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Rapid and automatic detection of micronuclei in binucleated lymphocytes image

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Abstract: In terms of radiation biological dose estimation, the cytokinesis block micronucleus (CBMN) assay is the internationally recognized dose estimation method. Due to the subjectivity and the time-consuming of manual detection, it cannot meet the needs of rapid standard assay of CBMN. Therefore, in this research work, we combined the convolutional neural network to design a software that can be used for rapid standard automatic detection of micronuclei in Giemsa stained binucleated lymphocytes image. The software analysis workflow is divided into four stages: cell acquisition, adhesive cell masses segmentation, cell type identification, micronucleus counting. After verification, our algorithm can quickly and effectively detect binucleated cells and micronucleus even when the cytoplasm is blurred, multiple micronucleus are attached to each other, or micronucleus is attached to the nucleus. In the test of a large number of random images, the software reached 99.4% of the manual detection in terms of the detection rate of binucleated cell, and the false positive rate of binucleated cell was 14.7%. In terms of micronucleus detection rate, the software reached 115.1% of manual detection, and its false positive rate was 26.2%. The analysis time of each picture is about 0.3s, an order of magnitude faster than conventional method.

Keywords: Cytokinesis block micronucleus assay, Automated detection, Image segmentation, Convolutional neural network

I. Introduction

In the event of a radiation accident involving a sizable of people exposed, it is imperative to evaluate their exposure dose in time to determine the priority of them for treatment and alleviate their unnecessary worries¹. Since ionizing radiation can cause chromosome damage, the biological dose can be estimated by cytogenetic methods. Among them, dicentric chromosome detection² is the most reliable method in biological dose detection, but its analysis process is very time-consuming and requires excellent professional skills and knowledge. Therefore, when a large number of samples need to be tested, other dose estimation methods³⁻⁷ have aroused the interest of researchers. Among these methods, CBMN analysis⁸⁻⁹ is the best alternative method.

CBMN analysis is a method of forming a large number of binucleate lymphocytes by adding appropriate amount of cytochalasin B in the process of cell culture to make the nucleus divide normally and the cell membrane not divide. Because micronucleus (MN) is a fragment of chromosome, the MN rate in binucleated cells (BNC) can be

correlated with the exposure dose to estimate the unknown dose¹⁰⁻¹³, similar to the detection of chromosome aberrations. MN is an entire chromosome or a partial fragment of a chromosome induced by ionizing radiation. When the cell is irradiated, MN is in a hysteresis state in the late stage of cell nuclear division. Therefore, MN is not contained in the main nucleus of the cell but forms a small round entity and remains in the cytoplasm¹⁴. Compared with dicentric chromosome detection, CBMN assay is easier and faster, so it is more suitable for rapid biological dosimetry in a huge number of people. In addition to radiation dose estimation, CBMN analysis is also an effective detection method for studying the potential genotoxicity and cytotoxicity of compounds¹⁵⁻¹⁶. Due to the subjectivity and long assay time of manual detection¹⁷⁻¹⁸, it cannot meet the needs of rapid standard detection. To overcome these problems and achieve better statistical data and higher repeatability, automated detection of MN has become an important issue.

At present, there are a variety of automated analysis methods for detecting BNC and its MN. One of the main differences between different automated analysis algorithms is whether the analyzed object contains cytoplasm or not. The first type of algorithm only analyzes the nucleus information and does not correlate the cytoplasmic information of related cells¹⁹⁻²⁵. The second type of algorithm is related to the cytoplasm, which uses the whole cell as a detection unit²⁶⁻³². Generally, different pixel thresholds are used to realize the separation of picture background, cytoplasm, and nucleus/MN.

The current research has proved that the automatic detection of MN has the value of engineering application, but the existing algorithms still have some limitations, such as the low detection rate of MN. In this research, a software for fast standard automatic analysis of Giemsa stained binucleated lymphocytes image was designed by combining with Convolutional Neural Network (CNN). And this algorithm can effectively detect binucleated cells (BNC) and MNs even when the cytoplasm is blurred and also has the function of detecting mononuclear cells (MOC) and polynuclear cells (POC).

