Detection System of Gram Types for Bacteria from Gram Stained Smears Images

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Abstract: In this paper, we develop the detection system of *Gram types* determined by stained colors and stained shapes for bacteria from Gram stained smears images. Here, we call four types of bacteria, that is, *Gram positive cocci (GPC)*, *Gram positive bacilli (GPB)*, *Gram negative cocci (GNC)* and *Gram negative bacilli (GPB) Gram types*, and then add to two types as *Gram positive unknown (GPU)*, and *Gram positive unknown (GNU)*. The system first infers the candidate regions of bacteria by using image processing. Next, it constructs a classifier dividing the candidate regions into Gram types by using SVM (support vetcor machine) and DNN (deep neural network). Finally, it detects the occurrences of Gram types in a newly input image and retrieves Gram stained smears images similar as the input image such that the occurrence ratio for the Gram types is similar.

1 INTRODUCTION

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

After Gram staining, we call the bacteria colored by purple or violet *Gram positive* and those by red or pink *Gram negative*. Also we call the bacteria stained as sphere-shape *cocci* and those as rod-shape *basilli*. Hence, in this paper, we call four kinds of bacteria as *Gram positive cocci (GPC)*, *Gram positive bacilli (GPB)*, *Gram negative cocci (GNC)* and *Gram negative bacilli (GNB)*[1] *Gram types*.

Since the Gram stain is applicable inexpensively and fast returns the results (within 30 min.), it is important for the initial medical care of infectious diseases (Mitsuda, 2004; Yamamoto, 2015). On the other hand, Gram staining is possible to stain not only bacteria but also non-bacteria substances such as leukocytes, dust, oil and crystals. Also, there exist many kinds of phlogogenic fungus for infectious diseases. Table 1 illustrates the relationship between Gram types and bacteria.

Table 1 shows that the bacteria as the phlogogenic fungus for hospital-acquired infection tend to belong to GPC or GNB. Then, the detected bacteria will determine the direction for culture and identification tests (Mitsuda, 2004; Yamamoto, 2015).

In the microscope test, Gram stained smears images are checked manually and visually and not automatically in general. The reason is that we can detect bacteria exactly by applying culture and identification tests after the microscope test. On the other hand, anaerobic bacteria are never lived in the culture test, they cannot be detected by the identification test. Hence, the detected bacteria through the culture and identification tests are the part of bacteria in smears.

Also, since the culture and identification tests spend one day, we cannot apply them to the initial medical care of infectious diseases. Furthermore, whereas expert skills are necessary to detect bacteria manually and visually from Gram stained smears

[1]Sometimes we call GPB and GNB *Gram positive rod* and *Gram negative rod* (Smith et al., 2018).

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Table 1: The relationship between Gram stain and bacteria.

Gram types (color, shape)	bacteria
Gram positive cocci (purple/violet, sphere)	Enterococcus faecalis, Staphylococcus aureus, Streptcoccus pyogenes, Streptcoccus pneumoniae
Gram positive bacilli (purple/violet, rod)	Clostridium (Clostridium perfringens, Clostridium tetani), Corynebacterium diphtheriae, Listeria monocytogenes
Gram negative cocci (red/pink, sphere)	Nesseria meningitidis, Nesseria gonorrhoeae
Gram negative bacilli (red/pink, rod)	Bacteroides spp., Klebsiella spp., Psuedomonas aeruginosa, Escherichia coli, Serratia spp.
Gram intermediate	Legionella spp., Mycobacterium spp.

images, such technicians with expert skills are not enough to apply the initial medical care in Japan. Hence, the automatic detection of bacteria from Gram stained smears images automatically is required.

In order to solve these problems, in this paper, as the prepossessing of detecting bacteria exactly from Gram stained smears images, we focus on the Gram types. Then, we develop the system to detect such types of bacteria and to compute the occurrence ratio of types in every image.

In this system, first we extract candidate regions of bacteria as the regions obtained by excluding the region not occurring bacteria from Gram stained smears images by using image processing. Then, we compute the features as colors, areas, aspect ratio, and so on. After classifying candidate regions by colors as Gram positive and Gram negative, we detect Gram types by using the classifier constructed from SVM (support vector machine) and DNN (deep neural network).

