An Automated Method for Bacterial Flora Counting Based on Image Analysis

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Abstract. How to quickly detect the characteristics of water quality flora, such as bacterial flora number, is a key problem of water quality safety, the traditional manual counting method has some serious defects such as low efficiency, low accuracy, easy to cause visual fatigue and hard to deal with large samples. This paper designs an automated method for bacterial flora counting based on image analysis, including such modules as pretreatment of bacterial image classification and colony counting of culture medium images by filter method (using Hough circle detection algorithm and a circle deduplication algorithm). The accuracy of experimental results is above 90%, which is in line with international standards, and the time complexity of the algorithms is up to the order of square. This method can solve the problems of heavy workload and low efficiency in traditional manual counting method.

1. Introduction
National standards for water quality security include quite a number of considerable water quality indicators such as bacterial flora number. Rapid detection of all the indicators of bacteria flora is of great significance for raising the operation efficiency of waterworks. Analyzing the structure of microbial community (e.g. coli group) which is under conventional treatment in a filter chamber can measure whether the water quality of waterworks meets the standards in terms of microbial community species.

The biological method for detecting coli group is to culture it in the medium and count the colonies. However, manual counting method has lots of serious defects such as low efficiency, low accuracy, easy to cause visual fatigue of inspectors and hard to deal with large samples. But other detection methods often have more using requirements and limitations[1,2]. Therefore, the paper comes up with an automated method for bacterial flora counting based on image analysis.

Inspectors, who apply the automated method proposed in the paper, only need to take photos of the culture results and use the photos as the input of the colony counting algorithm, so that the accurate colonies counting results will be obtained in a very short time.

2. An Automated Method for Bacterial Flora Counting Based on Image Analysis
An automated method of colony counting proposed in the paper is divided into two procedures: data pre-processing and colony counting of culture medium images by filter method.

In the data pre-processing module, when processing the filter medium image, manual clipping is required to prevent interference from other culture dishes in the image; when there is only the target medium in the image, an algorithm can be designed to determine the position of the target medium in the picture, and finally cut out the target medium and save it in a unified specification. The specific process is shown in figure 1.
Figure 1. Flow chart of outline design of medium image pre-treatment by the filter membrane method.

In the filter method, colonies grow within the effective inoculation range on the culture medium. According to the medium images obtained by the pretreatment, which have the uniform specifications, the algorithm needs to count within the effective range and deduplicate the counting results. The basic process is shown in figure 2.

Figure 2. The outline design flow chart of colony counting in the image of the filter medium.

Among them, the pseudo-code of the algorithm for deduplication of the colony counting results is shown in table 1.

Table 1. The pseudo-code of the algorithm for deduplication of the colony count results.

| Algorithm: | De-duplicate counting results |
|------------|------------------------------|
| Input:     | Sequence list of counting results of colony counting algorithm |
| Output:    | the result sequence circlelist after deduplication |
| 1:         | Set the flag array of the same length as the list, all set to 1 |
| 2:         | Set the circlelist of result sequence and set it to empty. |
| 3:         | for i ← 0 to list.length() do |
| 4:         | if flag[i] == 1 do |
| 5:         | for j ← i + 1 to list.length() do |
| 6:         | if flag[j] == 1 and the distance between list[i] and list[j] is less than the radius of one of the circles do |
| 7:         | flag[j] ← 0 |
| 8:         | end if |
| 9:         | end for |
| 10:        | end if |
| 11:        | end for |
| 12:        | for i ← 0 to list.length() do |
| 13:        | if flag[i] == 1 do |
| 14:        | circlelist.append(list[i]) |
| 15:        | end if |
| 16:        | end for |
3. Results and Analysis

In the pretreatment of culture medium images by filter method, manual clipping is required first to remove the non-target medium; when positioning the medium and unifying images’ specifications, we first need to convert the RGB image to a grayscale image in Python OpenCV and gamma correct the grayscale image of the medium with a nonlinear correction method to reduce the influence of the background and highlight the edges of the medium. Then we need to binarize the image, position the medium by the minimum enclosing circle algorithm\[3\], clip the image of the medium to a size of 800×800 according to the minimum enclosing rectangle and save the pre-processed image. The process and results of image processing are showed in figure 3.

![Figure 3](image-url)

Figure 3. The process and results of the pretreatment of culture medium images by filter method.

In the colony counting of culture medium images, we need to count the colonies of fecal coliform and colonies number in the culture medium images by filter method. The effective inoculation range of the medium should be determined before counting. The colony counting algorithm in this paper is designed based on the original Hough circle detection algorithm\[4,5\] and deduplicating the counting results is required after the counting work (as shown in figure 4).

![Figure 4](image-url)

Figure 4. The process and results of colony counting by filter method (fecal coliform).

In the colony counting of culture medium images by filter method for colonies number, there is no need to locate the effective inoculation portion of the culture medium when counting the total colony as the colonies grow everywhere in the culture medium. The results of binarization and counting are shown in figure 5.
Figure 5. The results of binarization and counting of culture medium images by filter method.

Comparing program counting with manual counting and analyze the data and calculate the errors, the maximum error of the algorithm of the colony counting for fecal coliform in culture medium images by filter method of is 14.8% and the average error is 6.8%, which is in line with the national standard that describes medium colony counting error should not exceed 15% when the number of colonies is no more than 300. The maximum error of the algorithm for colonies number in culture medium images by filter method is 8.0% and the average error is 5.4%, which is in line with the national standard that describes medium colony counting error should not exceed 30% when the number of colonies is more than 300. Table 2 shows more details.

Table 2. The results of program counting and manual counting for colonies number in culture medium images by filter method

| Image number | 1   | 2   | 3   | 4   | 5   | 6   |
|--------------|-----|-----|-----|-----|-----|-----|
| Manual counting | 334 | 423 | 378 | 384 | 387 | 430 |
| Program counting | 356 | 457 | 408 | 396 | 403 | 418 |

4. Conclusion

The paper designs an automated method for bacterial flora counting based on image analysis, including pretreatment of bacterial image classification, colony counting of culture medium images by filter method and so on, whose average accuracy rate is more than 90%, and the maximum time complexity of the algorithm is on the order of square. This method is able to address the difficulty of heavy workload and low efficiency in traditional manual counting.

5. References

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