II. MATERIALS AND METHODS

A. Software and hardware platform

To implement fast CBMN image analysis, high-definition microscopic images of the cells must be obtained first. The image acquisition system consists of three parts (Fig.1): (1) Color microscope (OLYMPUS, Japan) with 10x, 20x, and 40x objective lenses. (2) CCD camera (Lumenera, Canada) is connected to the computer through the USB interface to achieve real-time display and take pictures. (3) Motion console and storage system. The computer sends instructions through the Ethernet to make the motion console move according to the default path. The microscope moves up and down to take captured images and store them in the computer. According to the magnification and the size of the standard glass slide, 3200 images can be taken by scanning a complete standard-size glass slide with a 10x eyepiece and a 20x objective lens.

Software development and image analysis were performed on a desktop computer with a 6-core Intel Core i7-8700K 3.70GHz CPU (32G memory) and NVIDIA GeForce GTX 1080 Ti GPU (11G memory). The software development tool uses Visual Studio 2013 and PyCharm 2018. The designed software includes four modules: image acquisition module, image processing module, image analysis module and data storage management module. The software support library includes OpenCV and other third-party libraries, python scripts is called by C++ to realize the classification of various types of cells.

Figure 1. CBMN fast automatic assay system.

B. Slide source

Entrusted China Henan Institute of Occupational Disease Prevention and Control to make 100 samples of CBMN. Twenty healthy volunteers (10 males and 10 females) aged 25-40 were selected, and 5ML of peripheral blood was collected per person with informed consent. All donors are non-smokers, kept relatively healthy at the time of blood collection, have no obvious diseases such as cold, flu or pneumonia, and have not received medical exposure in the past 12 months. All blood samples received a 4 Gy gamma irradiation.

All experimental protocols of this research submitted in Scientific Reports were approved by the Ethic Committee of the China Henan Institute of Occupational Disease Prevention and Control and conformed to the Declaration of Helsinki, including:

- Collection of human blood.
- Cell culture and glass slide production.
- Collection of microscopic cell images.
- Automated detection and manual analysis of binuclear lymphocyte images.

The preparation of the glass slide samples is carried out in accordance with the guidelines and regulations promulgated by the International Atomic Energy Agency².

C. Image Acquisition

20x objective of OLYMPUS microscope was used to image the sample. One image was acquired for each field of view, and each image had a resolution of 2048x2048 pixels and was stored in BMP format. In total, 100 slides were marked for model training and testing. 80 out of 100 slides from 16 donors (8 males, 8 females) were used for CNN model training, whereas the residual 20 slides from the other 4 donors (2 males and 2 females) were kept untouched until the final testing. The training-marked slides were scanned and photographed by our system.

III. Design

The image analysis process is divided into four steps. First, extract the cells from the image background. Then, segment individual cells from the adhesive cell masses. Next, use the trained CNN model to identify and classify individual cells. Finally, count the number of MNs on BNC.

A. Cell acquisition

To obtain a complete cell image, threshold segmentation is performed on the collected image first. Before threshold segmenting, the original color image (Fig. 2(A)) is converted into grayscale images of R, G, and B channels according to the RGB channels. Since the sample is stained with Giemsa, the image of this sample will appear dark blue or reddish purple in human vision. Therefore, among the four images of R, G, B grayscale images and conventional grayscale images, the cytoplasm/nucleus pixel intensity in the G channel grayscale image (Fig.2(B)) is the highest. Then the iterative thresholding algorithm is used for the G-channel gray image and the image is thresholded. It takes about 0.2 seconds to use the iterative threshold classification algorithm for each image (2048×2048 pixels). After thresholding, the mask image is corroded and expanded to remove some small noise points and impurities. Finally, the mask image is synthesized with the original color image, and the result is shown in Fig.2(C).

Figure 2. Image thresholding segmentation process. (A)Color original image. (B) Grayscale image of G channel conversion. (C) A composite result obtained by using an iterative threshold algorithm for the G channel grayscale image.