1.1 Related Works

As related works to this paper, Carvajal *et al.* (Carvajal et al., 2014) have developed the system to learn the candidate areas from fixed-size (51×38 pixels) images applicable to the microscope test with high magnification. They have dealt with the Gram stained smear images per $64\times$ field. Note that we use images par $1,000\times$ field in the microscope test in general, which we deal with in this paper. Then, whereas they have dealt with Gram stained smear images, they have not achieved to detect Gram types or bacteria.

On the other hand, Smith *et al.* (Smith et al., 2018) have dealt with the Gram stained smear images provided from the sample of blood. Their target Gram types are GNB, GPC in clusters and GPC in pairs or chains, which are meaningful Gram types for blood. Then, after extracting fixed size (146×146 pixels) images, they have detected the above three Gram types by using CNN.

Note that the above researches of (Carvajal et al., 2014) and (Smith et al., 2018) have dealt with randomly selected fixed size images as training data, so their researches have not detect the area of bacteria in the whole images. On the other hand, Lejon and Andersson (Lejon and Andersson, 2016) have detected the area of bacteria in the image, as same as this paper, and then Gram types and then bacteria from the Gram stained smear images for the sample of blood by MATLAB. Whereas they have detected Gram types and bacteria without machine learning, we detect Gram types by using machine learning.

In (Lejon and Andersson, 2016), they have adopted the template matching to detect the areas of bacteria, which they have implicitly adopted the ideal assumption that every bacterium has the similar small size and there exist no substances such as dust with the similar size of bacteria. Furthermore, when dealing with the images for not only blood but also sputum and feces, more kinds of bacteria such as Table 1 containing the bacteria not occurring in the images for blood and many other substances except bacteria occur in the images for sputum and feces.

Hence, we can position this paper to develop a new system to detect the area of bacteria and then Gram types with machine learning applicable to the Gram stained smear images for not only the sample of blood but also other samples of sputum, feces, pus and urine uniformly.

2 DETECTING SYSTEM OF GRAM TYPES

In this section, we explain our detection system of Gram types from Gram stained smears images.

2.1 Data and Outline of System

First, we use the Gram stained smears images per $1,000\times$ field, provided from Osaka General Medical Center applied to our detecting system. Every image consists of $2,448 \times 1,920$ pixels and is assigned the ratio of the occurrences of GPC, GPB, GNC and GNB as percentage. For every sample, the number of im-

ages is 42 for blood, 40 for sputum, 10 for feces, 40 for pus and 69 for urine, respectively.

Our detection system of Gram types mainly consists of *the extraction of candidate regions of bacteria*, *learning phase* and *detecting and retrieving phase*. First, by using image processing, the system extracts *candidate regions of bacteria* from Gram stained smears images. Then, in the learning phase, the system constructs the classifier for Gram types from training data. Finally, in the detecting and retrieving phase, the system detects the regions of Gram types by using the classifier, and then outputs the input image with depicted such regions and retrieves similar images such that the occurrence ratios of Gram types are similar.

2.2 Candidate Regions of Bacteria

In order to extract candidate regions of bacteria, in our system, first we convert Gram stained smears images to *glayscale* images. Here, we adopt the NTSC (non-subsampled contoulet transform) coefficient method supported from OpenCV[2] and then compute *luminance*.

Next, we transform the grayscale images to the binary images by applying *binarization* consisting of 1 if a pixel has the luminance more than the threshold and 0 otherwise. Here, in our binarization, we adopt *adaptive thresholding* (Keahler and Bradski, 2013) to determine the threshold by considering pixels on neighbors at a current pixel. As a result, our system extracts the regions with clearer boundaries than around pixels. Here, the adaptive thresholding is computed by matrix and the coefficient of neighbor regions under Gaussian distribution minus a subtractive constant[3]. The constant reflects fluctuation such that noises tend to be ignored if the constant is large and pixels with small changes of luminance tend to be remained if it is small.

