Image processing tool for the Microorganism cell counting and its recognition

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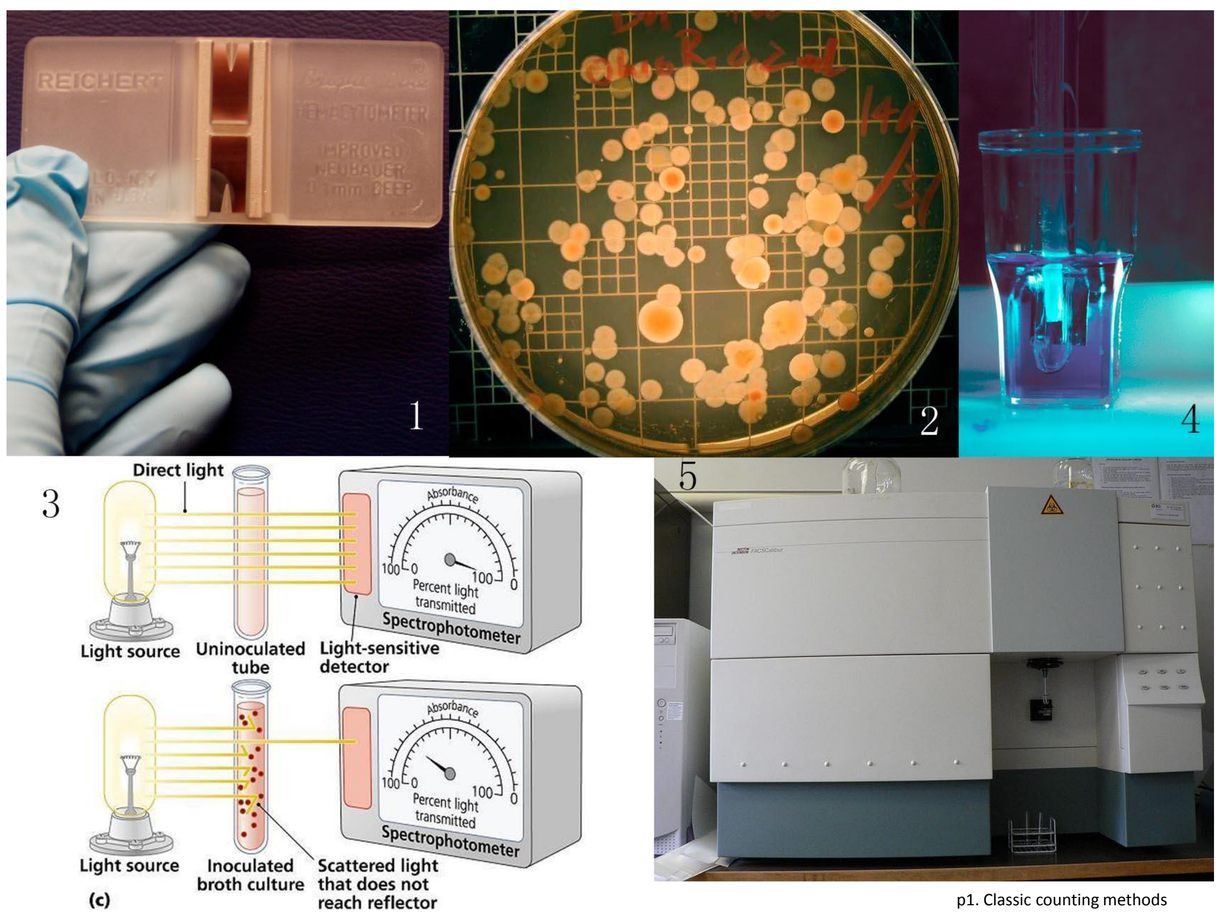
Abstract

Microorganism cell counting is a basic laboratory technique which was frequently used in Microbiology Lab, hospital and pharmaceutical company. The traditional technique which is called “Hemocytometer method” counts the cells smeared on Hemocytometer (A special micro slide) under a microscope. It is the most popular and cheapest way to count cells or microorganisms. However, Hemocytometer method is not always accurate enough and counting cells under microscopes is a tedious job. Therefore, the engineers have developed lots of new means to achieve higher accuracy and shorter processing times. But those new means require fastidious preparation and complicated operation. Furthermore, most basic labs cannot afford those equipments. Therefore, Hemocytometer method still is the top choice for most researchers. With my background and experience in computer science and biomedicine, I am developing simple software to improve the accuracy and reduce the counting time for Hemocytometer method. The general idea of this software is to digitally process the images taken under an optical microscope. The general steps are: Eliminating noise and impurities, Gridding recognition, Cell recognition, Cell counting and gathering the data. The benefit of this software is to achieve more accurate results while avoiding arduous tasks performed by the technicians and scientists.