B. Classification of individual cells and adhesive cell masses

The distribution of cells on the glass slide is not uniform, so there will be adhesive cell masses in the images obtained in the previous step. To increase the detection rate of BNC, it is necessary to segment the adhesive cell mass into individual cells. But, before segmentation, all the obtained cells need to be classified into individual cells and adherent cell clusters.

The classification method is considered from two aspects: 1. An individual cell should have a complete cytoplasm and roughly round; 2. The area value of the cell should be within a certain reasonable range. According to these considerations, the used judgment parameters include: cell area, cell extension rate, and cell defect rate.

1) Cells that are too large or too small will be eliminated first. The minimum acceptable number of cell pixels is 1000 pixels, which is equivalent to about $100\mu\text{m}^2$; the maximum acceptable number 8000 pixels, about $800\mu\text{m}^2$.

2) The calculation formula of the elongation rate parameter is:

$$\min(H, W) / \max(H, W) \quad (1)$$

where H is the width of the minimum circumscribed rectangle of the cell, and W is the length. The elongation rate describes how close the shape of an object is to a circle. For a circle, its elongation value is 1; for objects that are not so round, its elongation value will be less than 1, and as the shape of the object is closer to non-circular, the value of elongation will approach 0. In this algorithm, the acceptable value of the elongation rate is set to 0.5-1.0.

3) The formula for calculating the defect rate is:

$$(A - B) / B \quad (2)$$

where A is the area of the smallest circumscribed circle of the cell, and B is the area of the cell itself. The elongation rate describes how close the actual area of the object is to the circular area. For a circle, its defect rate will be 0; for cell objects with incomplete cytoplasm, its defect rate value will be greater. In this algorithm, the acceptable value of the defect rate is set to 0-0.5.

The above two judgment parameters are combined in a "parallel" manner, that is, when the elongation rate parameter and the defect rate parameter of an object are all within the acceptable range, the object will be judged as an individual cell. And it was found that the maximum cell detection rate can be obtained when both the elongation rate parameter and the defect rate parameter use the above threshold.

C. Adhesive cell masses segmentation

In this step, the watershed algorithm is used to segment the adhesive cell masses. Firstly, grayscale the image of the adhesive cell mass, and then continuously corrode the grayscale image to obtain its distance gradient map. Finally, the watershed algorithm is performed on the distance grayscale image to obtain the edge lines of each cell in the image of the adhesive cell mass. Since the shape and size of the cells are different, and some impurities and cells stick together, the use of conventional watershed algorithms will cause excessive segmentation. Therefore, the judgment of the seed point area threshold is added to the algorithm. The number of pixel points of the seed point is used as the criterion, and false seed points with too small area value are discarded, so as to avoid the occurrence of excessive segmentation. In this algorithm, the lowest threshold of seed points is set to 100 pixels. Segmentation results of adhesion cell clusters are shown on Fig.3.

Figure 3. Segmentation results of adhesion cell clusters. (A) Original image of adhesive cell mass. (B) Distance gradient map. (C) Seed points map. (D) The image after segmentation. (E-I) Individual cell images after segmentation.

D. Cell type recognition based on CNN model

As a deep learning algorithm, CNN is composed of image input layer, convolutional layer, maximum pool, average pool, comprehensive connection layer and other parts. It takes the original pixels of the input image by mapping as input, extracts feature in a hierarchical manner, and then further uses a fully connected layer to classify

the features. According to the size of the input image, the number of layers can be appropriately increased or decreased, and it is not necessary to use all the layers in the network. The advantage of CNN is that it does not require any artificial feature extraction by other methods.

A CNN model suitable for image classification of micronucleus cells is designed, and Fig. 4 gives an overview of the proposed CNN architecture. This CNN model contains 7 layers (4 convolutional layers, 3 fully connected layers). The first convolutional layer (Conv1) uses 8 kernels with a size of 3×3 to filter a $3 \times 132 \times 132$ input image. Conv2 has 16 filters with a size of 3×3 , and Conv 3 and Conv 4 have 32 and 48 filters with a size of 3×3 , respectively. The first fully connected layer (FC1) has 40 neurons, and each neuron uses a 50% loss rate during training. FC2 has 20 neurons, and each neuron also uses a 50% loss rate during training. After Conv1, Conv2, and Conv4, the largest pooling layer with a 2×2 kernel size is used. The rectified linear unit (ReLU) activation function is used after each convolution and fully connected layer. At the end, the softmax function is used to generate probability distributions on the five class labels.

