Detecting Geckler Classification from Gram Stained Smears Images for Sputum

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Abstract: A *Geckler classification* is a criterion how the smear image is quality based on the number of buccal squamous epithelial (BSE) cells and leukocytes in the Gram stained smears images per $100\times$ field for sputum. The Geckler classification then determines which of images is valuable to microscope testing for the Gram stained smears images per $1,000\times$ field for sputum. In this paper, we develop the system to detect the Geckler classification from Gram stained smears images per $100\times$ field for sputum. In this system, first we detect the regions of BSE cells and leukocytes and then construct the classifier of the BSE cells and leukocytes by SVM and DNN. Then, we detect the Geckler class of every test image by detecting the candidate regions and by applying the classifier.

1 INTRODUCTION

The *Gram stain* (Bartholomew and Mittwer, 1952) is the method for microbial smears test in microscope test *per* $1,000\times$ *field*, introduced by Hans Christian Gram (1853–1938) at 1884. For the Gram stain, we detect bacteria occurring in the smears for the samples of blood, sputum, feces, pus and urine based on the stained colors as purple/violet or red/pink and the stained shapes as sphere-shape, rod-shape, singles, pairs, chains, clusters, and so on.

In particular, for the sample of sputum, in order to provide a criterion how the Gram stained smears image per $1,000\times$ field is quality for the microscope testing, a *Geckler classification* has been introduced by Geckler *et al.* (Geckler et al., 1977) and developed by Wong *et al.* (Wong et al., 1982). The Geckler classification is defined by the number of *buccal squamous epithelial (BSE) cells* and *leukocytes* for the Gram stained smears images *per* $100\times$ *field*. Table 1 illustrates the definition of the six classes in the Geckler classification (Geckler et al., 1977; Mitsuda, 2004; Wong et al., 1982), which we call *Geckler classes*. In the Geckler classification, the Geckler classes 4 and 5 are valuable for microscope testing.

In this paper, we sometimes denote the Geckler classes from 1 to 3 by NG (no good), those of 4 and 5 by GE (good and excellent) and that of 6 by UN (unknown). By using these general Geckler classes, we say that the quality of the Geckler classification is NG if the number of BSE cells is greater than 25, GE if the number of BSE cells is less than 25 and the number of leukocytes is greater than 25 and UN if both the numbers of BSE cells and leukocytes are less than 25.

Table 1: The Geckler classification.

class	BSE cells	leukocytes	quality	
1	>25	<10	no good	NG
2	>25	$10-25$	no good	
3	>25	>25	no good	
4	$10-25$	>25	good	GE
5	<10	>25	excellent	
6	<25	<25	unknown	UN

In our laboratory, we are developing the detecting systems of Gram types for bacteria from Gram stained smears images per $1,000\times$ field (Iida et al., 2020). On the other hand, the Geckler classification is based on the Gram stained smears images per $100\times$ field. Note that the relationship of shapes between leukocytes and

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bacteria in the images per $1,000\times$ field is similar as the relationship of shapes between BSE cells and leukocytes in the images per $100\times$ field for sputum. Based on this observation, in this paper, we extend the method to detect Gram types from the images per $1,000\times$ field to the method to detect the Geckler classification from the images per $100\times$ field for sputum.

In this paper, we first detect the regions of BSE cells and leukocytes in the images for sputum per $100\times$ field like as those of bacteria and leukocytes for images per $1,000\times$ field (Iida et al., 2020). Then, after constructing training data for the Geckler classification, that is, assigning to the labels to BSE cells and leukocytes, we detect the Geckler class from a given image by using the machine learning of SVM (support vector machine) and DNN (deep neural network).

1.1 Related Works

Note that the method to detect bacteria or Gram types from the Gram stained smear images per $1,000\times$ field have developed by several researchers (Lejon and Andersson, 2016; Smith et al., 2018). However, their works have dealt with images for the sample of blood, not sputum.

