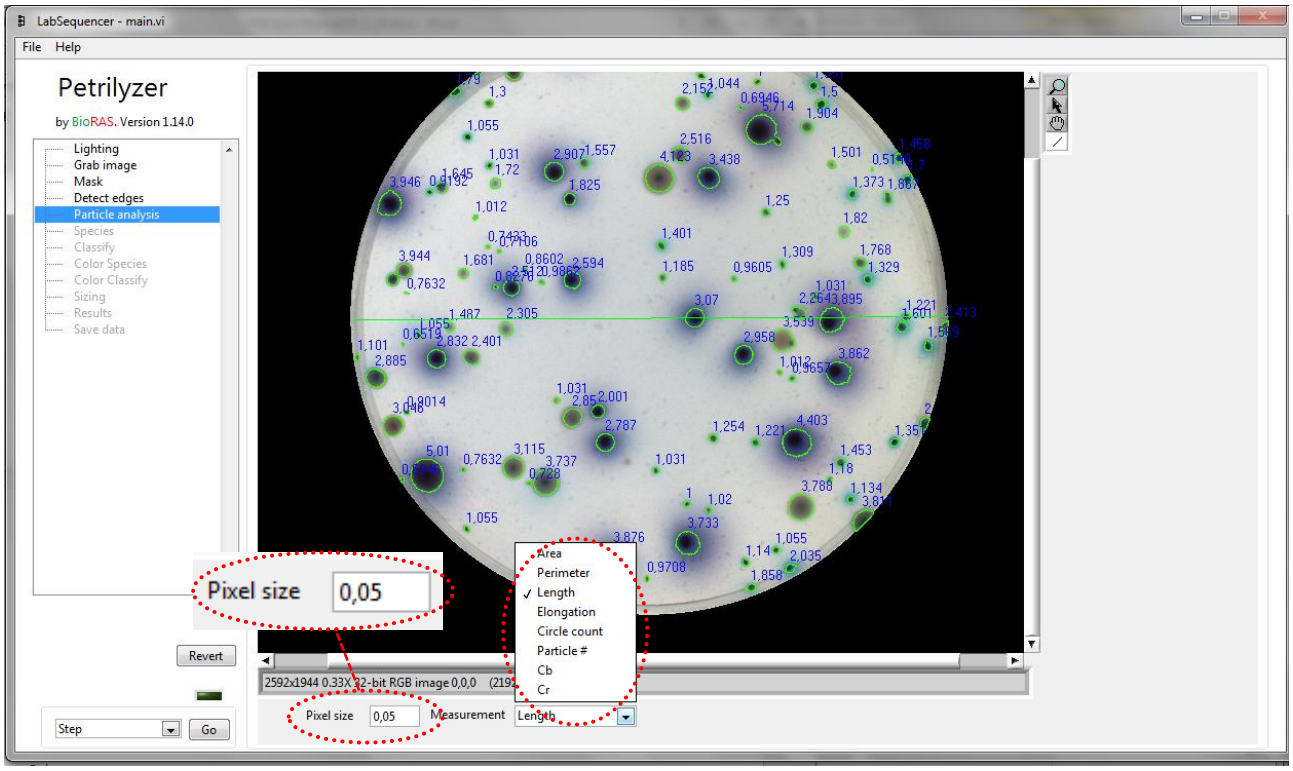
Split objects may remove the smallest colonies, and should be unchecked for counting of pinpoint colonies.

The colony counts are shown in an example below; 2 colonies were counted in the joint colony, 1 colony for others (image from the Particle analysis step, see below).



Particle analysis

In this step every colony is analysed, and measured. The measurements can be calibrated by adjusting the pixel size.

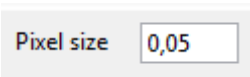


In the ”Measurement” box, it is possible to select which measurement is to be displayed. For each particle/colony the following data is available:

- Area
- Perimeter: circumference
- Length: diameter
- Elongation: Colony length/width ratio
- Circle count: total number of colonies including separated colonies
- Particle #: each particle or colony is numbered
- Cb: Color information
- Cr: Color information