Figure 4. Overview of the proposed CNN architecture.

After performing cell extraction and segmentation of adhesion cell clusters on the images of 80 slides used for training, approximately 280,000 images of individual cells were obtained. The classification was carried out by five experienced analysts according to standardized standards³³. Cell images are divided into five categories: 0.

BNC without MN, 1. Binucleated cell with Micronuclei (BNCMN), 2. MOC, 3. POC with three nuclei and above, 4. Any other cell or substance that do not belong to the first four types. In the end, about 24,000 BNC images without MN, 16,000 BNCMN images, 45,000 MOC images, 35,000 POC images, and 160,000 other types of images were obtained. Because the number of various types of images is not balanced, and the unbalanced number of images will lead to overfitting of the training model. Therefore, data enhancement methods were used to generate new images to balance the corresponding category images in the training set. The methods used include: horizontal flip, random addition of noise or blocks with occlusion effects on the original image, principal component assay (PCA) dimensionality reduction operations, etc. Each type of image is randomly classified into CNN training set, validation set, and test set according to the ratio of 7:2:1. Among them, the training set and the validation set participate in the CNN model training process, and the test set does not participate in the model training but only serves as a preliminary internal test.

Since the area values of different cell images are not the same, before training, all images need to be scaled to a 132×132 image without stretching (this size is close to the average area of BNC images). Then use TensorFlow's Keras function package to implement the CNN model. The learning rate is initialized to 0.001 and reduce it by a factor of 10 after half of the iterations. The momentum term is set to 0.9, and the weight decays to 0.0001. Except for the specified configuration, all parameter settings are standard configurations. The network was trained for 60 iterations and spent 40 hours on the NVIDIA GeForce GTX 1080 Ti GPU. During the training process, the convergence of the model was good and there was no obvious over-fitting phenomenon. In the end, the overall recognition accuracy of the model reached 85%, and the loss rate dropped to about 0.4.

The trained model was internally tested using the images of the test set. The recognition accuracy of BNC images without MN reached 84.5%, and the recognition accuracy of BNCMN images reached 87.1%. The test results of other categories are shown in Fig. 5.

Actual(%)	0 BNC without MN	84.5	11.0	1.3	1.1	2.2
	1 BNCMN	10.1	87.1	0.2	2.0	0.6
	2 MONOC	5.8	3.9	81.3	0.2	8.7
	3 POLYC	15.2	6.9	0.6	71.4	5.9
	4 Other	6.1	1.8	4.0	2.9	85.3
		0 BNC without MN	1 BNCMN	2 MONOC	3 POLYC	4 Other
		Predicted(%)				

Figure 5. Confusion matrix of recognition results

E. Micronucleus count

In the previous step, the CNN model can only identify cell types, but it cannot accurately identify the number of MN in BNCMN that contain multiple MNs. Therefore, the number of MN needs to be identified in this step of work. First, the nucleus and MN are extracted from the cytoplasm, and then the multiple MNs that are

attached together and the MNs that are attached to the nucleus are segmented, and finally the nuclei and MNs are sorted according to the area value to count the number of MNs.

There are many images of cells with blurry cytoplasm in the collected images, so the iterative threshold algorithm cannot effectively extract cell nuclei and MN from these images. Therefore, a threshold segmentation algorithm based on k-means clustering is designed. The flowchart is shown in Fig. 6.

After extracting the nucleus and MNs, import them into the watershed algorithm. The corrosion parameters of the watershed algorithm used here are different from the watershed algorithm in the previous step. In the previous step, the purpose is to segment the adhesive cell masses, so a larger corrosion parameter (value: 7) needs to be used. In this step, dividing the adhered nucleus into independent nuclei is not the goal (because the number of nuclei has been determined), the main goal is to separate the adhered NM into independent MNs. Therefore, in this step, the watershed algorithm uses a smaller corrosion parameter (value: 3) to strengthen the segmentation effect. The segmentation effect is shown in Fig.7. After the segmentation is completed, the area value and coordinate information of each cell nucleus and MN can be obtained.