On the other hand, as related works to this paper, Carvajal *et al.* (Carvajal et al., 2014) have developed the system to learn the candidate images from training data, consisting of fixed size (51×38 pixels) images, applicable to the microscope test with high magnification. Here, the training data are assigned to 4 labels, that is, (1) the candidate areas for high magnification, (2) those but pathologists observed no bacteria, (3) the dense and dark area difficult to diagnosis and (4) background areas or areas with non-bacterial substances. They have also dealt with the Gram stained smear images for the sample of blood, not sputum, per $64\times$ field.

Crossman *et al.* (Crossman et al., 2015) have developed the method to detect the regions of BSE cells and leukocytes by using multiple covariance approach with Kernel SVM from manually cropped images of BSE cells, leukocytes and other negative data for the Gram stained smears images par $65\times$ field. Since the shape and the size of BSE cells have wide variety in the Gram stained smear images for the sample of sputum, in order to achieve the Geckler classification by using their work, it is necessary to start to detect the regions of BSE cells carefully.

Then, in this paper, we first detect the candidate regions of BSE cells and leukocytes from Gram stained smear images for the sample of sputum, by using the similar method of (Iida et al., 2020) applicable to wide variety of the shape and the size of BSE cells. Then, after constructing the positive and negative data of BSE cells and leukocytes, we detect the Geckler class for every image.

2 DETECTING GECKLER CLASSIFICATION

In this paper, we use Gram stained smears images per $100\times$ field for sputum, provided from Osaka General Medical Center. Table 2 represents the number of images to construct training data (which we call *training images*) and to test the classifier for the Geckler classification (which we call *test images*) for every Geckler class.

Table 2: The number of training images and test images for every Geckler class.

Geckler class	1	2	3	4	5	6	total
training images	19	20	20	19	19	10	107
test images	20	14	28	18	16	11	118

2.1 Detecting Candidate Regions

In order to detect the regions of BSE cells and leukocytes from training images, we use the similar method as (Iida et al., 2020). First, as image processing, we apply the following processes to every training image:

1. Grayscale by the NTSC (nonsubsampled contoulet transform) coefficient method (Keahler and Bradski, 2013) under the following formula from the RGB values for every pixel:
$$Y = 0.298912R + 0.586611G + 0.114478B.$$
2. Binarization under the adaptive thresholding (Keahler and Bradski, 2013) with the size N of neighbor and the subtractive constant C.
3. Opening processing (Keahler and Bradski, 2013) at P times as applying the dilations after applying the erotions.
4. Edge detection by the Canny filter (Canny, 1986).
5. Detecting candidate regions of BSE cells and leukocytes within the ranges $[S_{min}, S_{max}]$ of areas, $[A_{min}, A_{max}]$ of aspect ratio and $[C_{min}, C_{max}]$ of circularity.

Note that the processes of the binarization and the opening processing are essential to detect wide variety of the shape and the size of BSE cells, because the opening processing complements to capture the regions not to capture the binarization.

Table 3 represents the above parameters adopted in this paper for image processing. Here, the rows of blood, sputum, faces, pus and urine denote the parameters to process images per $1,000\times$ field adopted by (Iida et al., 2020).

Table 3: The parameters for image processing.

	N	C	P	S_{min}	S_{max}
BSE cells	4,911	7	3	2,500	75,000
leukocytes	301	44	3	150	1,000
blood	257	37	2	200	30,000
sputum	431	37	2	130	30,000
feces	281	18	2	300	30,000
pus	301	38	2	150	15,000
urine	581	35	2	200	50,000

	A_{min}	A_{max}	C_{min}	C_{max}
BSE cells	1.0	100	0.05	1
leukocytes	1.0	3	0.05	1
blood	0.1	1	0.1	1
sputum	0.1	1	0.1	1
feces	0.1	1	0.1	1
pus	0.1	1	0.1	1
urine	0.1	1	0.1	1

Table 3 shows that the parameters for BSE cells and leukocytes are different from those for others. In particular, the parameters of N, S_{min}, S_{max} and A_{max} for BSE cells are much greater than those for others. This is because the size of BSE cells is much greater than the size of leukocytes per $100\times$ field and the corresponding size to the BSE cells, that is, the size of leukocytes, in the images per $1,000\times$ field is much greater than the size of bacteria.

