Two-way detection of image features and immunolabeling of lymphoma cells with one-step microarray analysis

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(Received 26 September 2018; accepted 10 December 2018; published online 28 December 2018)

Detecting the number of pathological lymphoma cells and lymphocyte subtypes in blood is helpful for clinical diagnosis and typing of lymphoma. In the current study, cell type is identified by cell morphological features and immunolabeled lymphocyte subtypes. Red blood cells and leukocytes were separated using a microfluidic cell chip based on physical blood cell parameters, and leukocytes were identified using five characteristic parameters: energy variance, entropy variance, moment of inertia variance, color mean, and cell area individually. The number of red blood cells that could come into contact with the leukocyte membrane was ≤ 2 based on the microfluidic injection flow rate of microfluidic chips. Anti-CD3 and anti-CD19 antibodies were used for immunofluorescence staining of T-lymphocyte and B-lymphocyte surface antigens, respectively. The results suggested that the microfluidic assay could detect lymphocyte surface antigen markers and intact leukocytes. Therefore, we report a one-step microfluidic chip for classifying hematological lymphoma cells based on the physical parameters of cells, which can simultaneously measure the overall morphology of blood cells and immunolabeling of lymphocyte surface antigens in one step, solving the current problem of detecting subtypes of hematological lymphoma cells based on multiple methods and multi-step detection. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5063369>

I. INTRODUCTION

Lymphoma is a malignant tumor that occurs in lymph nodes and/or extranodal lymphoid tissues. The incidence of lymphoma is the eighth highest of all tumors, and the mortality rate is the tenth highest in China.^{1,2} Summarizing the molecular genetics³ and molecular mechanism of lymphoma pathogenesis,^{4,5} the type of molecular marker on the surface of lymphocytes,^{6–8} the gene mutation characteristics of lymphoma cells,⁹ and the type and degree of nuclear expression biomolecules in lymphoma cells⁹ can assist in the clinical diagnosis of lymphoma. The World Health Organization (WHO) has developed a lymphoma classification standard based on the correlation between the biological phenotype and lymphoma disease.^{10–12} Therefore, the clinical examination of patients with lymphoma should contain the following items: the morphological characteristics of lymphoma cells in the patient's blood, the type of biomarker on the surface of lymphoma cells, the biogenetic characteristics of the cell's gene molecules, and the expression characteristics of cell

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molecules. Then, the clinical characteristics of patients for lymphoma diagnosis and subtype classification are combined. The clinical classification of lymphoma is currently divided into Hodgkin's lymphoma and non-Hodgkin's lymphoma. The latter is further divided into B cell and T/NK cell origin according to the immunophenotypic characteristics.

Blood cells include lymphocytes, granulocytes, and monocytes. When lymphoma disease occurs in the body, the pathological lymphoma cells can also be detected. The number of lymphoma cells and lymphocyte subsets can be a basis for diagnosing lymphoma.¹⁰ Therefore, a one-step method for examining the morphological characteristics of blood cells and detecting biomarkers lymphoma cells would aid in the clinical detection of hematological lymphoma.

Flow cytometry is the main method for analyzing single cell immunobiomarker types but is not able to systematically analyze cell markers and all cellular information.^{6–8} In cell biomarker analysis, histiocyte immunohistochemical staining can analyze the specific single marker immunogenicity expression degree of the cell being assayed.^{13,14} The tissue cell chip can be combined with immunolabeling technology to measure the expression of multiple biomarkers on the detected tissue cells simultaneously.¹⁵ Cellular gene amplification and DNA sequencing can determine the expression of cell biomolecular markers.^{16–21} However, the diagnosis of lymphoid tumors requires further identification of the T-lymphocyte markers (CD3), B-lymphocyte markers (CD19), NK cell markers (CD56), and R-S lymphoma cell markers (CD30) after determining the lymphocyte type.^{22–26}

Recently, cell types were reportedly classified according to physical cell parameters, which helped to achieve hydrodynamic analysis and lay the foundation for the analysis of hematological lymphoma cells using microfluidic techniques.²⁷ In the previous study, the study group extracted characteristic parameters of nuclear staining and immunohistochemical staining images to realize the identification of morphological characteristics of cervical endothelial cells, granulocytes, and monocytes, which provided a preliminary basis for the extraction of blood cell characteristic parameters.²⁸ Based on the above research, a microfluidic chip was used in the present study to classify leukocyte types according to the morphology of blood cells and immunolabeling of lymphocytes using anti-CD19 and anti-CD3 antibodies, achieving one-step detection of lymphocytes and further identification of lymphoma cell subtypes in combination with immunolabeling.