Figure 6. Flow chart of extracting nucleus and MN

After obtaining the area information of the cell nucleus and MNs, arrange them according to the numerical value. Then, based on the principle that the maximum acceptable MN area is 20% of the nuclear area value (if there are multiple nuclei, the average value of multiple nuclei is taken), the candidate MNs are screened out. Finally, calculate the number of MNs based on the principle that the minimum acceptable MN area is 20 pixels (to prevent some noise points from being misjudged as MNs).

Figure 7. The result graph after using k-means clustering threshold algorithm and improved watershed algorithm segmentation algorithm. (A, D) Original image. (B, E) The image after extracting cell nucleus and MN. (C, F) The image after segmentation.

IV. Results

A. Random image algorithm test

20 slides from 4 different donors (2 males and 2 females, A-D) were scanned and photographed, and then these images were used to verify the system. The images of these 20 slides did not participate in the training of the CNN model. After the image collection is completed, 100 images are randomly selected from the picture collection of each slide, that is, 500 images are selected for each donor, and a total of 2000 images are obtained. The proofreaders also performed artificial visual scoring on 2000 original images separately according to the standard method³³. The image analysis data obtained is shown in Table 1.

Donors	Slide number	BNC	BNCMN	MN	MN/BNC
A	A 1	106	36	39	0.368
	A 2	116	40	50	0.431
	A 3	107	37	43	0.402
	A 4	118	40	47	0.398
	A 5	119	42	52	0.437
B	B 1	136	47	62	0.456
	B 2	132	46	57	0.432
	B 3	139	48	67	0.482
	B 4	138	48	64	0.464
	B 5	140	49	62	0.443
C	C 1	164	57	76	0.463
	C 2	171	59	63	0.368
	C 3	161	55	79	0.491
	C 4	163	56	83	0.509
	C 5	165	57	80	0.485
D	D 1	177	60	88	0.497
	D 2	179	59	75	0.419
	D 3	183	63	90	0.492
	D 4	180	66	89	0.494
	D 5	184	64	93	0.505
Total number		2978	1029	1359	

TABLE 1. The number of Binucleated Cells (BNC), Binucleated Cells with Micronuclei (BNCMN), Micronuclei (MN) and the number of Micronuclei per Binucleated Cell (MN/BNC) by manual scoring

As shown in Table 1, five experienced inspectors found 1949 BNCs without MN, 1029 BNCMNs and 1359 MNs.

The manual detection took about 7.5 hours of proofreading time in total. After that, these images are automatically analyzed, and the resulting data are shown in Table 2. According to statistics, automatic image analysis took about 620s in total (about 0.3s per image on average), which is 44 times the efficiency of manual detection. As shown in Table 2, the software automatically detected 2041 binucleated cells without MN and 920 BNCMNs. The total number of MNs is 1564.

Donors	Slide number	BNC	BNCMN	MN	MN/BNC
A	A 1	127	39	63	0.496
	A 2	130	40	65	0.500
	A 3	122	37	60	0.492
	A 4	125	39	56	0.448
	A 5	124	38	53	0.427
B	B 1	131	41	73	0.557
	B 2	125	38	74	0.592

	B 3	129	40	67	0.519
	B 4	136	42	70	0.515
	B 5	130	40	60	0.462
C	C 1	150	46	90	0.600
	C 2	156	48	74	0.474
	C 3	145	45	82	0.579
	C 4	156	49	84	0.538
	C 5	148	45	76	0.514
D	D 1	183	57	101	0.552
	D 2	184	63	93	0.505
	D 3	183	56	103	0.563
	D 4	190	59	113	0.595
	D 5	187	58	107	0.572
Total number		2961	920	1564	

TABLE 2. The number of BNC, BNCMN, MN and MN/BNC through automatic scoring

	BNC	BNCMN	MN
Automated scoring	2961	920	1564
Manual diagnosis	2525	816	1154
False positive incidence of BNC			14.7%
False positive incidence of BNCMN			11.3%
False positive incidence of MN			26.2%

TABLE 3. Relation between the automatically scored data and correct diagnosis by observers

After the automated analysis of these images, the same proofreaders use the gallery in the system to visually proofread the BNC images obtained from the automated analysis and count the error values of each data. The data results after proofreading are shown in Table 3. The comparison result of manual detection data and automatic analysis data is shown in Table 4.