Then, by using *morphorogical operation* (Keahler and Bradski, 2013), we separate connected pixels from other pixels and then eliminate noises. Here, we adopt *opening processing* as applying the *dilation* at *n* times after applying the *erotions* at *n* times.

Finally, we extract *edges* as boundaries as pixels with large changes of luminances. We call the set of edges for a substance an *outline*. In our system, we adopt a Canny filter (Canny, 1986) as a robust edge detection filter to noises. By applying the edge detection filter, we exclude regions as noises with small changes of luminances.

After determining the regions consisting of substances but not noises, we regard the regions such that every value of areas, aspect ratio and circularity satisfies every threshold as *bacterial regions*. As a result, we extract such bacterial regions as *candidate regions of bacteria*.

2.3 Training Data

In order to construct training data, we design the tool as Figure 1 (whose GUI is in Japanese) to represent the rectangle as candidate regions of bacteria, and then assign the correct Gram type to the rectangle manually through GUI by the fourth author who is the medical technologist for clinical microbial testing.

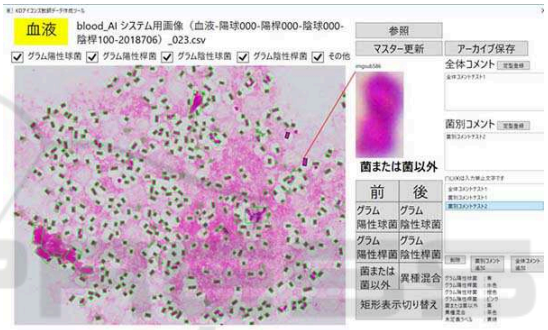

Figure 1: Tool for constructing training data.

As a result, Table 2 illustrates the number of training data as images transferring a single bacterium. Here, since there exist regions not determining whether cocci or bacilli, we add those as *Gram positive unknown* (GPU) and *Gram negative unknown* (GNU) and extend Gram types to six kinds of GPC, GPB, GPU, GNC, GNB and GNU.

Table 2: The number of training data for samples.

sample	num.	color	cocci	bacilli	unknown	total
blood	6,216	pos.	1,062	512	406	1,980
		neg.	0	3,424	812	4,236
sputum	10,308	pos.	1,118	665	1,821	3,604
		neg.	826	2,522	3,356	6,704
feces	2,905	pos.	20	74	154	248
		neg.	56	2,029	572	2,657
pus	8,178	pos.	296	534	1,388	2,218
		neg.	0	4,225	1,735	5,960
urine	6,052	pos.	14	50	395	459
		neg.	0	4,397	1,196	5,593

[2]https://docs.opencv.org/3.1.0/de/d25/imgproc_color_conversions.html

[3]https://docs.opencv.org/3.0-beta/modules/imgproc/doc/miscellaneous_transformations.html?highlight=cv2.adaptive#cv2.adaptiveThreshold

2.4 Learning Phase

From training data illustrated as Table 2, in the learning phase, we construct the classifiers by using SVM (support vector machine) and DNN (deep neural network). 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).

For SVM, we construct the classifier to detect Gram types by using *feature values* as features for candidate regions of bacteria. Here, we adopt features as the *area*, the *aspect size*, the *aspect ratio*, the *color* and the *circularity* of the region, and also the *number of detected bacteria* in the region.

On the other hand, for DNN, we construct the classifier to detect Gram types by using images for candidate regions of bacteria as training data.

2.5 Detecting and Retrieving Phase

In the detecting and retrieving phase, after setting a sample and a file path, referring to the button "Refer," on GUI, our system starts to detect four Gram types. Then, it outputs, for each of SVM and DNN, the occurrence ratio of every Gram type, the main image such that the substance is enclosed by a colored rectangle if it belongs to one Gram type and the three subimages whose occurrence ratio is similar. Here, we can change the main image or subimages by SVM and DNN.

Figure 2 illustrates the output of our detection system for the sample of blood and DNN. Here, in the main image, the Gram types of GPC, GPB, GNC, GNB, GPU and GNU are enclosed by frames colored by blue, light blue, red, pink, light green and black, respectively.