2.2 Training Data

For training images, the medical technologist (the fourth author) has assigned every candidate region to the label of a BSE cell, a leukocyte or others.

As a result, Table 4 represents the number of regions of BSE cells and leukocytes as positive data and others as negative data. Here, the candidate region of BSE cells (*resp.*, leukocytes) but not labeled by BSE cells (*resp.*, leukocytes) are negative data for BSE cells (*resp.*, leukocytes).

We call all the positive and negative data for BSE cells and leukocytes represented in Table 4 *training data*. Then, by applying SVM and DNN to the training data, we construct the classifier of Geckler classes. Here, we adopt SVM as the library provided from OpenCV (Keahler and Bradski, 2013) whose kernel is CHI2 (Li et al., 2010). Also we adopt DNN as Caffe (convolutional architecture for fast feature embedding) based on the rayer structure of AlexNet (Krizhevsky et al., 2017).

Table 4: The number of regions of BSE cells and leukocytes as positive data (pos.) and others as negative data (neg.).

	pos.	neg.
BSE cells	2,624	2,952
leukocytes	10,222	16,009

3 EXPERIMENTAL RESULTS

In this section, we give experimental results for detecting a Geckler class.

3.1 Detecting Geckler Class

First of all, Table 5 represents the number of detected regions of BSE cells and leukocytes in test images.

Table 5: The number of detected regions of BSE cells and leukocytes in test images.

BSE cell	leukocyte
5,337	21,877

From the regions in Table 5, Table 6 represents the average running time (msec) to learn whether or not the detected region in test images is the region of the BSE cells and leukocytes by SVM and DNN.

Table 6: The average running time (msec) to learn whether or not the detected region in test images is the region of the BSE cells and leukocytes by SVM and DNN.

	BSE cell	leukocyte
SVM	0.10	0.38
DNN	221.61	223.69

Table 7 represents the results of the detected Geckler class by applying the classifiers constructed by SVM and DNN from the training images with the correct Geckler class to the test images.

Table 8 represents the results in Table 7 summarizing the Geckler classes from 1 to 3 as NG, those of 4 and 5 as GE and that of 6 as UN.

Tables 7 and 8 show that the classifier constructed by SVM does not achieve the correct Geckler class, because it cannot detect BSE cells well, whereas it

Table 7: The result of the detected Geckler class from the test images by classifiers constructed by SVM and DNN from the training images.

SVM	correct					
detect	1	2	3	4	5	6
1	0	0	0	0	0	0
2	1	0	0	0	0	0
3	0	0	0	0	0	0
4	1	2	0	0	0	0
5	3	15	19	20	16	0
6	15	3	0	0	3	20

DNN	correct					
detect	1	2	3	4	5	6
1	9	0	0	0	0	0
2	10	10	2	0	0	0
3	1	8	14	**1**	0	0
4	0	0	**1**	8	2	0
5	0	0	0	0	12	0
6	0	2	2	11	5	20

Table 8: The results in Table 7 summarizing the Geckler classes.

SVM	correct		
detect	NG	GE	UN
NG	1	0	0
GE	37	36	0
UN	18	3	20

DNN	correct		
detect	NG	GE	UN
NG	54	**1**	0
GE	**1**	22	0
UN	4	16	20

can detect leukocytes. On the other hand, the classifier constructed by DNN succeed to detect the correct Geckler class.

In the remainder of this section, we focus on the Gram stained smears images per 100× field for sputum that (1) the correct Geckler class is 3 (NG) but the detected one is 4 (GE) and (2) the correct Geckler class is 4 (GE) but the detected one is 3 (NG), that are depicted by bold faces in Tables 7 and 8.

Figure 1 illustrates the Gram stained smears image that (1) the correct Geckler class is 3 (NG) but the detected one is 4 (GE) and the results of detecting BSE cells and leukocytes.

Figure 2 illustrates the Gram stained smears image that (2) the correct Geckler class is 4 (GE) but the detected one is 3 (NG) and the results of detecting BSE cells and leukocytes.