As shown in Table 3 and Table 4, the detection rates of BNC and BNCMN of the automated CBMN analysis algorithm reached 99.4% and 89.4% of manual detection, and the false positive rates were 14.7% and 11.3%, respectively. The detection rate of MN reached 115.1% of manual detection, and its false positive rate was 26.2%.

It can be found that the BNC and MN detection rate of the automatic analysis algorithm based on the CNN model is much higher than the conventional image recognition algorithm. Because conventional image recognition algorithms are essentially a "screening" mechanism, which pursues high accuracy by continuously eliminating candidate targets. However, this algorithm retains more than 95% of all types of cells, so this algorithm can identify more BNCs and MNs.

Since the difference between cases and controls is not the main goal of this study, there is no adjustment for age, sample storage time, etc., nor for the treated or untreated cases of patients. Because it will not affect the direct comparison between visual counting and automatic counting.

	BNC	BNCMN	MN
Automated scoring	2961	920	1564
Manual scoring	2978	1029	1359
BNC yield relative to manual scoring			99.4%
BNCMN yield relative to manual scoring			89.4%
MN yield relative to manual scoring			115.1%

TABLE 4. Relation between the automatically scored data and manual scored data

B. Assay of test results by SPSS

For studying the difference between the results of automatic analysis and manual detection, SPSS (Statistical Product and Service Solutions, version 19.0) was used to perform statistical analysis on the MN rate(MN/BNC) data in Table 1 and Table 2. The 20 MN rates in Table 1 are regarded as the "Manual" group, and the 20 MN rates in Table 2 are regarded as the "Automatic" group. Since the amount of data is not large, and the two sets of data do not obey the normal distribution, the Mann-Whitney U is first used to test whether there is a statistical difference between the MN rate data of manual visual inspection and automatic analysis.

In the Mann-Whitney U test results, the average rank of the manual detection data is 13.30, and the average rank of the automatic analysis data is 27.70. The exact P value is <0.001 (The specific data can be viewed in Supplementary Fig.S1). Therefore, the difference between manual detection and automatic analysis was deemed to be statistically significant. According to calculations, it can be found that the mean and median values of the data of the automatic analysis group are higher than the manual detection group, indicating that the value of the automatic grouping is generally higher than the manual detection group.

Then, the 20 MN rates in Table 1 are divided into four groups from A to D according to their respective donors (the number of samples in each group is 5). Since the amount of data is not large, and each group of data does not follow a normal distribution, the Kruskal-Wallis H test is performed. The same operation is also performed on the data in Table 2. On the one hand, the Kruskal-Wallis H test can detect whether there are statistically significant differences between the 4 groups of data. On the other hand, through the Kruskal-Wallis H test on the MN rate data of manual detection and automatic analysis, two conclusions can be obtained respectively. Then the consistency of the two conclusions can be observed. If the inspection conclusions of the two are the same, it proves that the automatic analysis has the detection ability roughly equivalent to the manual detection. The test result data of the MN rate of manual detection is shown in Fig.8.

It can be found that the distribution of the manually detected MN rate data is not the same, and the difference is statistically significant ($P = 0.039 < 0.05$, in Supplementary Fig.S2). The average rank of Donor A group data is 4.50, the average rank of Donor B group data is 10.20, the average rank of Donor C group data is 12.50, and the average rank of Donor D group data is 14.80. After the significance level was adjusted by Bonferroni method, the data of each group was compared in pairs, and it was found that the data distribution of Donor A group and Donor D group ($P = 0.035$ after adjustment, $P < 0.05$) was statistically different, and the difference between the other groups was not statistically significant (the comparative data of other groups can be viewed in Supplementary Fig.S3). Similarly, the 20 MN rate data in Table 2 are also divided into four groups from A to D ($n = 5$) according to their respective donors. The test results are shown in Fig. 9.

