Computer-Aided Somatic Cells Counting on Three Different Milk Staining Condition

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Abstract. The maximum amount of somatic cell in a millilitre of milk defined by National Standardization Agency is $4x10^5$ cells/ml. The condition of milk will be excluded from a decent quality if the amount of somatic cell is greater than it. Microscopic image of somatic cells counting is a conventional method which utilized by farmer or researcher to count the number of somatic cells from dairy milk sample. The problems of this method are: 1) The counting process is manually conducted which prone to miscalculation, 2) The different colour of staining (purplish, bluish, and greenish) may complicate the calculation. In this research, a computer-based approach is proposed to calculate the number of somatic cells from sample of milk and to eliminate the difficulty of different colour due to staining technique. Image processing knowledge i.e. erosion, dilation, colour conversion, and BLOB Analysis are involved and utilized to achieve the objective. Overall, the correctness of the system in detecting the number of somatic cells is 94%. From this study, a comprehensive system to calculate the number of somatic cells from cow's milk will be closer to be implemented.

1. Introduction

The quality determination process of cow's milk according to SNI standards involves fifteen characteristics that must be fulfilled by the standard. One of these characteristics is the maximum number of somatic cells found in milk. Somatic cells in milk are epithelial cells that secrete milk, these cells are released from the lining of glands and white blood cells (leukocytes) and enter the mammary gland [1]. Somatic cells occur in response to injury or infection in the cow's milk glands. The number of somatic cells contained in cow's milk is widely used as an index for determining the health of mammary glands and the quality of cow's milk. The number of normal somatic cells is less than 1 x 105 cells / ml, the number of somatic cells will increase if an infection occurs [2].

One type of infection in the cow's milk glands is mastitis, mastitis is inflammation that can be caused by a pathogenic bacteria infection such as Staphylococcus aureus. Low hygiene, environmental condition, and unproper nutrition might lead to this condition. The existence of mastitis reduces the quality of cow's milk, one result is that cow's milk is broken and unhygienic. Mastitis is an inflammatory disease in goats that can cause directly or indirectly [3]. While some losses occur if cows and goats have mastitis, excreted milk production per quarter per day decreases between 9% - 45.5% [4][5], the quality of milk that needs to be avoided Milk loss reaches 30% -40% and the quality of processed milk products [6] as well as increased care and care costs[7][8].

The selection of dairy animals for greater milk production and the disposal of the milk by the milking machine puts unnatural stress on the udder of the cow [9]. This has increased the chances of breast infection in these animals. To defend against breast infection, somatic cells (SC) are released into the milk. These cells not only fight infection but also repair damaged tissue. All developed countries use milk somatic cell counts (SCCs) as a marker to monitor the prevalence of mastitis in dairy farming, as an indicator of raw milk quality for processors, and as a more general indicator of hygiene. milk production conditions on the farm [10][2].

Based on problem, BLOB analysis method is used to see the somatic number of cells in livestock, so in this study will use image processing using BLOB Analysis. BLOB analysis aims to extract objects from each binary image space so that each object can be selected by somatic cells. BLOB analysis also succeeded in increasing the segmentation results of images that have a lot of noise [11]. BLOB analysis is commonly used to determine the properties of object in the image especially for object detection and recognition. Among the BLOB properties for the object such as areas, diameter, major and minor axis length, shape, location, and perimeter. In the study of [12] which utilize BLOB analysis, three types of properties were used such as area, centroid and boundary. For the BLOB area properties, the size of the fish larvae can be determined in pixel values. Meanwhile the BLOB centroid properties are used to determine the centre of the fish larvae and counting the number of somatic cell in the image.

Generally, the somatic cells number is counted using the Fossomatic tool, which is relatively expensive tool. Otherwise, the measurement of the maximum number of somatic cells are generally performed by manual estimation using the help of microscope. However, this kind of task would be not efficient if dealing with many samples. A better method using computer system automation in analysing the liquid sample is required. The implementation of image processing technology enables the automatic counting of the somatic cells based on the sample image which are given.

2. Material and Method

This research uses the tools and method used describes.

2.1 Image Acquisition

Sample of cow's milk was taken in Desa Limpakkuwus, Baturraden, Banyumas. And then, the milk liquid is observed on a slide under microscope device to be seized its image by using Optilab Tool. The images format was stored in .JPG files, 850x850 of resolution, and RGB channel. There only 1 condition of background colour of the images i.e. bluish from the original image acquisition. Since this research attempts to solve the problem of background colour variation, replication of images is necessary in order to create the image in purplish and greenish background colour. By using Adobe Photoshop and designed by professional artist, images are reproduced cautiously without removing any component which appeared in the image.