Adjusting the pixel size

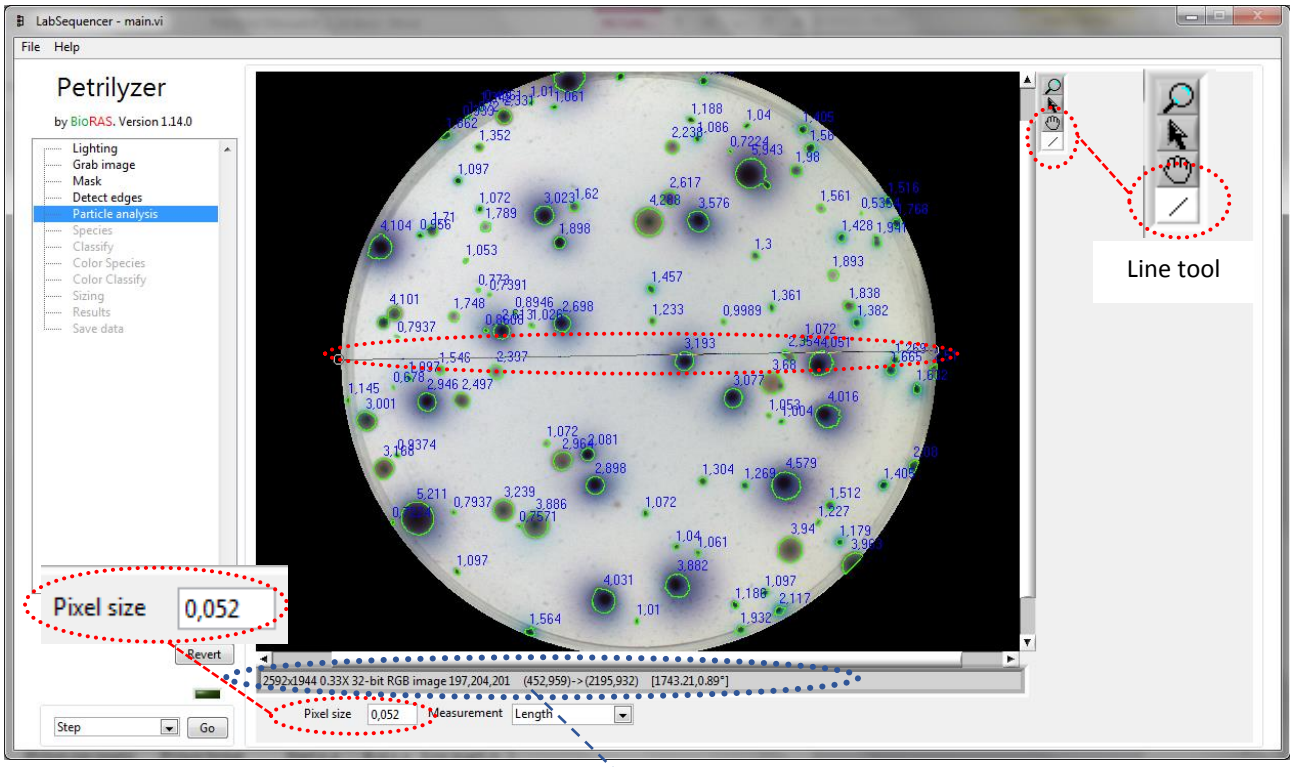
Pixel size is set to 0,05 pixels/mm by default. Pixel size can be adjusted. To calibrate pixel size, the diameter of a petri-dish can be used together with a measurement taken using the line tool, which gives the length of the line in pixels.

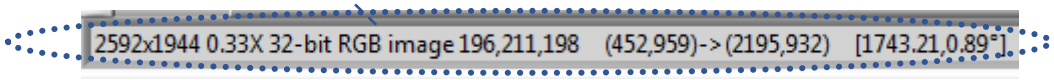


Calibration of Petrilyzer through the measurement of the diameter of a petri-dish:

- Select the line tool
- Draw across the diameter of a petri dish

- Check that the ends match correctly by zooming in. The line can be adjusted by pulling the little round circle at the ends
- Measure the line length by clicking on the line with the line or arrow tool
- The line's length will be displayed in the field under the picture (2302 pixels in the example on the right)
- Calculate the pixel size:
- Pixel size = line length (mm) / line length (pixels)
- Pixel size = 90 mm/1743 pixels = 0,052
- Set pixel size in the pixel size field. Whole numbers and decimals are accepted; see the example to the right.

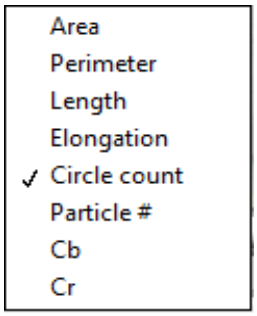


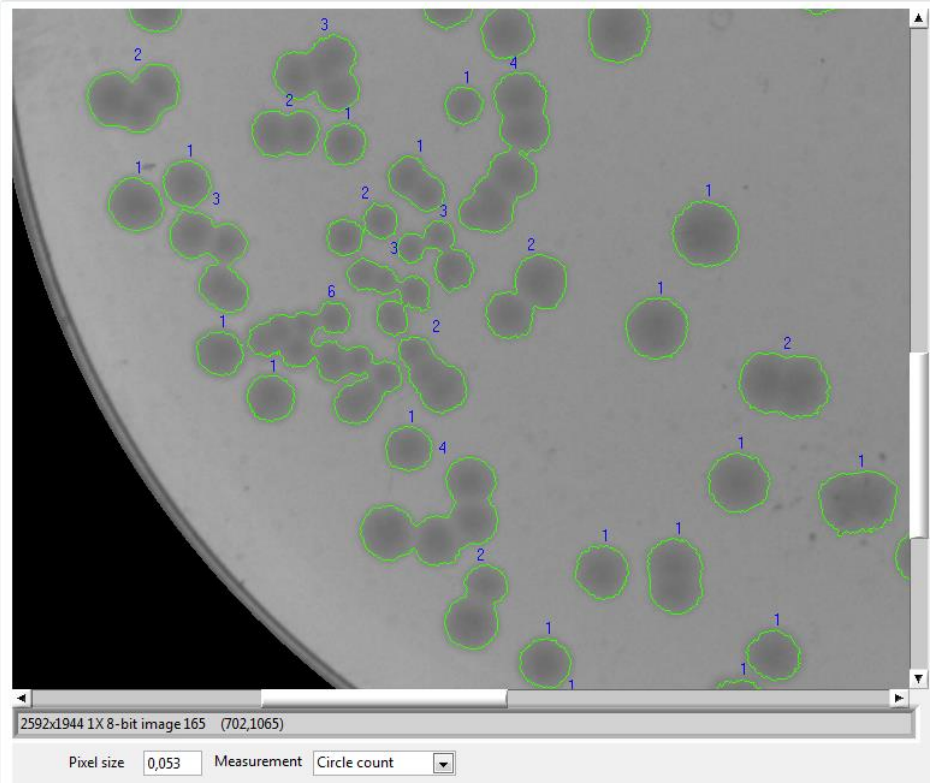


Picture size (pixels)	Picture format	Start x, y position	End x, y position	Line length in pixels and line angle