It can be found that the distribution of the data of each group of the automatic analysis result is not completely the same, and the difference is statistically significant ($P = 0.042 < 0.05$, in Supplementary Fig.S4). The average rank of Donor A group data is 4.20, the average rank of Donor B group data is 11.20, the average rank of Donor C group data is 12.40, and the average rank of Donor D group data is 14.20. After the significance level was adjusted by Bonferroni method, the data of each group was compared in pairs, and it was found that the data distribution of Donor A group and Donor D group ($P = 0.045$ after adjustment, $P < 0.05$) was statistically different, and the

difference between the other groups was not statistically significant (The comparative data of other groups can be viewed in Supplementary Fig.S5).

Pairwise Comparisons of Donor

Each node shows the sample average rank of Donor.

Figure 8. Pairwise comparison result of manually detected MN rate data

In Fig.8, the value next to the dot represents the average rank of the group. The connecting line represents the result of the pairwise comparison, the black connecting line represents the difference between the two groups is not statistically significant, and the orange connecting line represents the statistical significance of the difference between the two groups.

Pairwise Comparisons of Donor

Each node shows the sample average rank of Donor.

Figure 9. Pairwise comparison result of automatically analyzed MN rate data

The Kruskal-Wallis H test result of manually detected data and automatically analyzed data are the same. Both concluded that the data distribution of Donor A group and Donor D group is statistically significantly different. Therefore, this shows that the automatic CBMN analysis algorithm basically has the detection ability roughly equivalent to manual detection.

V. Discussion

The purpose of this research is to design a system that can automatically analyze Giemsa stained CBMN images, which can simultaneously analyze the images while shooting. Unlike conventional analysis algorithms, this algorithm retains all cells to the greatest extent, so it can identify more BNC and MN. Conventional image recognition steps such as manual feature extraction, dimensionality reduction and feature ranking are also not needed. This is because CNN can intelligently adjust network parameters to obtain the highest classification performance, and automatically extract the elements by generating feature maps after each convolutional layer, so as to determine the best performance elements by itself. To solve the problem that CNN cannot realize the MN counting of BNC with multiple MNs, a K-means clustering-based cell nucleus/MN extraction algorithm is designed. And the algorithm can effectively deal with the adhesion of multiple MNs to each other and the adhesion of MN to the nucleus.

The detection rates of BNC and BNCMN of the automated CBMN analysis algorithm reached 99.4% and 89.4% of manual detection, and the false positive rates were 14.7% and 11.3%, respectively. Most of the pseudo-BNCs come from cells with complex nuclei overlap. Even experienced observers can hardly determine whether the nuclei overlap or connect in space when encountering such images. The detection rate of MN reached 115.1% of manual detection, and its false positive rate was 26.2%. The incidence of false positives in MN is mainly due to Giemsa dye, which will cause blurred cytoplasmic background and produce some artifacts similar to MNs. These artifacts are also very difficult for manual detection. On the other hand, some MNs with lighter colors are missed by the algorithm, because these MNs are difficult to be effectively separated by the k-means clustering threshold algorithm. The same Kruskal-Wallis H test result of the MN rate data of manual detection and automatic analysis shows that the automatic CBMN analysis algorithm basically has the detection ability roughly equivalent to that of manual detection. With the continuous increase of CNN training samples, the detection accuracy and detection rate of the algorithm can be further improved, and finally reach a level that is completely equivalent to that of experienced human observers.

In previous similar work, the first type of algorithm only analyzes the nucleus information. Therefore, this type of algorithm not only needs to locate the cell boundary and estimate the number of nuclei in each cell, but also need to analyze the relative position and relative size of the nucleus. This type of algorithm is suitable for images acquired by automated microscopes¹⁹⁻²² or imaging flow cytometers²³⁻²⁵. The algorithm generally searches for the cell nucleus first, and then judges the two adjacent nuclei as a BNC, then defines a circular area around the two nuclei of the BNC as the area of the cell, and finally searches for MN in this area. The determination parameters used are based on morphological criteria, such as area value²², circularity²³, degree of overlap of two nuclei²⁵, etc. The MN detection rate obtained by this kind of algorithm analysis is about 20-35% of manual counting^{20,24-25}. When using this algorithm, it is usually required to give priority to the use of fluorescent stains in the manufacturing process of the slides²⁰⁻²¹ and to reduce the cell density as much as possible. Because when the cell density is high, it may happen that some nuclei are assigned to the same cell by mistake²².

