Università degli Studi di Brescia

Digital Image Processing

Individual Project

AUTOMATIC COUNTING OF BACTERIAL COLONIES

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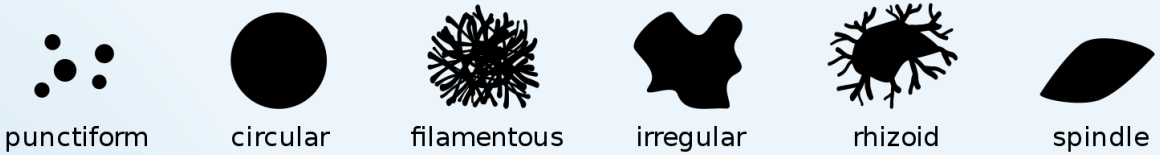
Introduction: bacterial colonies

- **Bacteria** are unicellular organisms and are considered to be primitive living things. They have a ring of genetic material (one circular chromosome), a cell membrane, and a cell wall. However, they are prokaryotic cells (cells that lack a nuclear membrane).

- A **bacterial colony** is a visible bunch of microorganisms that sprang from a single mother cell, which makes each member genetically identical.

Introduction:

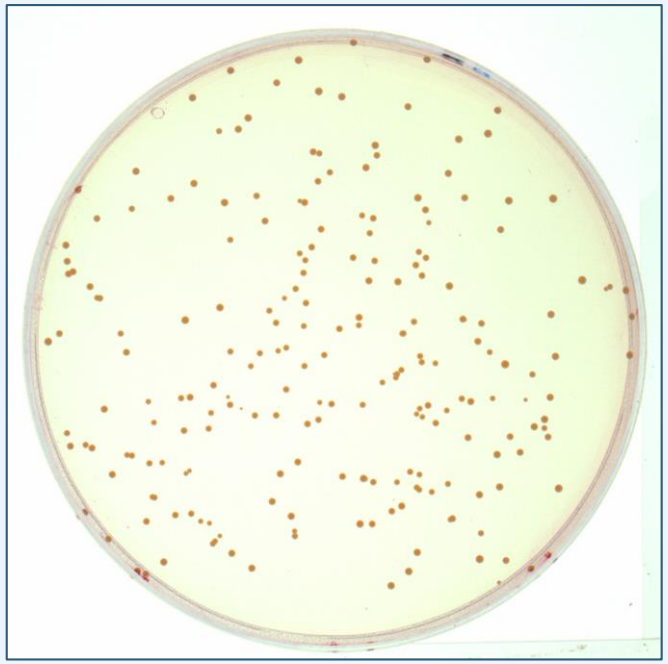
- Bacterial colonies differ from each other with respect to their morphology. The main forms in which they appear are the following:



- **Colony forming units**, usually abbreviated as **CFU**, refer to individual colonies of bacteria. A colony of bacteria refers to a mass of individual cells of same organism, growing together. Colony forming units are used as a measure of the number of microorganisms present on surface of a sample.

Introduction

- To determine the number of colony forming units, a sample is prepared and spread or poured uniformly on a surface of an agar plate and then incubated at some suitable temperature for a number of days. The colonies that form are counted. CFU is not a measure for individual cells or spores as a colony may be formed from a single or a mass of cells or spores.



Example of bacterial colonies on an agar plate.