Separation of joined colonies

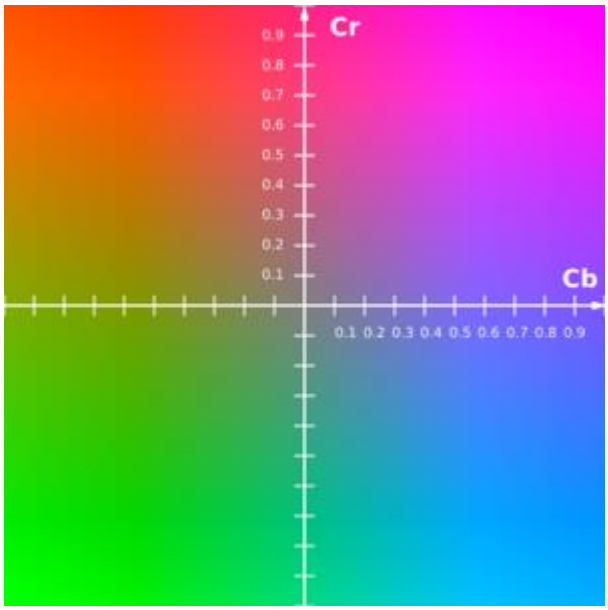
Colonies within a cluster of joined colonies are counted separately. The Particle analysis step shows the number of sub-colonies within a cluster if Circle count is selected. The sub-colony number is written above the colony. The data is saved and included in the results as Sub-colonies (see below).





Colors

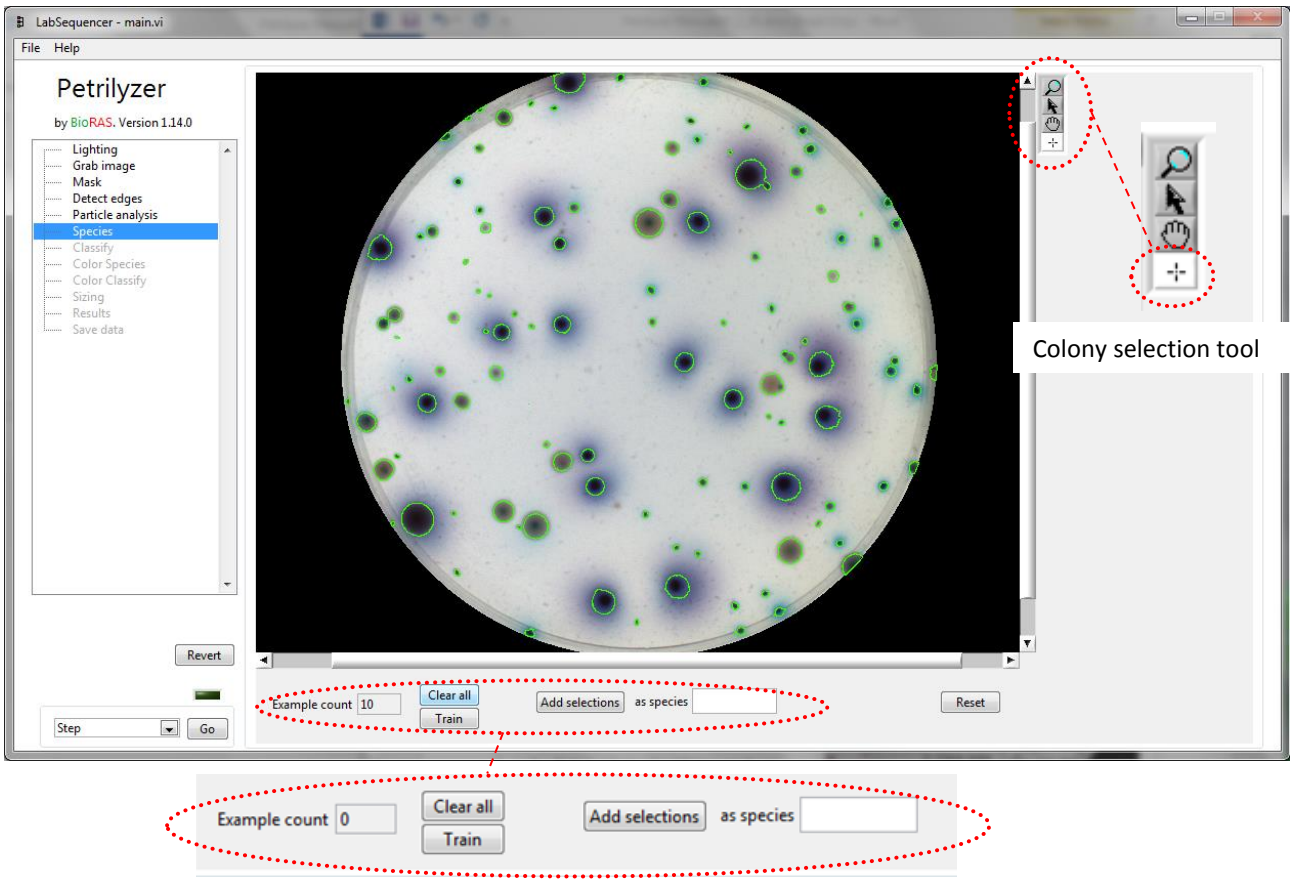
Colors are described by Cr and Cb. These are two of the three parameters in the YCbCr color space which together describe color independently of intensity (Y). They are used for color classification and for quantifying color in general.