II. MATERIALS AND METHODS

A. Cell lines

Jurkat (T-lymphoma cell line, BeNa Culture Collection) and Mino (B-lymphoma cell line, BeNa Culture Collection) cells were used to analyze the image features of pathological lymphoma cells. Both cell lines were cultured in RPMI 1640 medium (Gibco) containing 10% fetal bovine serum (Biological Industries) in a 37 °C, 5% CO₂ incubator.

B. Peripheral blood cells

Healthy volunteer blood leukocytes were used to analyze the morphological characteristics. According to the WHO diagnostic criteria for lymphoma, ten patients with lymphoma were diagnosed and their venous blood were collected. The morphological detection of blood leukocytes was performed on an SP-1000i Slide Preparation Unit (SSPU, SYSMEX). The proportion of leukocytes in blood was measured one by one, and the number of pathological lymphoma cells was calculated. Informed consent was obtained prior to patient inclusion, and this study was approved by the Ethics Committees of Tianjin Medical University (TMUhmec2017012).

C. Set microfluidic cell chip analysis conditions

The microfluidic chip was fabricated using soft lithography techniques, including two microfluidic channel inlets for A and B. The cell separation solution (pH 7.4, 0.01 mol of phosphate buffer) entered the microfluidic chip through the A-channel, and the solution containing the detected cells entered the chip through the B-channel. The rate of injection in the B-channel was

300-1100 $\mu\text{l/h}$, and the ratio of the injection rate of the A-channel to the injection rate of the B-channel was 1:0.85. The three cell collection pools collected various types of leukocytes.

D. DAPI staining

After the cells were fixed in 4% paraformaldehyde (Biosharp) in Eppendorf tubes for 10 min, 100 μl of the ready-to-use DAPI (10 $\mu\text{g/ml}$, Solarbio) was added and incubated in the dark for 10 min at room temperature. The prepared cell sample was passed into a microfluidic chip, and then, the image of the cell was observed using an upright fluorescence microscope (Nikon).

E. Blood cell for immunostaining and imaging

Blood leukocytes were immunostained with two fluorescent antibodies: CD19-PE (Tianjin Sanjian), labeled with PE red fluorescent group, and CD3-488 (Biolegend), labeled with 488 green fluorophore. A cocktail of antibodies containing 2 μl CD19-PE and 2 μl CD3-488 was added to 100 μl of anticoagulated (EDTA) blood and then incubated in darkness for 30 min on ice. Next, 200 μl of paraformaldehyde (4%) was added and incubated for 10 min to fix the cells. Then, 300 μl of DAPI was added in darkness for 10 min for nuclear staining. Finally, the cell image was observed using an upright Nikon fluorescence microscope with the microfluidic chip.

F. Image analysis

The self-designed Matlab image analysis software extracted a gray-level co-occurrence matrix and color histogram characteristic parameters of the three RGB channels and cell area features outside the RGB channels for normal blood leukocytes and lymphoma cells (Fig. S1).

The gray-level co-occurrence matrix is a common method for representing image texture features. The co-occurrence matrix is a joint distribution of two pairs of pixels that maintain a certain distance on an image, and it can reflect the comprehensive information of the image gray-scale on the adjacent interval, direction, and amplitude change, among others. It is a better analysis method for irregular natural texture. In order to extract the texture feature expression in the co-occurrence matrix, a statistical calculation is needed. We selected the following characteristic parameters.

Energy: Reflecting the uniformity of the grayscale distribution of the image:

$$\text{Ene} = \sum_{i=0}^{L-1} \sum_{j=0}^{L-1} [P(i, j)]^2. \quad (1)$$

Entropy: Measuring the amount of information in the image:

$$\text{Ent} = - \sum_{i=1}^k \sum_{j=1}^k G(i, j) \log G(i, j). \quad (2)$$

Moment of inertia: Reflecting the depth and sharpness of image texture grooves:

$$\text{Contrast} = \sum_{i=0}^{l-1} j \sum_{j=0}^{l-1} (i - j)^2 P(i, j). \quad (3)$$

Color histogram is a common method of characterizing color distribution and describing the proportion of different colors in the entire image. Color mean, which is the average pixel value of the image, was selected to express the color features of the image:

$$H(i) = \frac{n_i}{N}, i = 0, 1, \dots, \quad (4)$$

$$u = \sum_{i=0}^{L-1} iH(i). \quad (5)$$

These characteristic parameters of the cell image were extracted according to the obtained characteristic parameters to set the range of various blood cell parameters. These cells contained normal granulocytes, normal B-lymphocytes, normal T-lymphocytes, normal monocytes, pathological T-lymphoma cells, and pathological B-lymphoma cells.