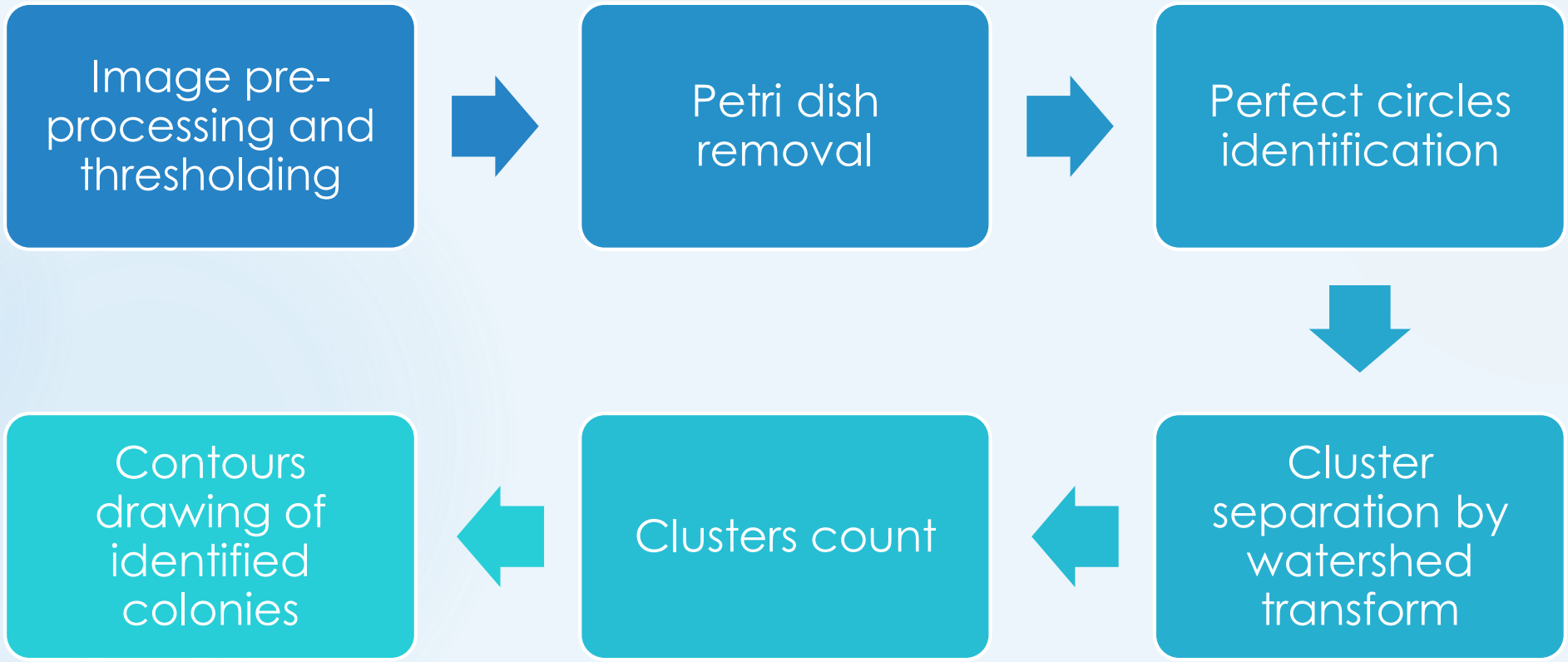
Introduction

- Counting colonies is traditionally performed manually using a pen and a click-counter. This process is **time-consuming**, tedious and **error prone**. There is a tendency to analyse only high dilutions of the initial culture as these have fewer colonies to count. Unfortunately, in low count assays minor counting errors have significant effects on the calculated concentration in the primary liquid medium. On the other hand, accurate counting of plates with high numbers of CFUs is error prone since it requires a high level of attention by the performer.
- These reasons led to efforts to develop **image processing-based software** able to detect and count CFUs automatically.