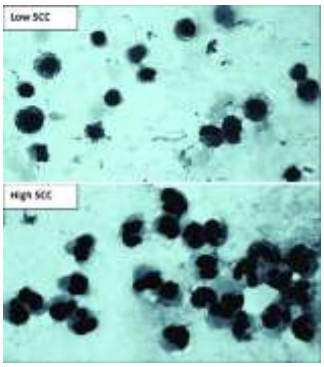


Figure 1. Sample microscopic somatic cell image [9]

2.2 Binarization and Colour Reversion

Binarization step will convert the image into black and white colour. This research utilizes Otsu Thresholding Binarization method. The background of the image is the major area which represent the plasma cell. It has blue colour in bluish image, purple colour in purplish image, and soft green colour in greenish image. After binarization process, the background will shift to white and the remainder components to black. The conversion to negative image is followed to revert black-to-white and white-to-black. Black area will dominate the background colour, meanwhile white objects will be the component of images which possible to be proceed by using morphological image processing in the latter phase.

2.3 Morphological Operation

Morphological operation is involved in this series of method. This step is solely accomplished to enhance the image quality by removing noisy small objects which known as artefact or unused object. It can be observed by bare eye that somatic cells have larger size compared to artefacts in which its size is very small. Hence, by adjusting the right opening and closing value, this procedure may eliminate the existence of artifacts. Apart from that, this step is also enhancing the visibility of image boldness on its edge.

2.4 Blob Analysis and Somatic Cell Counting

BLOB Analysis is a simple technique in image processing to count an independent element of white colour which separated one-another. A cluster of pixels which connected each other which formed into a single area will be counted as an object. These objects are spread on the surface of the image and it presents the existence of somatic cells. BLOB analysis commits the location of each objects with its physical parameter, such as centre of mass, area, perimeter, and the location in the image. The whole developed system is considered as capable to count the somatic cells by recognizing the location of each object on the image.

3. Result and Discussion

3.1 Image Replication

This step is conducted by professional artist in order to reproduce the image without losing its originality. The bluish image is converted into purplish and greenish image. From Figure 2, it is stated that the little spots scattered on the image are known as objects, possibly the somatic cells or the artefacts. One thing to be noticed is artefacts has very small size compared to the somatic cells. Hence, in this image, it is called as noise.

(a) (b) (c)

Figure 2. (a) original image in bluish colour
sample of replication in (b) purplish colour, (c) greenish colour

3.2 Converting to Binary Image

The phase of converting the image to binary channel require a specific value to define the ‘border’ or floating value between upper and lower value. Upper value means the area above the border value which is going to be converted into 1 or white. Vice versa, lower value means the area below the

border value that will be changed into 0. This will produce the black area. All objects in white and blacks are essential element since it contains all information required by system to detect and count the somatic cells.

(a) (b) (c)

Figure 3. Binarization result of (a) bluish, (b) purplish, (c) greenish

3.3 Negative Image / Complementary Image

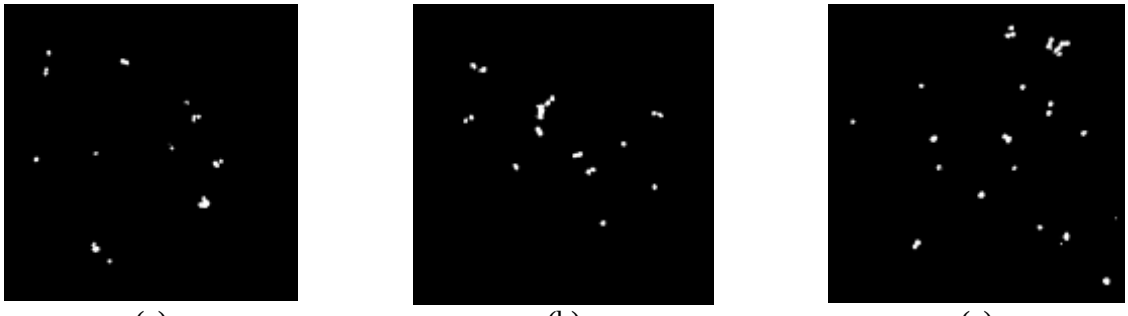
Converting the result to negative image implies a changing colour of the binary-based image from 1 to 0 and from 0 to 1. In short, this process transforms the white colour objects to black, and in opposite, black to white colour. Since, the objects in white colour is spread around the image, it is stated that those white objects are the somatic cells and the artifacts.

(a) (b) (c)

Figure 4. Negative Image result of (a) bluish, (b) purplish, (c) greenish

3.4 Operation of Morphologic to Eliminate the Existence of Noise

Converting the result to negative image implies a changing colour of the binary-based image from 1 to 0 and from 0 to 1. In short, this process transforms the white colour objects to black, and in opposite, black to white colour. Since, the objects in white colour is spread around the image, it is stated that those white objects are the somatic cells and the artifacts.