Species

Shape Classification

The default shape recognition can be changed or improved by additional training of the classifier. This is typically necessary if the default training set does not recognize colonies correctly. The panel below the image window is used for selection of different types of colonies, and training of the classifier.



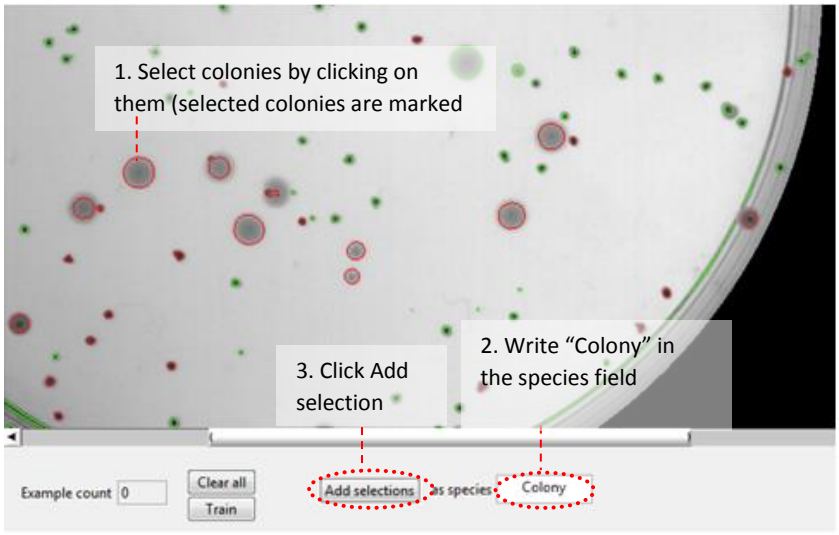
Select colonies by simply clicking on them with the colony selection tool (the small cross).

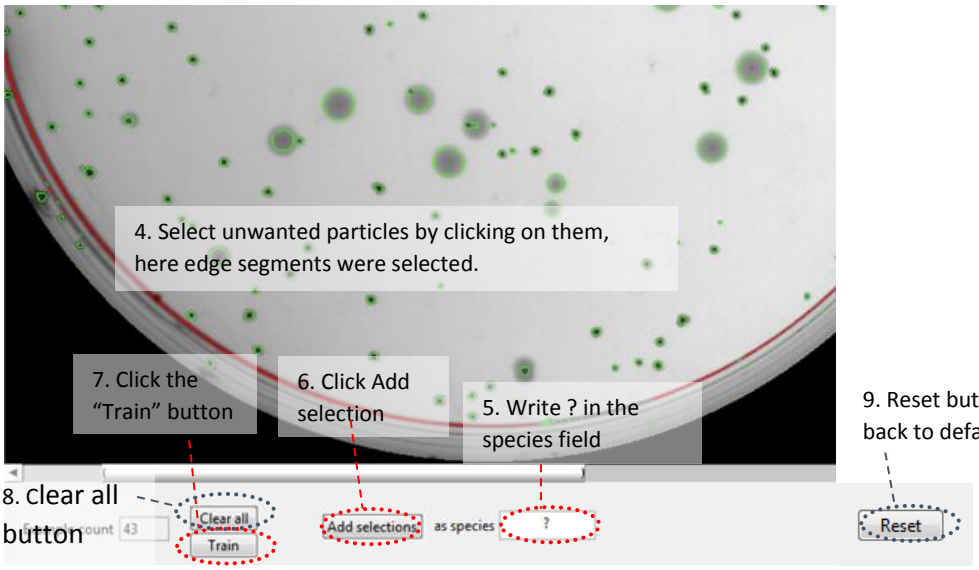
Deselect a colony by clicking on it a second time.

Shape classification

Selection of colonies and unwanted shapes, and training of the classifier:

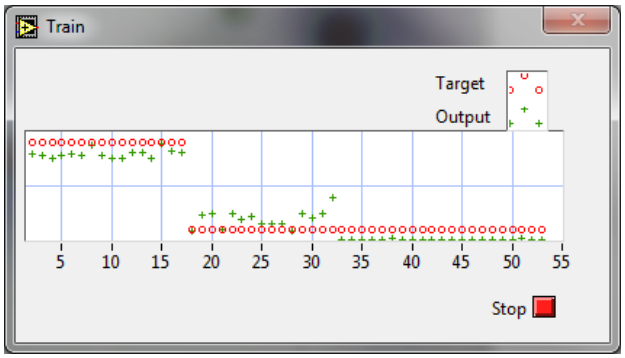
1. Select colonies by clicking on them. Selected colonies or shapes are marked with a red contour
2. Write “Colony” (or another label) in the species field
3. Click Add selections
4. Select unwanted particles
5. Write “?”in the species field
6. Click Add selections
7. Train the Neural Network by clicking the “Train” button. A Training-panel will appear, see below.
8. If mistakes are made during selection of colonies, the training set can be cleared by clicking the “Clear all” button.
9. The Reset button resets the Neural Network settings back to default settings.
10. Other classes can be added in the same way.





After adding colonies and unwanted particles to training set, the classifier is ready to be trained by simply clicking the Train button (see above).