The second type of algorithm uses the whole cell as a detection unit. In terms of staining, the dye must not only mark the nucleus/MN, but also the whole cell. The visualized nucleus and cytoplasm can match the nucleus/MN with the corresponding cells, so that BNC and MN can be more accurately identified. This type of algorithm first recognizes the cytoplasm of the cell to determine whether it is a complete cell²⁶⁻²⁷;

then detects the number of nuclei in the cell; and finally detects the number of MNs. The used judgment parameters include the circle (ellipse) shape of the cell and the nucleus²⁸, area²⁹ and so on. To obtain clearer images and improve the recognition effect of such algorithms, techniques such as double fluorescent dyeing²⁸ and high magnification imaging³¹⁻³² are applied to the image acquisition process. The MN detection rate obtained by this kind of algorithm analysis is about 65% of manual counting^{26,31}.

Compared with the previous similar work, our algorithm flow is similar to the second type of algorithm, but the segmentation steps of adhesion cell clusters and binucleated cell recognition method based on CNN in this algorithm are not available in other algorithms. These differences are the reason why the detection rate of this system is much higher than that of the previous system^{20, 26}. In terms of the applicability of slides and the clinical applicability of the system, this system can achieve good recognition results for slides prepared by conventional methods, without high requirements on the staining effect²⁹ and the cell density²⁷. And without using a high microscope magnification³⁰, the system can also detect BNC and MN with blurred cytoplasmic, which means that it has both excellent detection capabilities and rapid analysis capabilities. Unlike conventional image analysis algorithms, this software uses a CNN model for cell recognition. Therefore, there is no need to perform tedious manual feature selection and threshold parameter setting, and only need to manually provide enough training samples for CNN.

According to statistics, the analysis time of the system is about 620s for every 2000 photos, which is 44 times the efficiency of manual detection. If the biological dose estimation is performed according to the requirement of analyzing a minimum of 1,000 BNCs, the average analysis time only takes 7-10 minutes with enough BNCs. Compared with manual visual inspection, this undoubtedly greatly improves the speed of CBMN analysis. By deploying the software on high-end workstations and further optimizing system performance, faster image processing speeds can be achieved.

In the process of detecting MN, not only BNC is involved, but also a sizable of MOCs and POCs are produced. Another advantage of the software is that it allows the detection of cells with different nuclei counts. The information of MOC and POC can be used to calculate the cell proliferation index³⁴, the ratio of trinuclear cells to tetranuclear cells³⁵ and other information, which can help estimate high radiation doses.

VI. Conclusion

This article introduces a method for fast and automatic analysis of Giemsa stained CBMN microscopic images, which overcomes many limitations. The slides used in this system do not need to specifically control the density of the cells and the degree of staining, so the user can use it more easily. The detection rates of BNC and BNCMN of this system reached 99.4% and 89.4% of manual detection and the analysis speed is 40-50 times faster than manual detection. Even when the cells are stained deeply, the system can detect BNC and MN, and this system has the function of detecting MOC and POC. Because of the CNN model used in this system, the accuracy and detection rate of MN detection can be continuously improved as the number of training samples increases.

In future work, system testing and analysis will be performed on samples irradiated with different doses to continually improve the performance of the system. In addition, the detection functions of the software will be further expanded, such as the detection of MOC containing MN, POC containing MN, nucleoplasmic bridges, necrotic cells and apoptotic cells.

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Author contributions

All authors contributed to the study conception and design. X.S. and Y.C. were instrumental in idea and study design of this research. X.S., C.L. and F.Y. was involved in the programming, data analysis, the main manuscript text. Z.W., J.Z. and Z.Z. were instrumental in manuscript preparation, critical manuscript revision. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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