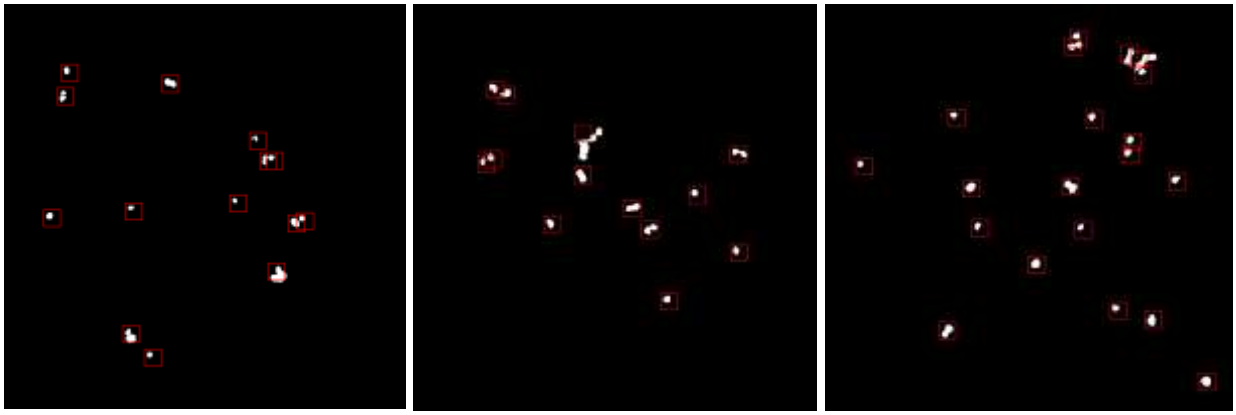


(a) (b) (c)

Figure 5. Opening and Closing result of (a) bluish, (b) purplish, (c) greenish

3.5 BLOB Analysis

BLOB analysis is utilized by system to recognize the identity of each object like centroid, perimeter, area, etc. In this phase, this technique has successfully recognized the existence of white objects, which is the somatic cells, and also provide the information needed of every object lied on the image.



(a) (b) (c)

Figure 6. BLOB analysis result of (a) bluish, (b) purplish, (c) greenish)

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banyak_kandidat =

    13

|Candidate Num| Centroid  |
|          1 |    151.9     329.4 |
|          2 |    167.7     320.4 |
|          3 |    173.5     175.9 |
|          4 |    200.3     187.0 |
|          5 |    292.2     458.5 |
|          6 |    357.0     359.3 |
|          7 |    370.6     293.1 |
|          8 |    464.8     425.3 |
|          9 |    501.0     470.0 |
|         10 |    535.6     616.3 |
|         11 |    593.2     394.4 |
|         12 |    687.5     311.2 |
|         13 |    679.7     515.0 |
```

Figure 7. BLOB analysis result with the number of somatic cells detected and its centroid

Colorization of cow's milk object on microscopic slide (staining method) may affect to the foremost colour of image. By using methylene blue, the colour of image stained is changed into bluish colour. And, as the consequences, the image captured by using Optilab tool is also appeared in bluish colour. However, the colorization process of object on a slide can also be visible in purplish and greenish colour depend on staining method. In general, on many cases, Coomassie brilliant blue staining may produce image with purplish background, meanwhile staining with fluorescent may be resulted in greenish colour.

By performing a sequence of image processing, the captured images are ready to be carried on by system to detect and calculate the number of somatic cells. After binarization process, the next step is performing morphological image processing. This phase is solely committed to eliminate the existence of artefact. It is noticeable that the size of artefact is very small compared to the somatic cell itself on the surface. Hence, opening and closing technique will remove the artifacts and thicken the edge of somatic. This state of image will help the system to recognize the somatic cells easier.

Table 1. BLOB Accuracy

No	Predicted Object Detected	Actual Number	Percentage
1	14	14	100%
2	18	20	90%
3	12	13	100%
Average			94%

As the last step of developed system, BLOB analysis counts the number of somatic cells lied on the surface of the image. Counting process is started from top-left to bottom-right to identify the location of each cell. The process is followed by giving label or marker to tag the location of every identified objects. As for, tagging coordinates is based on the centroid (centre of mass) of every object which obtained from BLOB analysis. Up to this last process, the system has counted the number of somatic cells on the image.

Developing this system to this stage does not imply that the system is flawless. One problem explored from this sequence of method is that the system has not able to identify imminent somatic cells object which stacked each other. There might be conditions where somatic cells are not separated clearly, attached to each other, and stacked. The system which only applying conventional.

4. Conclusion

From the series of method constructed to count the number of somatic cells, it is affirmed that the system is capable to detect the location of each cells on every condition. On bluish background which mostly produced by staining of methylene blue, the system delivers a good result of 96% accuracy of object counting. Likewise, when the image is shifted into purplish and greenish background, the system can detect the location of somatic cells as well and effortlessly. To put an end, this finding may have implication on computer-aided somatic cells counting with less problem which following the method.

Morphological image operation performed a vital role in this research. First, image colour conversion successfully converts RGB dataset image into binary channel which separate the somatic cell from another objects. Then, morphological operation will help separating imminent objects, highlighting each object, and eliminating any useless artifacts. BLOB Analysis positively identify the physical characteristics of objects hence the system can count the number of somatic cells in the image. To put an end, series of method in this research successfully demonstrates the counting of Somatic Cells in Cow's Milk up to 94% accurate identification.

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