Figure 3 illustrates the Gram stained smears image that (3) both the correct Geckler class and the detected one is 3 (NG) and the results of detecting BSE cells and leukocytes.

Figure 4 illustrates the Gram stained smears image that (4) both the correct Geckler class and the detected one is 4 (GE) and the results of detecting BSE cells and leukocytes.

Here, for the result of detecting BSE cells, the rectangle enclosed by blue line is the region of BSE cells and that by block line is not in the candidate regions. Also, for the result of detecting leukocytes, the rectangle enclosed by blue line is the region of leukocytes and that by block line is not in the candidate regions.

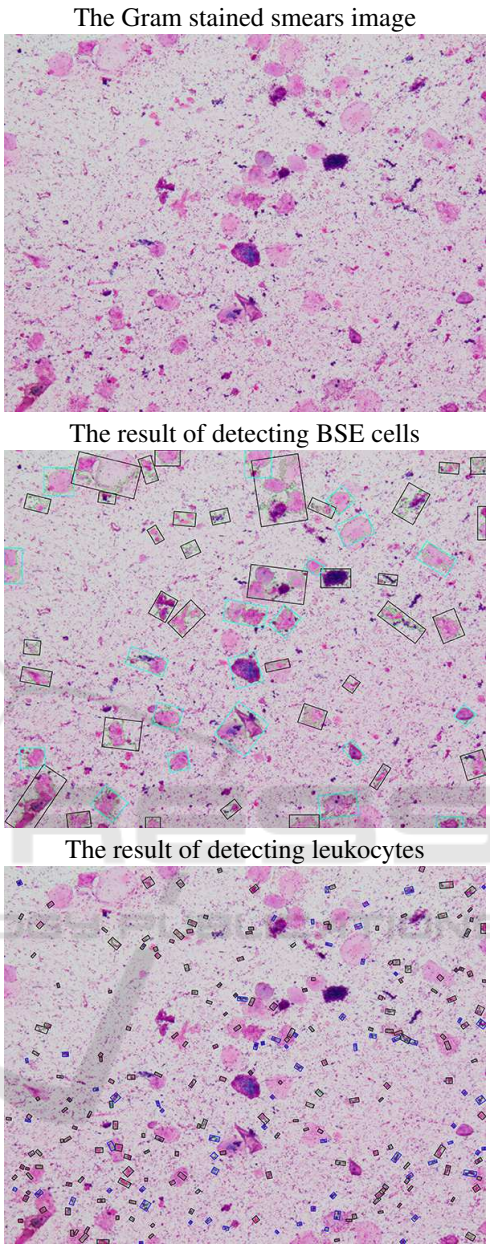

Figure 1: The Gram stained smears image (1) whose correct Geckler class is 3 (NG) and detected one is 4 (GE) and the result of detecting BSE cells and leukocytes.

Table 9 represents the correct Geckler class (CG) and the number of BSE cells (#BSE), the number of leukocytes (#leu) and the detected Geckler class (DG) by DNN and additionally SVM for the images (1), (2), (3) and (4).

Table 9 shows that the classifier constructed by SVM tends to classify the candidate regions that are BSE cells to the regions that are not BSE cells and