G. Statistical analysis

All experimental data were measured three times and presented as mean $\pm$ standard. Data were performed on GraphPad Prism Version 5.0 Software and SPSS Version 19.0 Software.

III. RESULTS AND DISCUSSION

A. Detection of blood leukocyte type using the SP-1000i slide preparation unit

Ten healthy volunteers and ten lymphoma patients were recruited, and 2 ml of intravenous peripheral blood was collected from each investigator. Every blood cell in the blood was classified using the SP-1000i Slide Preparation Unit (SSPU, SYSMEX). For each of the investigators, there were a total of 200 cells, including blood granulocytes, lymphocytes, and monocytes, which were calculated three times (Table I). No pathological lymphoma cells were found in peripheral venous blood from any healthy volunteer. Lymphoma cells were found in each patient's peripheral blood, and the proportion of lymphoma cells accounted for 1%-55% of the total cells. Two sets of blood cells were selected to verify the accuracy of the microfluidic chip for detecting various types of leukocytes in blood.

B. Set analysis conditions of microfluidic cell chip to test blood cells

The number of red blood cells was $3.0\text{-}5.5 \times 10^{12} \text{ l}^{-1}$, and the number of leukocytes was $4.0\text{-}10 \times 10^9 \text{ l}^{-1}$ under physiological conditions. The ratio of red blood cells to white blood cells (RBC/WBC) was approximately 550-750. If lymphocytes were measured separately, the proportion of other cells increased to (1800-2500):1. In this way, the process of measuring blood leukocyte biomarkers, other cells, and cell debris had the potential to interfere with the detection of leukocyte markers. A large number of reports have reported that microfluidic assays could help to detect circulating tumor cells, but no systematic study had evaluated the microfluidic detection of lymphoma cells.²⁹⁻³² This study was designed to assist in the examination of hematological lymphoma cells based on a microfluidic cell chip system. The chip structure consisted of two channels, microfluidic A-channel and microfluidic B-channel (Fig. S2). After the blood cells passed through the microchip, the blood leukocytes and red blood cells followed the microfluidics into the different channels on the chip according to the physical parameters of the cells [Fig. 1(a)]. The flow rate of the B-channel was designed to be 300-1100 $\mu\text{l/h}$, and the microfluidic flow rate of the A-channel was fixed. The microfluidic flow rate of the B-channel was adjusted, and the number of red blood cells at the junctions with the leukocytes was calculated under the conditions of each microfluidic flow rate. At 300 $\mu\text{l/h}$ conditions, 0-2 red cells were found around leukocytes, and the no cell area

TABLE I. The proportion of leukocytes and lymphoma cells in blood cells measured by SSPU.

Cell (%)	Healthy volunteer (n = 4)				Lymphoma (n = 4)			
	Number							
	1	2	3	4	1	2	3	4
Granulocyte	55 $\pm$ 0.94	60 $\pm$ 0.82	58 $\pm$ 0.47	60 $\pm$ 0.47	40 $\pm$ 0.47	28 $\pm$ 1.63	65 $\pm$ 2.05	69 $\pm$ 0.82
Lymphocyte	39 $\pm$ 0.82	34 $\pm$ 0.82	37 $\pm$ 0.47	35 $\pm$ 0.82	4 $\pm$ 0.47	65 $\pm$ 2.16	33 $\pm$ 1.41	16 $\pm$ 2.87
Monocyte	6 $\pm$ 1.25	6 $\pm$ 0.82	5 $\pm$ 0.82	5 $\pm$ 0.47	1 $\pm$ 0.00	4 $\pm$ 0.82	1 $\pm$ 0.82	8 $\pm$ 0.94
Lymphoma	0	0	0	0	55 $\pm$ 0.82	3 $\pm$ 0.82	1 $\pm$ 0.47	7 $\pm$ 1.41

FIG. 1. Microfluidic chip analysis system. (a) Microfluidic image analysis system includes microfluidic chip, cell image acquisition CCD, and microscope. Blood cell separation schematic: dark gray ball—white cell, light gray ball—red cell. (b) The number of red blood cells at the junctions with the leukocytes was calculated by adjusting the flow rate into the microchip microfluid. The ratio of the A-channel flow rate to the B-channel flow rate is 1:0.85, and the B channel is 300-1100 $\mu\text{l/h}$. When the fluid flow rate of the B microchannel is 300 $\mu\text{l/h}$, the number of red blood cells contacting with the leukocyte membrane was ≤ 2 .

surrounding some leukocytes was larger than the red blood cell diameter, and no red blood cell aggregation was observed [Fig. 1(b)]. The experimental conditions were thought to reduce the interference of other non-detected cells with the analyzed leukocytes.