In Figure 2, the left windows in the detection system represents the occurrence ratio for every Gram type. The upper three images are the original image, the result by SVM and the result by DNN from left to right. The right three images are similar images as the original image.

3 EXPERIMENTAL RESULTS

In this section, we give experimental results for our detecting system of Gram types. Here, computer environment to the learning phase is CPU Intel(R) Xeon(R) E5-1603 v3 @2.80GHz, RAM 32.0GB and OS Windows 10 Pro for Workstations. The samples consist of blood, sputum, feces, pus and urine. In this section, we use the images to assigned the occurrence ratio of Gram types by the professional technician, which we call the *images to assigned ratios*. Then, the number of the images to assigned ratios for blood, sputum, feces, pus and urine is 42, 40, 10, 40 and 69, respectively, and the total number of the images to assigned ratios is 201.

3.1 Parameters

Table 3 illustrates the parameters for image processing for Section 2.2 applied to experimental results. Here, we denote the size of neighbor for adaptive thresholding by N, the subtractive constant by C and the number of opening processings by P. Also we denote the upperbound an the lowerbound for the area as the candidate regions of bacteria by S_{min} and S_{max}, those for the aspect ratio by A_{min} and A_{max}, and those for the circularity by C_{min} and C_{max}.

Table 3: The parameters for image processing applied to experimental results.

sample	N	C	P	S_{min}	S_{max}	A_{min}	A_{max}	C_{min}	C_{max}
blood	257	37	2	200	30000	0.1	1.0	0.1	1.0
sputum	431	37	2	130	30000	0.1	1.0	0.1	1.0
feces	281	18	2	300	30000	0.1	1.0	0.1	1.0
pus	301	38	2	150	15000	0.1	1.0	0.1	1.0
urine	581	35	2	200	50000	0.1	1.0	0.1	1.0

3.2 Detection of Gram Classes

Figures 3, 4 and 5 illustrate the results obtained by detecting Gram types by SVM and DNN from arbitrary selected images for blood, sputum and feces and by searching their similar images.

The images in Figures 3, 4 and 5 are those to assigned ratios. Then, Table 4 illustrates the assigned and detected occurrence ratios of Gram types for blood (Figure 3), sputum (Figure 4) and feces (Figure 5) by SVM and DNN.

Table 4 shows that, in these images, whereas the detected occurrence ratios by SVM is more similar as the assigned occurrence ratios than those by DNN, the accuracy is too insufficient to detect Gram types exactly. For all the cases, every detected Gram type contains GPU and GNU. A large ratio of GNU follows that the stained dusts are detected as GNU.

3.3 Evaluation for Detection

In order to evaluate the method to detect the Gram types, by using the assigned ratio of the occurrences