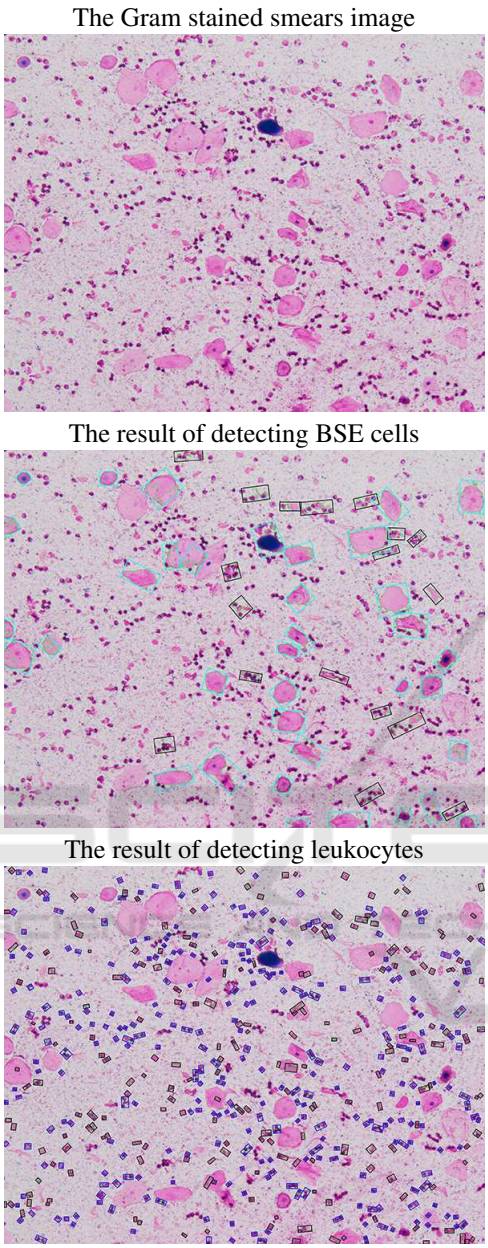

Figure 2: The Gram stained smears image (2) whose correct Geckler class is 4 (GE) and detected one is 3 (NG) and the result of detecting BSE cells and leukocytes.

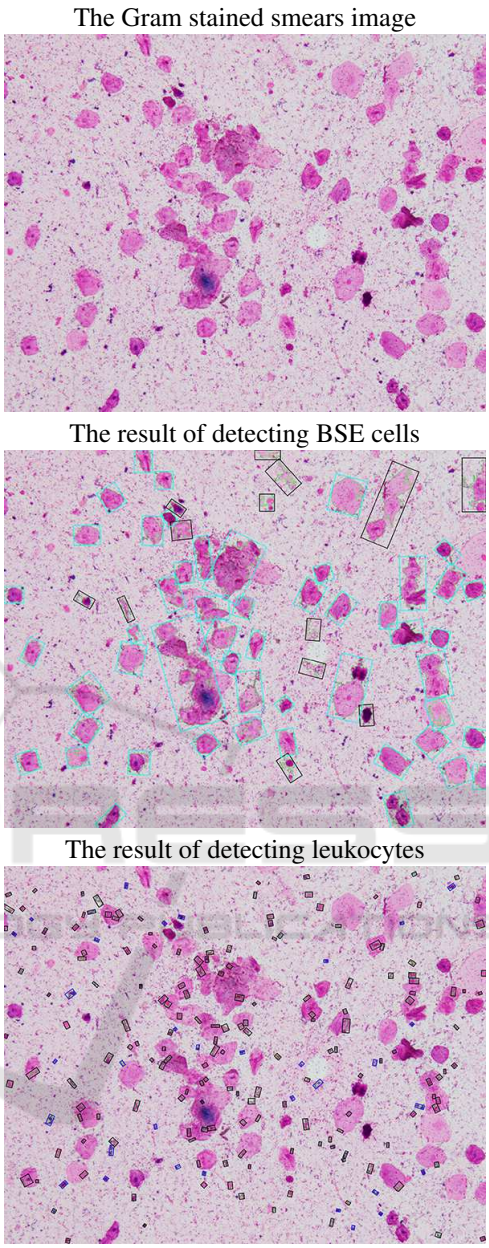

Figure 3: The Gram stained smears image (3) whose correct Geckler class and detected one is 3 (NG) and the result of detecting BSE cells and leukocytes.

the candidate regions that are not leukocytes to the regions that are leukocytes.

3.2 Evaluation of Training Images

As shown in Table 9, the test images have just the label of the correct Geckler class and do not have the number of BSE cells and leukocytes. Then, in this section, by applying the training images (with both the correct Geckler class and the number of BSE cells and leukocytes) as test images, we evaluate the method to detect the Geckler class.