Introduction

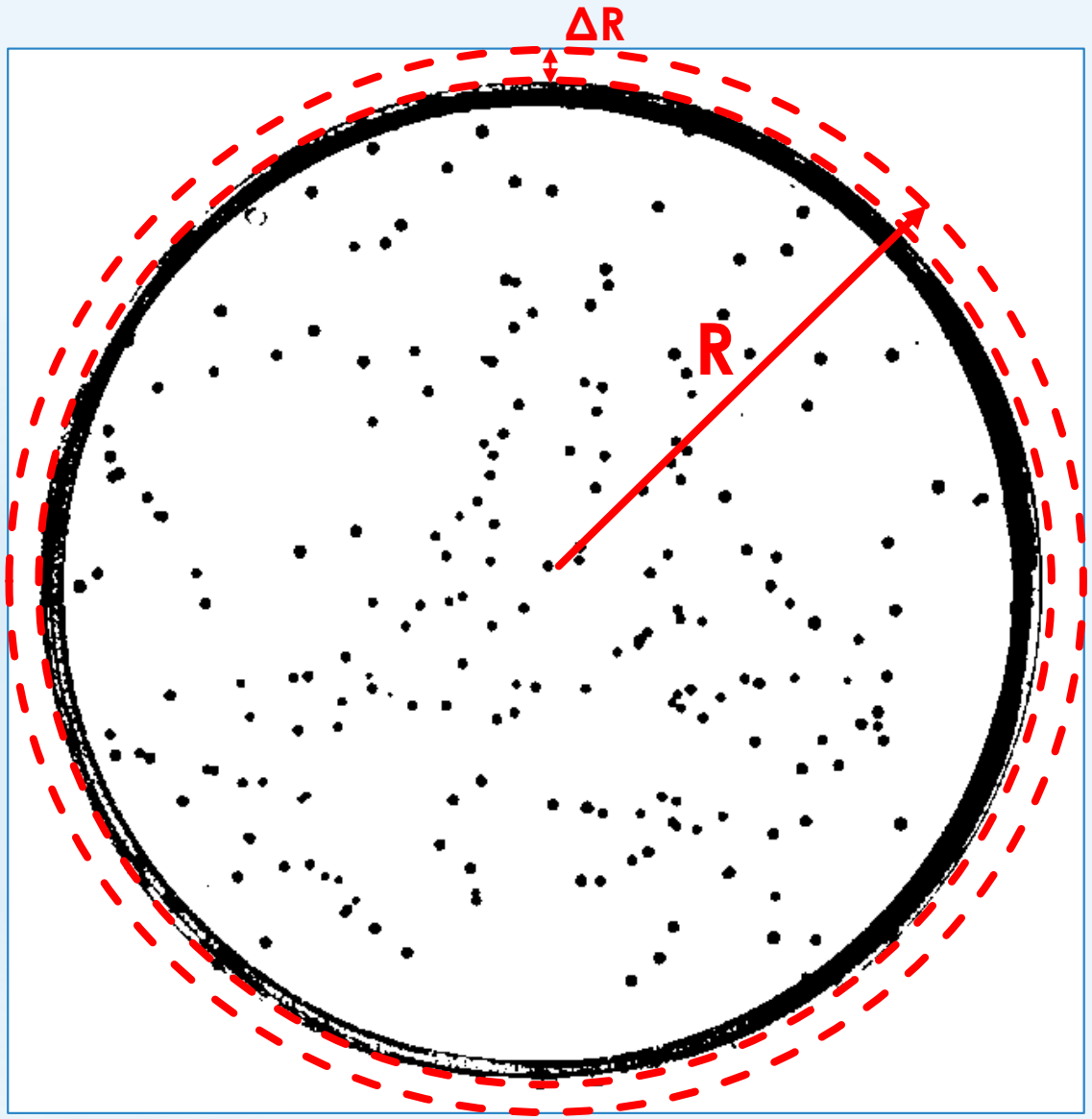
- My goal for this project was to develop a software in order to perform the aforementioned task. The software was written in **python**, based on a simplified version of the algorithm proposed by S. D. Brugger, C. Baumberger, M. Jost, W. Jenni, U. Brugger and K. Muhlemann in their paper *Automated Counting of Bacterial Colony Forming Units on Agar Plates* (2012).
- I employed various libraries for the project, in particular **Numpy** for scientific operations, **OpenCv** for image processing and visualization, and **PyQt** for the user interface.