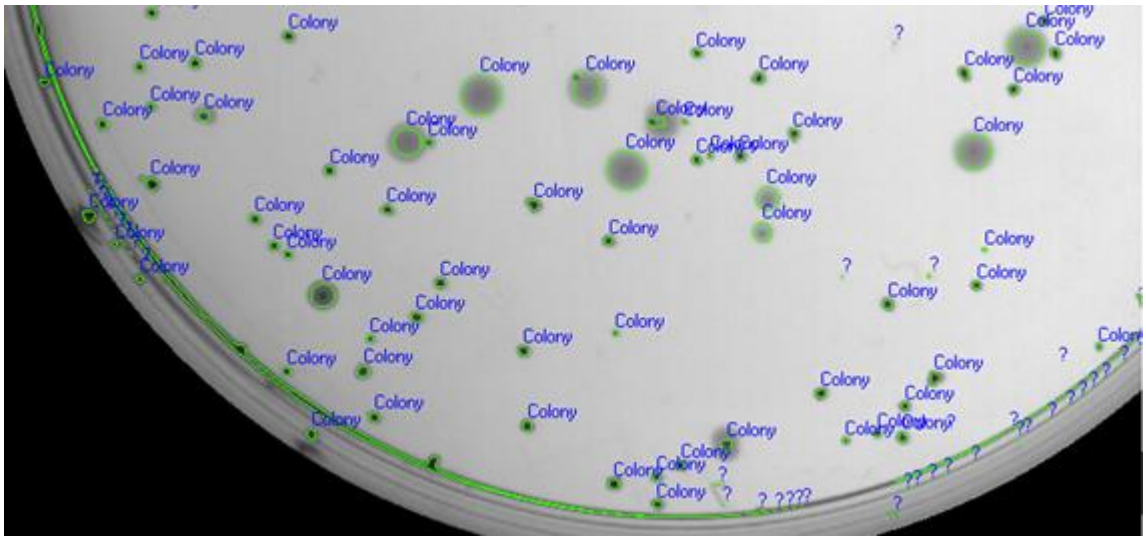
A Train panel will appear for a few seconds. Output should be reasonably well aligned with targets.



The training set can be extended, and the system can be retrained as often as required.

Classify

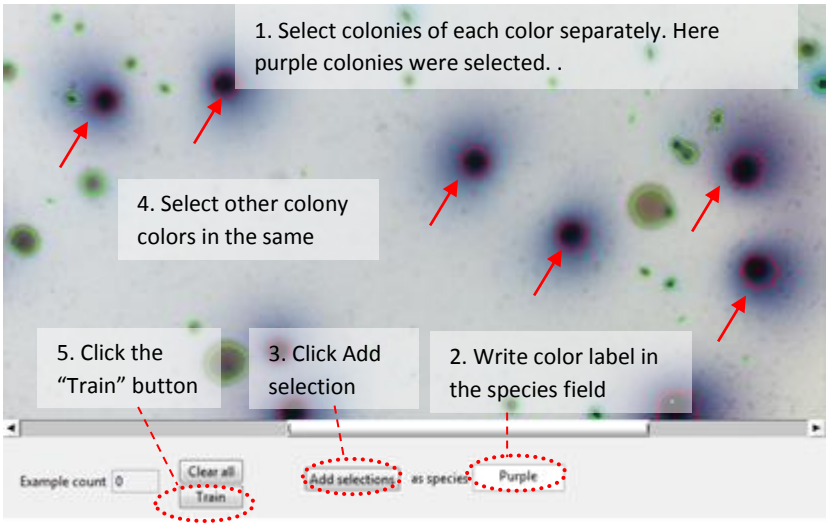
The classifier classifies colonies and other objects depending on how it was trained. In the example below Colonies are marked, and unwanted objects are marked with "?".



Colour species

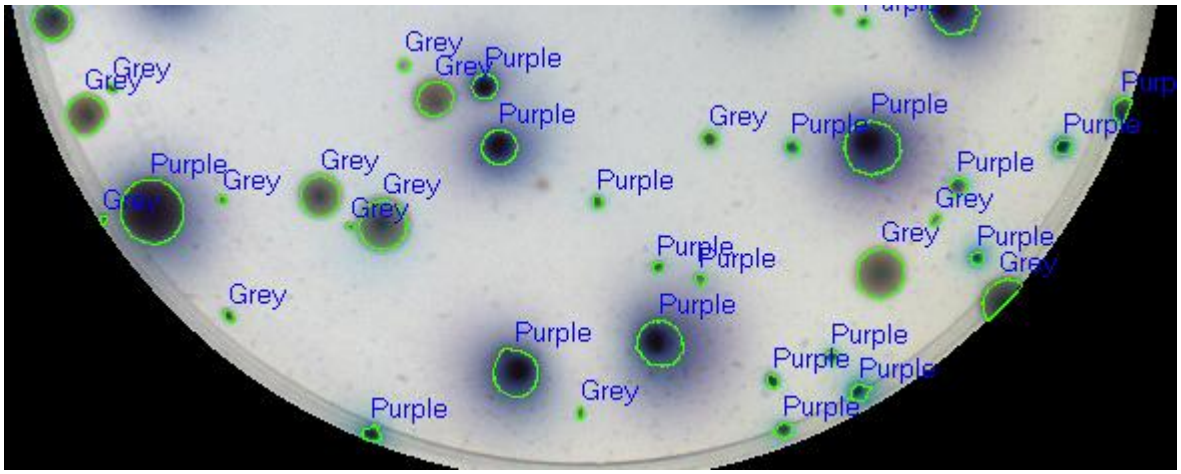
Colour classification is done in a separate step. By default colour information is ignored.