First of all, the Geckler class in the provided training images contains some errors, because both the numbers of BSE cells and leukocytes are counted visually, not automatically. Then, Table 10 represents the number of training images (the first row, which is same as that in Table 2) and training images whose

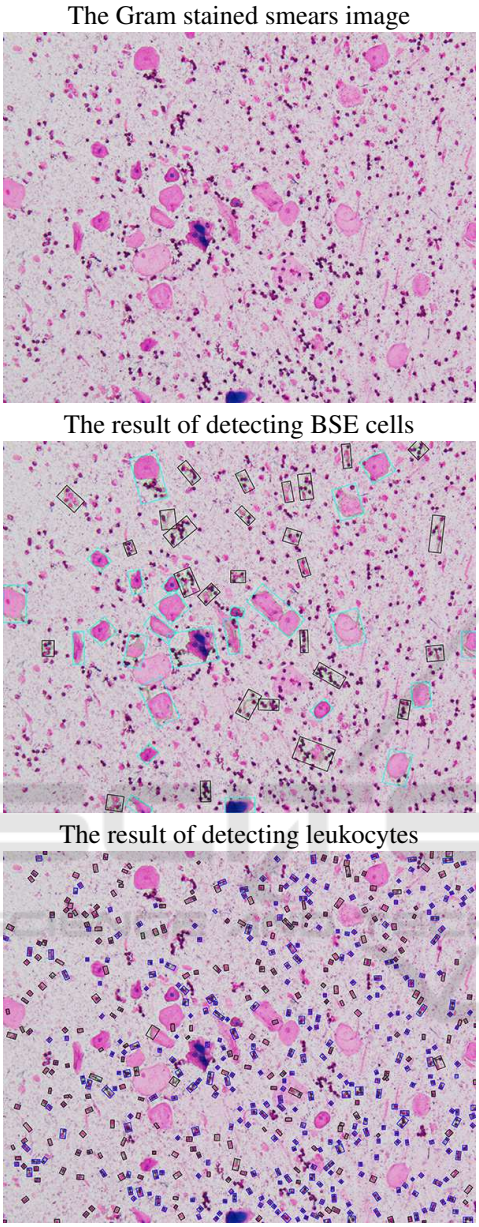

Figure 4: The Gram stained smears image (4) whose correct Geckler class and detected one is 4 (GE) and the result of detecting BSE cells and leukocytes.

Geckler class is relabeled by counting the number of assigned labels of BSE cells and leukocytes in Section 2.2 (which we call *relabeled images*) for every Geckler class.

In the remainder of this section, we regard the label of the relabeled images as the correct Geckler class. Then, Table 11 represents the result of the detected Geckler class by classifiers constructed by SVM and DNN from the same training data in Section 2.2.

Table 9: The correct Geckler class (CG) and the number of BSE cells (#BSE), the number of leukocytes (#leu) and the detected Geckler class (DG) by DNN and SVM for the images (1), (2), (3) and (4).

		DNN			SVM		
	CG	#BSE	#leu	DG	#BSE	#leu	DG
(1)	3	20	67	4	1	79	5
(2)	4	36	295	3	2	368	5
(3)	3	51	33	3	0	60	5
(4)	4	25	305	4	2	404	5

Table 10: The number of training images and relabeled images for every Geckler class.

Geckler class	1	2	3	4	5	6	total
training images	19	20	20	19	19	10	107
relabeled images	20	14	28	18	16	11	107

Also Table 12 represents the results in Table 11 summarizing the Geckler classes from 1 to 3 as NG, those of 4 and 5 as GE and that of 6 as UN.

As same as Section 3.1, Tables 11 and 12 show that, whereas SVM fails to detect the Geckler class, DNN succeeds to detect.

In order to evaluate the method to detect the Geckler class, we compare the number of BSE cells and leukocytes presented by bold faces in Tables 11 and 12. In other words, we investigate the number of BSE cells and leukocytes as follows:

(1) 1 image such that the correct Geckler class is 2 but the detected one is 4;

(2) 8 images such that the correct Geckler class is 3 but the detected one is 4;

(3) 2 images such that the correct Geckler class is 1 but the detected one is 6.