Microorganism cell counting



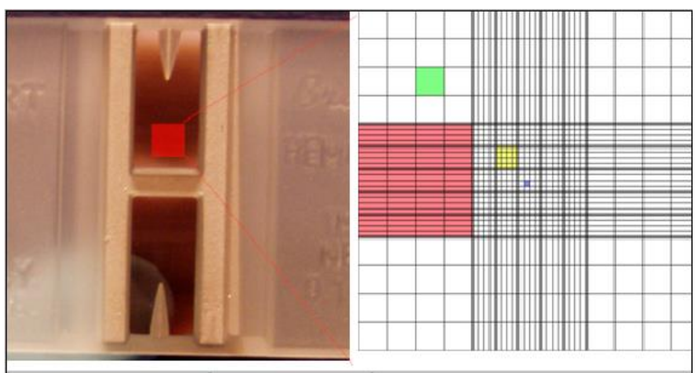
p1. Classic counting methods

Classic methods:

1. **Hemocytometer method** is economical and accessible, but counting is onerous and accuracy depends on operators.
2. **Colony-forming unit(CFU)** is easy to count, but takes a long time to get results and only could count living cells.
3. **Spectrophotometer(OD)** is fast, but has limited accuracy.
4. **Electrical resistance** has great accuracy, but is rather costly.
5. **Flow cytometry** has great accuracy and multifunctional, but procedure is perplexing, as well asexpensive

Hemocytometer method

Hemocytometer method is a counting-chamber method invented by Louis-Charles Malassez. A counting chamber consists of a thick glass microscope slide with grids of perpendicular lines. Each grid can hold specific volume of fluid. After samples are loaded properly on slides, cells can be counted in each grid, and divided based on the volume. The grid has specific dimensions so that the area is known, therefore the concentration of samples can be calculated.



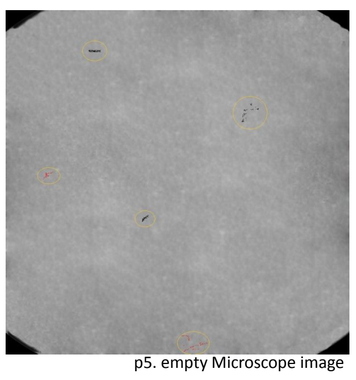
Dimensions	Area	Volume at 0.1 mm depth
1 x 1 mm	1 mm^2	100 nL
0.25 x 0.25 mm	0.0625 mm^2	6.25 nL
0.25 x 0.20 mm	0.05 mm^2	5 nL
0.20 x 0.20 mm	0.04 mm^2	4 nL
0.05 x 0.05 mm	0.0025 mm^2	0.25 nL

p2. Hemocytometer method

Count the cell in computer

Digital imaging of microscopy is widely used in the labs. Modern digital cameras provide great resolution which our naked eyes can never achieve. Also, we are able to process digital images with a specific algorithm to get specific kinds of results as needed.

p4. Microscope with camera

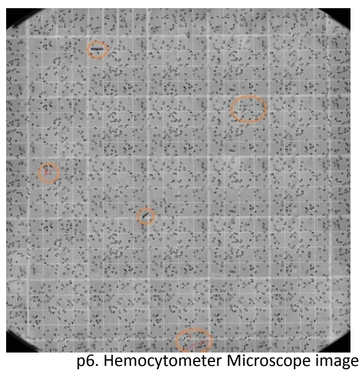


p5. empty Microscope image

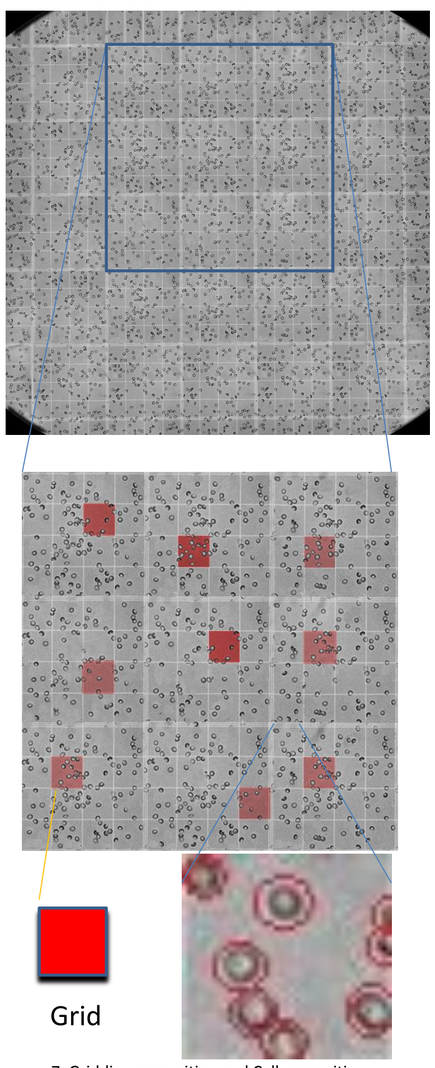
Five steps of this counting program :