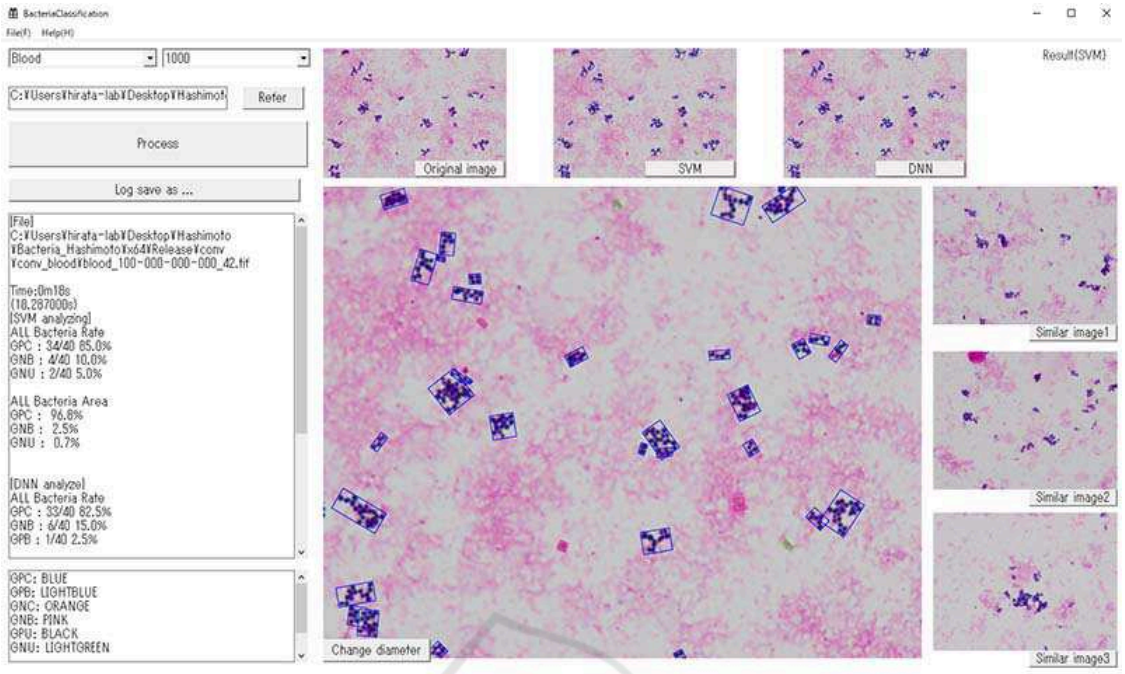

Figure 2: The detection system of Gram types for the sample of blood.

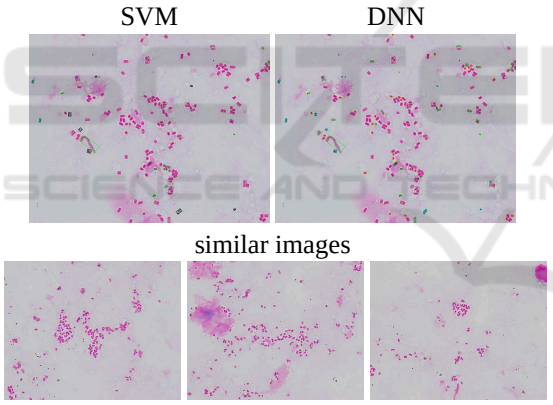

Figure 3: The images for blood by detecting Gram types by SVM and DNN (upper) and its similar images (lower).

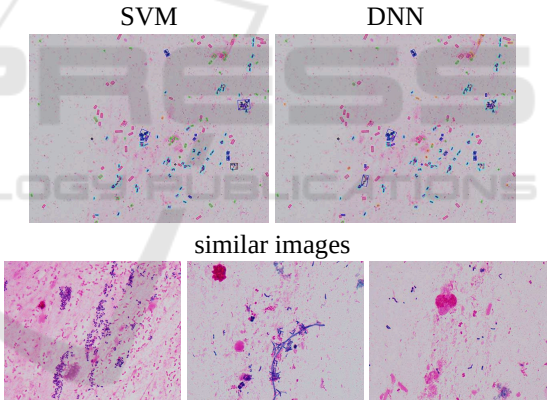

Figure 4: The images for sputum by detecting Gram types by SVM and DNN (upper) and its similar images (lower).

of GPC, GPB, GNC and GNB as percentage, we apply the following test for sputum and pus.

1. First, we divide all the images for a sample into two groups in half randomly. We call one group *training images* and another group *test images*. Here, we obtain 21 training images and 21 test images for blood, 20 training images and 20 test images for sputum and pus.
2. From the training images, we construct the training data (regions) with the classes of GPC, GPB, GPU, GNC, GNB and GNU as same as Sections 2.2 and 2.3.
3. By using SVM and DNN, we construct classifier from training data and apply it to test images. Then, for every test image, we compare the assigned ratio of the occurrences of GPC, GPB, GNC and GNB to the image with the detected ratio of the occurrences of GPC, GPB, GPU, GNC, GNB and GNU from the image.
4. Repeat the above procedures at five times.