The algorithm: overview

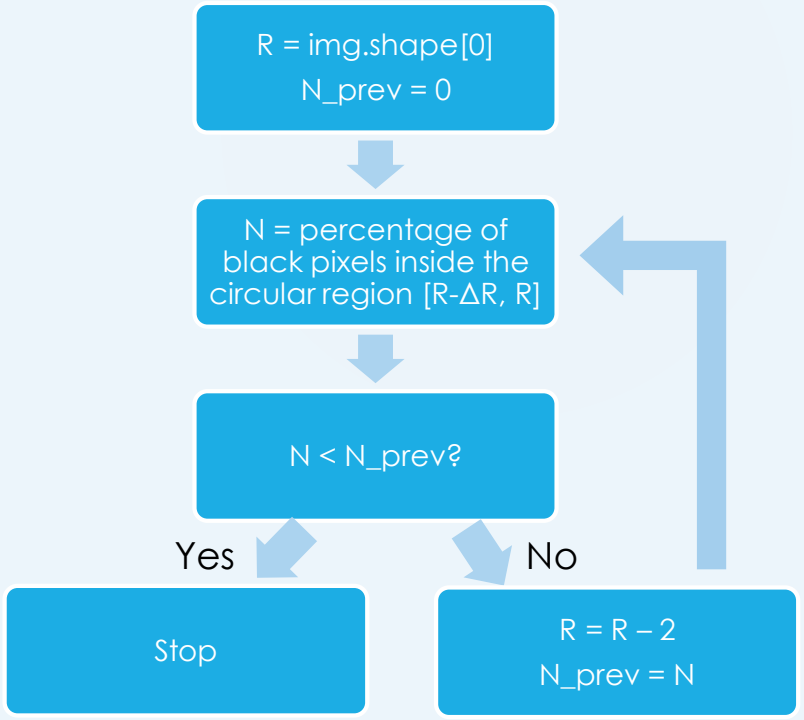


The algorithm: image pre-processing

The algorithm: Petri dish removal

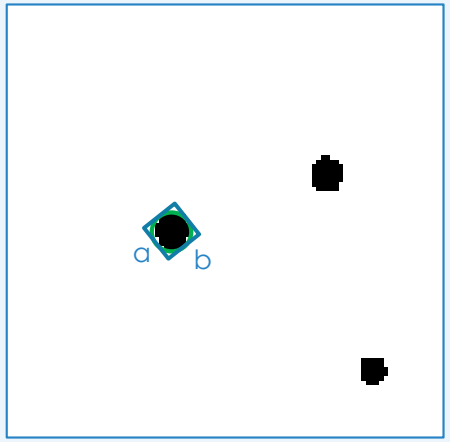


ΔR = constant (5 pixels)



The algorithm: Perfect circles identification

$a \cdot b > 0$ + $0.8 < \frac{a}{b} < 1.2$ + $0.7\frac{\pi}{4} < \frac{Area}{a \cdot b} < 1.3\frac{\pi}{4}$ = Perfect circle



The algorithm: cluster separation

- After having removed all perfect circles from the image, the next step is the evaluation of **clusters**, i.e. of groups of colonies (two or more) which touch one another.
- In order to figure out the number of colonies in each cluster, the **watershed transformation** is employed.
- A **watershed** is a transformation defined on a grayscale image. The name refers metaphorically to a geological *watershed*, or drainage divide, which separates adjacent drainage basins. The watershed transformation treats the image it operates upon like a topographic map, with the brightness of each point representing its height, and finds the lines that run along the tops of ridges.

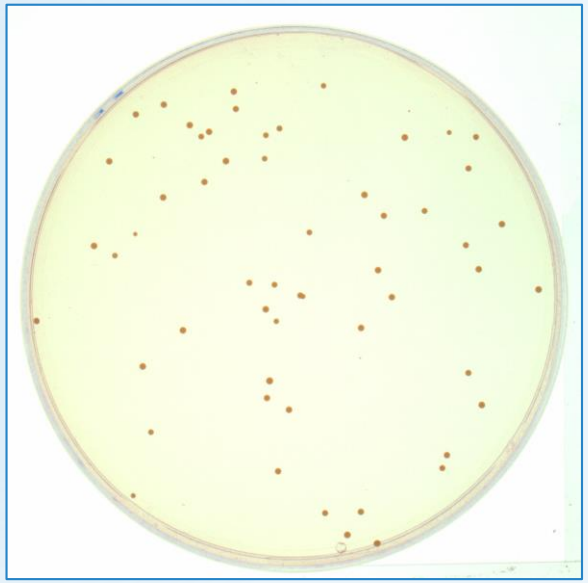
The algorithm: clusters count

- After having applied the watershed transform, the new contours are then analyzed in the same way as the **perfect circles** (with **relaxed constraints**, due to the imperfections of contours given by the watershed transformation).

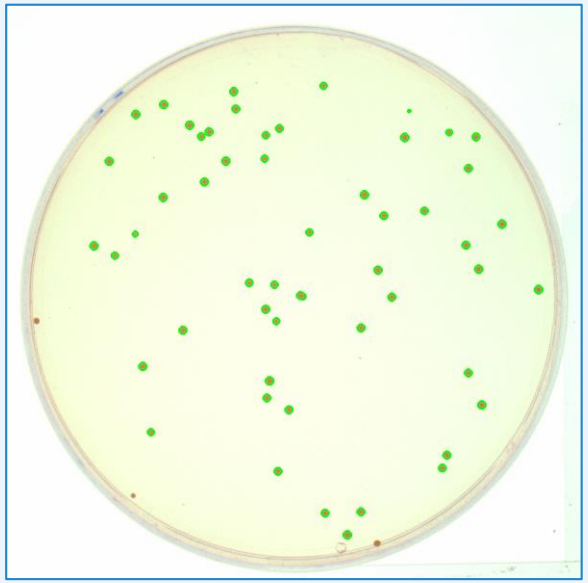
- Finally, all the perfect circles individuated during the process are counted and displayed to the user as the total number of colonies in the image.