1. Select a number of colonies of each colour by clicking on them with the selection tool. Selected colonies will be highlighted with a red ring.
2. Add a name in the Species field. Purple colonies were named “Purple” (red arrows indicate marked colonies).
3. Click “Add selections, and the selected colonies will be added to the Example count, and will be deselected (red rings disappear).
4. Blue and Grey colonies were selected likewise. The total Example count for colonies in this image was 54 (see below).
5. Train the classifier by clicking the Train button, as described above.



Colour Classification

The neural network classifies colonies according to the training set. In the example below purple and grey colonies are marked.



Sizing of colonies

Results are displayed as a histogram. The histogram shows the size distribution of the counted particles.

x-axis: Calibrated length measurement

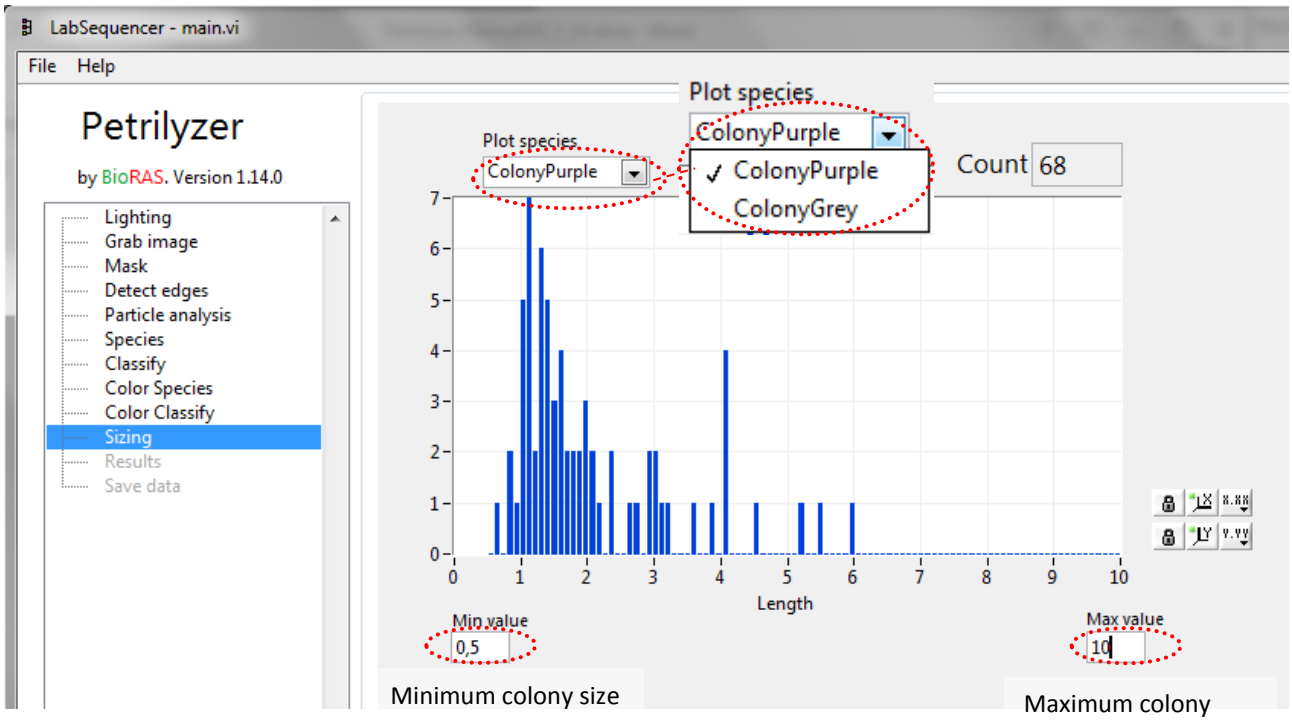
y-axis: Number of colonies in each size fraction

The total colony or particle count is shown in the Count indicator in upper right corner

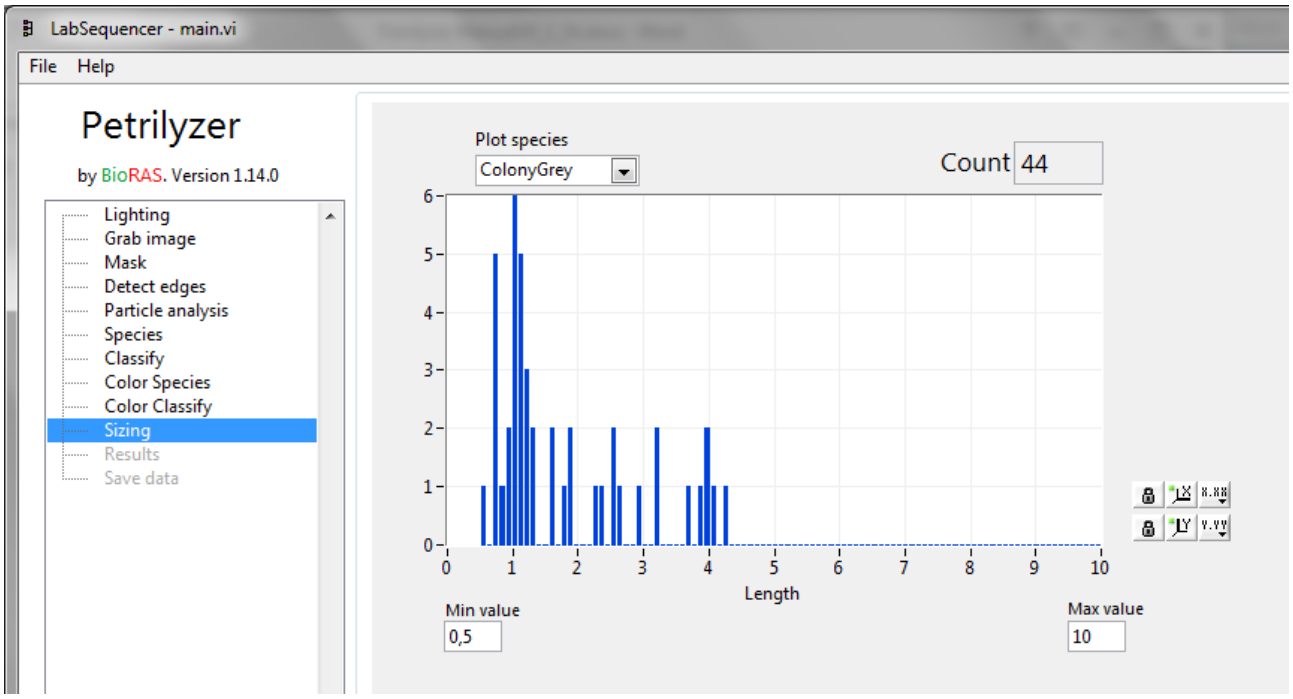
In “Plot species” the category of particles can be chosen from a drop down menu.