In order to evaluate the assigned ratio to the image and the detected ratio from the image, we first compare the occurrences for color. Let p_a and p_d be the assigned ratio and the detected ratio of the occurrences of Gram positive bacteria, respectively. Then,

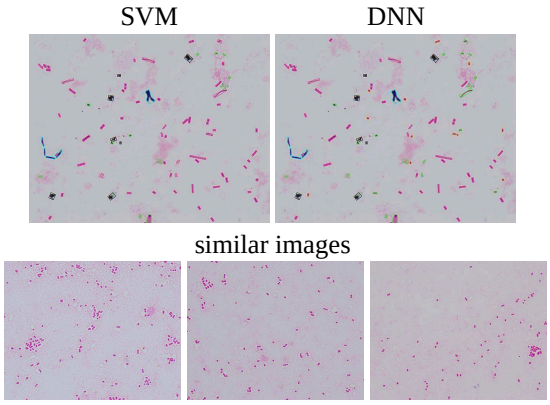

Figure 5: The images for feces by detecting Gram types by SVM and DNN (upper) and its similar images (lower).

Table 4: The assigned (ass.) and detected occurrence ratios (occ. ratio) of Gram types (GCs) for blood (Figure 3), sputum (Figure 4) and feces (Figure 5) by SVM and DNN.

blood (Figure 3)			
GCs	occ. ratio		
	ass.	SVM	DNN
GPC	0	0.00	0.00
GPR	0	0.00	2.74
GNC	0	0.00	17.81
GNR	100	83.56	41.10
GPU	–	2.74	0.00
GNU	–	13.70	38.36

sputum (Figure 4)			
GCs	occ. ratio		
	ass.	SVM	DNN
GPC	10	6.92	10.77
GPR	50	33.85	31.54
GNC	0	0.77	10.77
CNR	40	26.15	30.00
GPU	–	2.31	0.77
GNU	–	30.00	16.15

feces (Figure 5)			
GCs	occ. ratio		
	ass.	SVM	DNN
GPC	0	0.00	0.00
GPR	10	6.25	6.25
GNC	0	0.00	13.75
GNR	90	70.00	46.25
GPU	–	8.75	8.75
GNU	–	15.00	25.00

we say that a test image has an *admissible color* if $|p_a - p_d| \leq 20\%$.

Table 5 illustrates the number of images with an admissible color in the test images for blood, sputum and pus. Here, the number of the test images for blood is 21 and that for sputum and pus is 20.

Table 5 shows that the ratio for the test images with an admissible color is more than 50% and, in particular, it is about 90% for pus by DNN.

Next, we compare the occurrences for shapes. Let c_a and c_d be the assigned ratio and the detected ratio of the occurrences of cossi, respectively. Also let b_a and b_d be the assigned ratio and the detected ratio of the occurrences of basilli, respectively. Then, we say that a test image has *admissible cossi* if $|c_a - c_d| \leq$ 20% and *admissible basilli* if $|b_a - b_d| \leq 20\%$. Furthermore, we say that a test image has *admissible shapes* if $|c_a - c_d| \leq 20\%$ and $|b_a - b_d| \leq 20\%$.

Table 5: The number of images with an admissible color in the test images for blood, sputum and pus.

blood, SVM (21)					
1	2	3	4	5	ave.
12	10	14	14	12	12.4

blood, DNN (21)					
1	2	3	4	5	ave.
12	12	13	13	13	12.6

sputum, SVM (20)					
1	2	3	4	5	ave.
14	11	9	8	11	10.6

sputum, DNN (20)					
1	2	3	4	5	ave.
9	12	12	11	9	10.6

pus, SVN (20)					
1	2	3	4	5	ave.
9	12	12	11	9	10.6

pus, DNN (20)					
1	2	3	4	5	ave.
18	18	17	17	18	17.6

Table 6 illustrates the number of images with admissible cossi, basilli and shapes in the test images for sputum and pus. Here, the number of the test images is 20, respectively. Also we do not count the number of GPU and GNU.