The algorithm: contour drawing

- At the end of the process, to make it easy for the user to aknowledge which colonies have been effectively counted, a new image of the Petri dish is displayed with **all the counted colonies highlighted**.



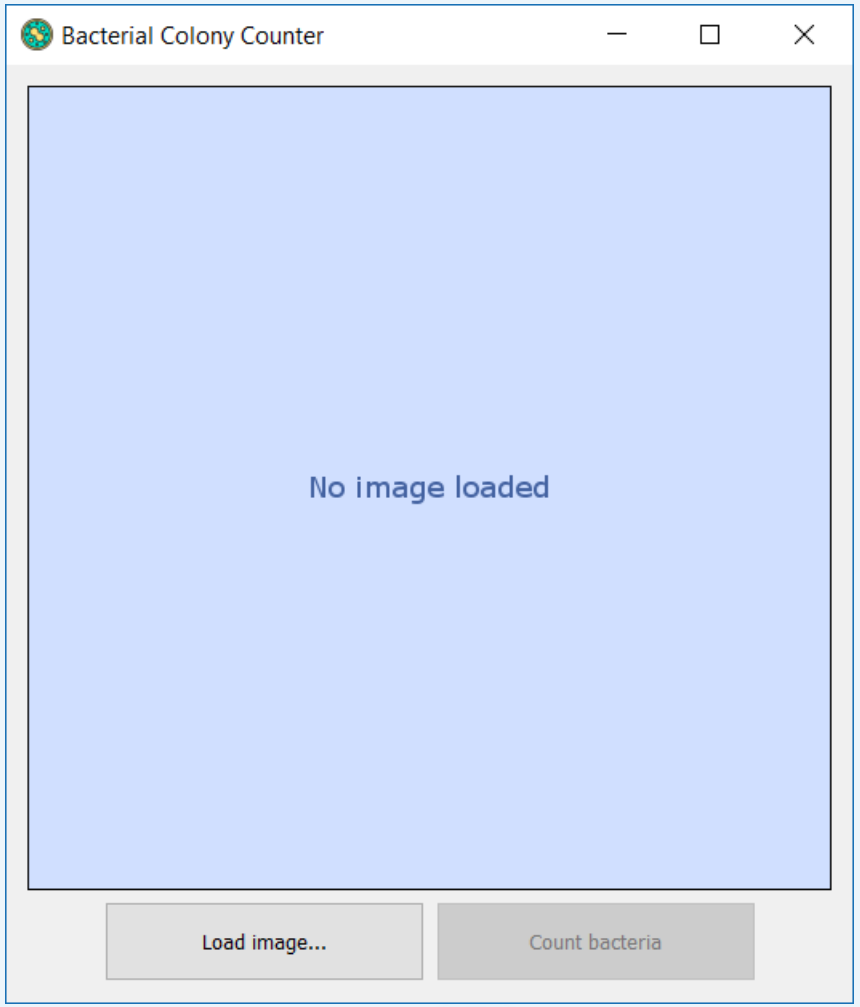
Initial image



Final image

User interface (1)