The minimum and maximum countable colony size (length) is defined in the fields below the graph. Here the minimum and maximum size were set to 0,5 mm and 10 mm.

Purple colonies:



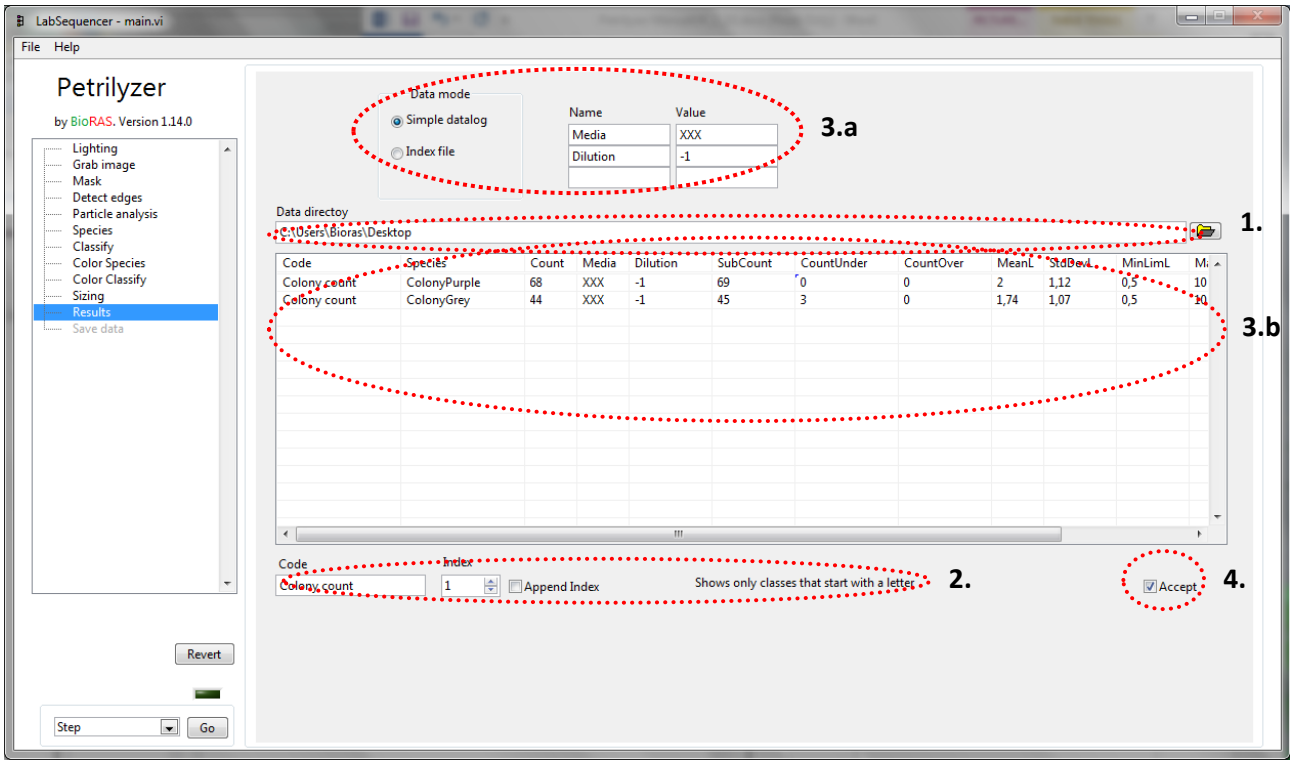
Grey colonies:



Results

In the results stage, the following is displayed:

1. Data directory
2. File name
3. Data
   a) Data mode, Index file
   b) Data display
4. Accept or reject result



1. Data Directory: Location of the Result Files

Choose the location on the computer for images and result files from the analysis. Click on the folder icon and choose the location on the computer. Place the result in a folder under Documents (e.g. a folder with the day's date or similar).

Results from one analysis session are added to a file called Data.txt

In order to start a new data file, specify a new empty directory or rename or move Data.txt to another folder, and a new Data.txt will be created.

2. File Name (Code and Index)

Code field. The Code field plus the Index field (optional) is the name under which results of analysis and the image of the sample will be saved.

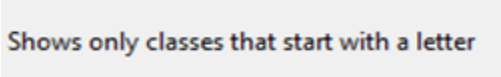
The code name can be added manually or it can be populated by scanning a barcode. The Code field is given focus automatically. A bar code scanner is typically connected to a USB port on the computer and should be programmed to output a CR/LF after reading, which is default for most scanners.