Table 6: The number of images with admissible cossi (C), basilli (B) and shapes (S) in the test images for blood, sputum and pus.

blood, SVM (21)						
	1	2	3	4	5	ave.
C	18	13	16	18	16	16.2
B	12	13	12	10	14	12.2
S	9	6	7	8	9	7.8

blood, DNN (21)						
	1	2	3	4	5	ave.
C	18	5	18	10	15	13.2
B	16	6	20	11	18	14.2
S	15	5	18	10	15	12.6

sputum, SVM (20)						
	1	2	3	4	5	ave.
C	7	9	6	5	6	6.6
B	8	7	9	10	10	8.8
S	0	0	0	0	0	0

sputum, DNN (20)						
	1	2	3	4	5	ave.
C	8	5	7	5	5	6
B	8	13	8	7	8	8.8
S	5	5	5	5	5	5

pus, SVN (20)						
	1	2	3	4	5	ave.
C	6	6	6	6	5	5.8
B	9	13	7	7	8	8.8
S	4	6	4	6	5	5

pus, DNN (20)						
	1	2	3	4	5	ave.
C	4	5	7	6	9	6.2
B	5	2	4	9	3	4.6
S	2	0	2	4	3	2.2

Table 6 shows that, for blood, the accuracy of detecting shapes (cossi and basilli) tends to be higher than the accuracy of detecting colors and the ratio for the test images with admissible cossi and basilli is greater than 50%. Since the second round for DNN detects all the assigned labels of GNB to GNC, the number of the images with admissible cossi and

basilli is very small.

On the other hand, for sputum and pus, the accuracy of detecting shapes (cossi and basilli) is much lower than the accuracy of detecting colors in Table 5. In all the cases, the ratio for the test images with admissible cossi, basilli and shapes for sputum and pus is less than 50%.

In particular, for sputum by SVM, the reason why the number of test images with admissible shapes is 0 is that the detection of cossi (*resp.*, basilli) succeeds when the ratio of the occurrences of cossi (*resp.*, basilli) is near to 0%. Also, for pus by SVM, either the detected ratio of the occurrences of GNB is 0% and that of GNC is 100% or the detected ratio of the occurrences of GNC is 0% and that of GNB is 100%. For pus by DNN, the detected ratio of the occurrences of GNB is always 0% and GNB is determined as GNC in the detection.

Hence, it is necessary to improve our detection system of detecting shapes rather than color for sputum and pus.

3.4 Running Time

Finally, Table 7 illustrates the average running time to detect the Gram type for a single candidate region of bacteria by SVM and DNN for every sample.

Table 7: The average running time (ms) to detect the Gram types for a single candidate region of bacteria by SVM and DNN for every sample.

	SVM	DNN
blood	0.033	240.509
sputum	0.143	247.572
feces	0.030	242.059
pus	0.081	246.837
urine	0.039	245.090

Table 7 shows that the running time to detect by SVM is much faster than that by DNN.

4 CONCLUSION

In this paper, we have developed the detecting system of Gram types for bacteria from Gram stained smears images. By applying our system to 201 Gram stained smears images to assigned the occurrence ratios of Gram types, we have given the experimental results for our system.

Since our system is still proto-typing and there exist many future works to improve our system. First of all, we have just applied standard image processing to our system and then not designed the method appropriate to Gram stained smears images, so it is an important future work to design such a method and embed it to our system.

As stated in Section 3.3, it is necessary to improve the detection of shapes rather than color for sputum and pus. Then, it is a future work to apply the image processing methods such as black top-hat transform, label connected component and template matching proposed by (Lejon and Andersson, 2016) and then to analyze which of them is useful of our system. Also, since the labels consist of GPC, GPB, GNC and GNB, it is a future work to design the method to evaluate the detection.

Furthermore, our system cannot avoid to detect dust as bacteria completely illustrated in Figure 6 (left), so it is a future work to improve our system to avoid to this situation. Also it is necessary to detect *leukocyte phagocystosis* in Figure 6 (right), which is an important future work.

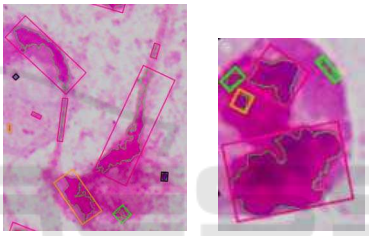

Figure 6: The detection of dusts as bacteria (left) and leukocyte phagocystosis (right).

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