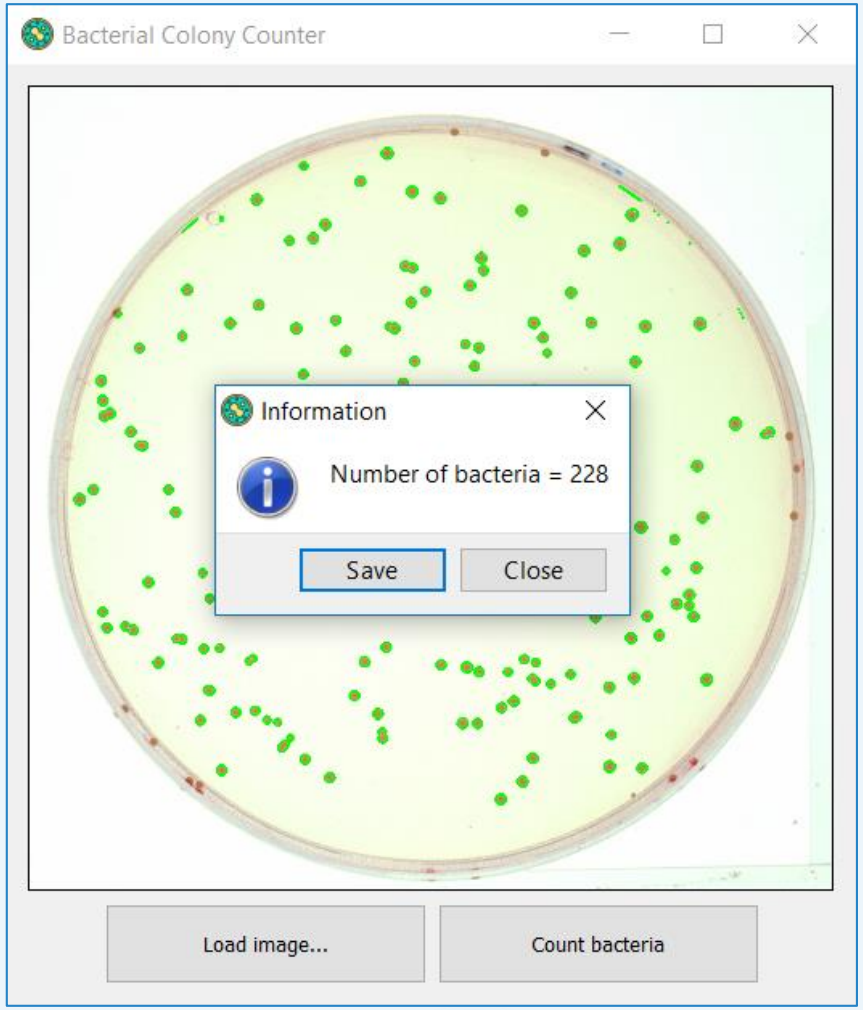
- The user interface was created with the python library **PyQt4**, the python version of the famous C++ library **Qt**.
- The main window is very minimal, including only the framework where the Petri dish image is displayed, at the center of the window, and two buttons on the bottom, the left one used to find and **load** the image file from the PC, and the right one (which becomes clickable only after an image is loaded) used to **start the algorithm** and count the bacterial colonies.



Main window

User interface (2)

- When the algorithm has finished his job, the total number of colonies is shown to the user by a **pop-up window** which appears automatically.
- From this window, the user can choose to **save** the number of colonies or simply **close** the pop-up window and continue to use the software.
- If the user chooses to save the data, a string including the **file path** and the **number of colonies** is saved in the *LOG.txt* file which is found in the program folder.



Main window with the pop-up window in the foreground

Critical issues

1. **Petri dish identification**

   The process of indentifying the Petri dish is automatic (unlike many other algorithms of the same kind), however it causes the non-classification of colonies along the borders of the Petri dish. Additional work can be done to improve this step of the algorithm.

2. **Importance of the dataset**

   The cleanliness of the images which undergo the process is crucial for the accuracy of the colony count. So in order to obtain reliable results, much attention must be put in the hardware-based process of acquisition of the images of the Petri dishes.

3. **Watershed transformation**

   The method employed to separate clusters of colonies in the algorithm is a watershed transformation. However, the very small size of the colonies in the images (which are often not big either) is not optimal for the transformation, which often gives not-so-good results. Additional work can be done to improve this step of the process, by developing a new technique expressly designed for this purpose.

Comparison of results

- To analyse the effectiveness of this algorithm, its results can be compared to those obtained with reliable softwares which are already available. I chose to use the software **OpenCFU**, which is considered one of the best on the market. The comparison is shown in the table below.

Image file	OpenCFU count	Our count
data10.jpg	28	27
data9.jpg	49	44
data18.jpg	206	189
data16.jpg	525	424

- While our software was significantly **faster then OpenCFU**, the latter is more precise. As it is shown above, the greater the number of colonies, the bigger is the difference. This is manly due to our software's **difficulty** in counting colonies **near the Petri dish border**, and on the correct separation of **clusters**.

Bibliography and webliography

- Algorithm based on: **S. D. Brugger, C. Baumberger, M. Jost, W. Jenni, U. Brugger and K. Muhlemann, *Automated Counting of Bacterial Colony Forming Units on Agar Plates* (2012).**
- Dataset: **https://sourceforge.net/projects/opencfu/files/samples/plosPicHQ.zip/download**
- https://www.cliffsnotes.com/cliffsnotes/subjects/sciences/what-is-a-bacterial-colony
- http://www.moldbacteriaconsulting.com/fungi/colony-forming-units-cfu.html
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