Manually added code:

Automatic Code from Barcode reading:

Append Index can be checked in order to automatically add an index suffix to each sample. For every analysis, the index number increases (this applies in the Run sequence and Loop mode. In step index numbers do not increase). The start number can be added manually at any time.

Select Append Index, if a series of samples with the same Code name is analysed.



NOTE: Only classes that start with a letter will be shown and saved. Classes that start with a number or non-letter symbol, like e.g. “?” will be excluded.

3. Data Mode

The following data modes can be used:

- Simple datalog
- Datalog with an index file
- Database connection (custom feature)

The following data are saved:

- Code: File name (see 2.)
- Species: Per default counted colonies are named “Colony”. Other species can be added by training the classifier (see above)
- Count: Number of colonies
- SubCount: The total number of colonies including separated colonies
- If Index is selected: Any columns in the index file
- Optional data from Name and Value fields
- Count Under: Number of colonies smaller than the minimum colony size (see “Sizing of colonies” above)

- Count Over: Number of colonies larger than the maximum colony size (see “Sizing of colonies” above)
- MeanL: Mean colony length of single colonies, joined colonies are excluded
- StDevL: Standard deviation of colony length of single colonies
- MeanLimL: Minimum colony size (length)
- MaxLimL: Maximum colony size (length)
- Time: Time of analysis (dd-mm-yyyy hh:mm:ss)

Simple datalog

Data is saved in the selected Data directory, and will be named Data.txt.

In the Name and Value field, extra information about the samples can be entered. “Name” is the column header, “Value” is the row value. The information will be a part of the data set.

Name	Value
Media	XXX
Dilution	-1
Batch	YYY

Fields and values can be letters, numbers or symbols. Number formats should be compatible with the spread sheet format or database which the results are imported into.

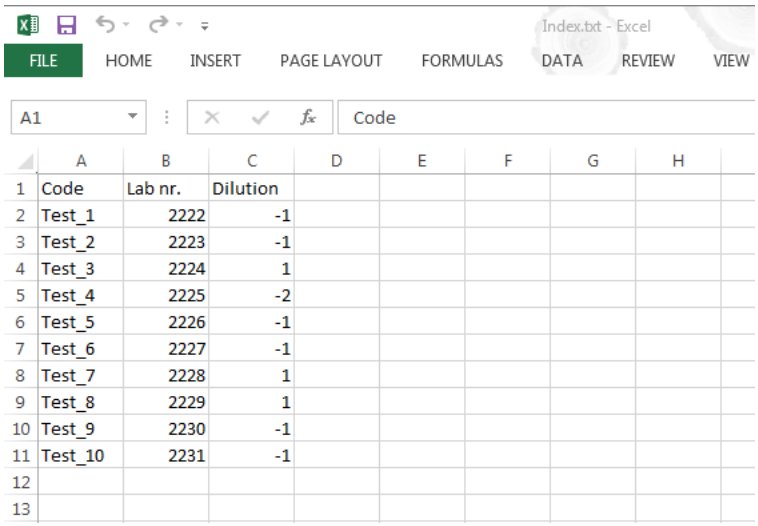
The results table will display the following data:

Code	Species	Count	Media	Dilution	Batch	SubCount	CountUnder	CountOver	MeanL	StdDevL	MinLi
Colony count	ColonyPurple	68	XXX	-1	YYY	69	0	0	2	1,12	0,5
Colony count	ColonyGrey	44	XXX	-1	YYY	45	3	0	1,74	1,07	0,5

Index File

An Index file is a file that is prepared beforehand, and which contains information about all the samples. The information from the Index file will be saved together with the results from the analysis.

The Index file is a table with rows describing a series of samples. The Index file will be read row by row during analysis of a series of samples. Petrilyzer looks for the code field in the first column and associates the corresponding row values to the data set.



	A	B	C
1	Code	Lab nr.	Dilution
2	Test_1	2222	-1
3	Test_2	2223	-1
4	Test_3	2224	1
5	Test_4	2225	-2
6	Test_5	2226	-1
7	Test_6	2227	-1
8	Test_7	2228	1
9	Test_8	2229	1
10	Test_9	2230	-1
11	Test_10	2231	-1
12			
13			

Note: The index file must be placed in the selected data directory!

When Index file is selected Petrilyzer will look for an Index file in the selected data directory.

Preparation of an Index file:

- Prepare the Index file in a spread sheet program, f.eks. Excel or Open office.
- The file format must be saved as tab delimited text (txt)
- Name of the Index file must be: Index.txt.

The Index file should contain the following information:

Column headers: Column 1 should be named Code, the following columns names are optional

Column 1: Code_index. In the example to the right the Code is "Test" and the Index is the figures 1 through **10.**

Column 2 and following columns: Additional data can be added here. Values can be letters, numbers or symbols. The number of columns is unlimited. Number formats should be compatible with the spread sheet format or database which the results are imported into. Results and image files will be named by the Code.

The results table from the example above will display the following data:

Code	Species	Count	Code	Lab nr.	Dilution	SubCount	CountUnder	CountOver	MeanL	StdDevL	MinLimL	MaxLim
Test_1	Colony	122	Test_1	2222	-1	124	0	0	1,32	0,723	0,2	50

4. Accept Data

Accept is checked by default, and allows data to be added to the data file.

If Accept is un-checked the data is not added to the data file. Choose this setting if the analysis is unsatisfactory, and should not be saved. The program will automatically check "Accept" in the next analysis.