1. Eliminating noise and impurities
   Dust and scratch inevitable occur in most microscope line system. To eliminate those noise and impurities , we could take a control image which is a empty microscope image.

Compare the control image with experimental image to eliminate noise and impurities. Also better results can be obtained from changing contrast ratio or running images through a convolution matrix.



p6. Hemocytometer Microscope image

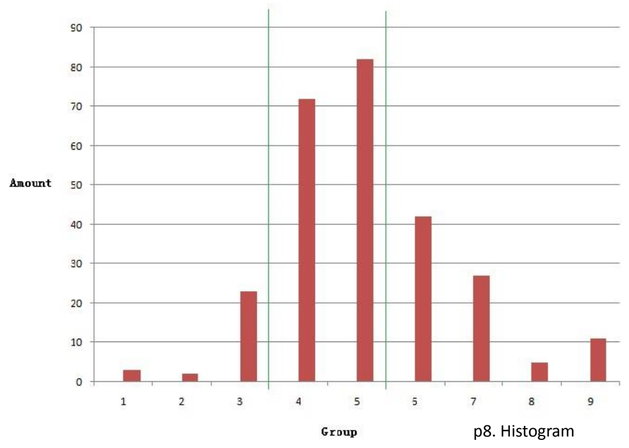


p7. Gridding recognition and Cell recognition

2. Gridding recognition
   Grid is the most important part in Hemocytometer. It is the measuring scale for cell counting.Average size of girds could be determined from several randomly selected grids. The more grids calculated, the more accurate results are.

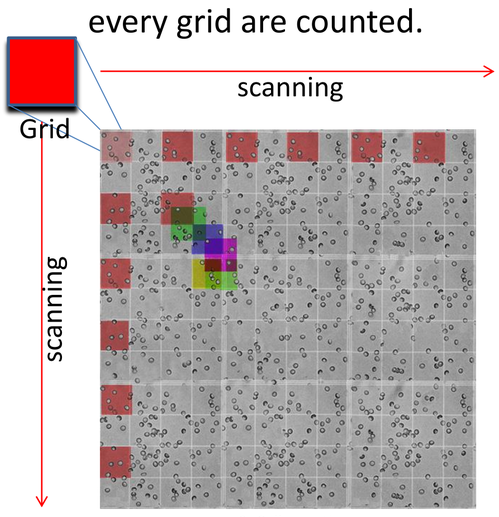
Average Volume$_G$= (V_{G1}+ V_{G2}+……+ V_{Gn})/n

3. Cell recognition
   Microorganisms on the images are measured and grouped according to the measurement of size. Then a histogram is generated based on the groups. Users are able to choose certain size ranges to count. Colors and shapes can also be set as screening parameters.



p8. Histogram

4. Cell counting
   After we decide which cells are to be counted. The processed images are scanned with grid. Number of cells in each every grid are counted.



p9. Cell counting

5. Result
   After average volume of grids is calculated as step 2. The whole image is scanned regardless of the original grids. In this way, significantly bigger area on the slide is covered for cell counting, comparing to manually counting. Hence, significantly more data contributes to a more precise result of a possible range of cell concentration.

$$\text{concentration of cells in original mixture} = \left(\frac{\text{number of cells counted}}{(\text{proportion of chamber counted})(\text{volume of squares counted})}\right)\left(\frac{\text{volume of diluted sample}}{\text{volume of original mixture in sample}}\right)$$

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References

1. Strober W (2001). "Monitoring cell growth". In Coligan JE, Bierer BE, Margulies DH, Sherach EM, Strober W. *Current Protocols in Immunology* **5**. USA: John Wiley & Sons. p. A.2A.1.
2. **Zephyris** (Richard Wheeler)) and the English language Wikipedia, http://commons.wikimedia.org/wiki/File:Haemocytometer_Grid.png