(4) 1 image such that the correct Geckler class is 3 but the detected one is 6.

Table 11: The result of the detected Geckler class by classifiers constructed by SVM and DNN from the same training data as Section 2.2.

SVM	correct					
detect	1	2	3	4	5	6
1	1	0	0	0	0	0
2	3	0	0	0	0	0
3	0	0	0	0	0	0
4	1	1	5	0	0	0
5	4	11	23	18	14	1
6	11	2	0	0	2	10

DNN	correct					
detect	1	2	3	4	5	6
1	10	2	1	0	0	0
2	5	3	2	0	0	0
3	3	8	16	0	0	0
4	0	**1**	**8**	11	0	0
5	0	0	0	6	13	0
6	**2**	0	**1**	**1**	**3**	11

Table 12: The results in Table 11 summarizing the Geckler classes.

SVM detect	correct NG	GE	UN	DNN detect	correct NG	GE	UN
NG	4	0	0	NG	50	0	0
GE	45	32	1	GE	**9**	30	0
UN	13	2	10	UN	**3**	**4**	10

(5) 1 image such that the correct Geckler class is 4 but the detected one is 6.

(6) 3 images such that the correct Geckler class is 5 but the detected one is 6.

Then, Table 13 represents the number of BSE cells (#BSE), the number of leukocytes (#leu), the correct Geckler class (CG) and the detected Geckler class (DG) for the above images from (1) to (6) and for detecting by DNN.

Table 13: The number of BSE cells (#BES), the number of leukocytes (#leu), the correct Geckler class (CG) and the detected Geckler class (DG) for the above images from (1) to (6) and for detecting by DNN.

	correct			DNN		
	#BSE	#leu	CG	#BSE	#leu	DG
(1)	43	11	2	17	40	4
(2)	41	32	3	23	39	4
	44	108	3	25	72	4
	34	212	3	17	184	4
	50	582	3	21	512	4
	49	339	3	22	333	4
	32	122	3	20	138	4
	27	347	3	25	387	4
	38	351	3	23	311	4
(3)	38	0	1	25	16	6
	56	8	1	18	24	6
(4)	45	26	3	19	18	6
(5)	14	32	4	5	22	6
(6)	0	53	5	0	13	6
	1	54	5	1	0	6
	1	91	5	1	1	6

Table 13 shows that DNN detects the smaller number of BSE cells than the correct number for the cases (2) and (3), the larger number of leukocytes for the case (3) and the smaller number of leukocytes than the correct number for the cases from (4) to (6).

4 CONCLUSION

In this paper, we have developed the system to detect the Geckler classification from Gram stained smears images for sputum. Then, we have given the experimental results to succeed to the Geckler classification for sputum by using DNN, whereas not to succeed by using SVM.

Concerned with Section 3.2, it is necessary to collect more training data and then evaluate our method by using cross validation, for example, which is a future work. Also it is a future work to improve the detection method with higher accuracy to avoid to the situation represented by Table 13, for example, introducing a proper method to detect BSE cells and leukocytes. Furthermore, it is a future work to apply the image processing methods proposed by (Lejon and Andersson, 2016) and then to analyze which of them is useful of our system.

The Geckler classification in Table 1 cannot determine the class when the number of BSE cells is just 25 and the number of leukocytes is less than or equal to 25. Since the Geckler classification is based on visual observation, it may not require the exact number of BSE cells and leukocytes. On the other hand, when we develop the detection system for Geckler classification, it is necessary to determine the class for every case. Then, it is a future work to redefine the Geckler classification without ambiguity from the medical viewpoint.

As discussed in (Carvajal et al., 2014), the ratio of the occurrences of BSE cells and leukocytes determines whether or not the images for not only sputum but also other samples is quality for the microscope testing of the Gram stained smears images per $1,000\times$ field. Hence, it is an important future work from the microbial viewpoint to provide the criterion for other samples to determine how the Gram stained smear image is quality for the microscope testing per $1,000\times$ field.

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