C. Effect of the microfluidic chip on the isolation of lymphoma cells

Mino cells (B-lymphoma) and Jurkat cells (T-lymphoma) were cultured with fluorescence nuclear DAPI staining, and the morphology of the cell nucleus was observed under a microscope. Normal blood leukocytes were simultaneously observed for their nuclear DAPI staining. The lymphoma cell lines with uniform size and detailed nuclear chromatin were consistent with the clinical

pathological lymphoma cell (Fig. S3). Anti-CD3 and anti-CD19 antibodies were used to perform immunofluorescence staining of blood cells on the microfluidic chip. CD19⁺ Mino cells were consistent with B-lymphocyte immune markers, and CD3⁺ Jurkat cells were consistent with T-lymphocyte immune markers [Fig. 2(a)].^{7,33} The same immune markers indicated that the

FIG. 2. The effect of immunofluorescence staining of blood cells in a one-step method based on microfluidic cell chip technology. DAPI and immunohistochemical staining were performed on blood cells of healthy volunteers and lymphoma patients in a microfluidic system. The morphology of the cells was observed by fluorescence microscopy. (a) Mino lymphoma cells, Jurkat lymphoma cells, and healthy volunteers' blood cells were stained with anti-CD3 and anti-CD19 antibodies. The CD3 antibody was green, and the CD19 antibody was red. (b) The lymphoma cells were mixed with healthy volunteer cells in a ratio of 10% to 30%. Leukocytes and lymphoma cells passed through the chip II-IV channels. There were no lymphoma cells and leukocytes in the I channel.

microfluidic analysis conditions were suitable for the analysis of lymphoma cell immune markers. In addition, Mino and Jurkat cells were added to the blood cells of healthy volunteers to achieve a ratio of lymphoma cells to blood leukocytes of 10% to 30%. The prepared sample was passed into a microfluidic chip. The lymphoma cells appeared between channels II, III, and IV of the chip and did not appear in channel I. The results suggested that the analysis conditions can concentrate the white blood cells on chip channels II-IV to collect cells [Fig. 2(b)].

D. Extraction of the characterization parameters of various types of leukocytes and lymphoma cells

Self-designed Matlab image analysis software was used to investigate the characteristic parameters of lymphoma cells and normal lymphocytes (Fig. 3). Mino and Jurkat cells were cultured at 8×10^{11} cells/l. A total of $300 \mu\text{l}$ of cells were used for Wright's staining of cell smears, and 200 cells were observed under the microscope. Another $300 \mu\text{l}$ of lymphoma cells underwent nuclear and immunofluorescent staining. We extracted the energy variance, entropy variance, moment of inertia variance, color mean, and cell area from each cell of the normal control neutrophils, monocytes, lymphocytes, and lymphoma cells. The five morphological parameters were analyzed, and the varied degree of the same characteristic parameters among the various types of cells was significant (Table II). According to the diagnosis standards of lymphoma cell, special cell identification characteristics were selected to set threshold. Currently, blood cell image recognition is a research hotspot based on individual differences of white blood cells.^{34–36} It is thought that intelligent selection of cell parameters and identification procedures can be set based on the pathological differentiation of the cells.

E. Selective cell characteristic parameters and design identification procedures

By labeling normal blood granulocytes, monocytes, lymphocytes, pathological T-lymphoma cells, and pathological B-lymphoma cells, the characteristics of three RGB channels were analyzed for each cell to extract their energy variance, entropy variance, moment of inertia, color mean, and

FIG. 3. Self-designed Matlab image analysis software. Each cell was extracted energy variance, entropy variance, moment of inertia variance, and color mean characteristic parameters of the RGB three-channel. The four feature parameters for each channel were stored as three-dimensional images.

TABLE II. Five characteristic parameters of various types of blood leukocytes measured.

Channel	Cell type	Energy variance	Entropy variance	Moment of inertia variance	Color mean	Area (no RGB channel)
R	Mino	0.0765 ± 0.0293	0.3062 ± 0.1433	0.0603 ± 0.0529	0.0722 ± 0.0337	450 ± 103
	Jurkat	0.0288 ± 0.0335	0.0505 ± 0.0581	0.0061 ± 0.0078	0.0351 ± 0.0056	435 ± 117
	B-L	0.0718 ± 0.0375	0.4910 ± 0.1728	0.2329 ± 0.1799	0.1208 ± 0.0494	174 ± 18
	T-L	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0058 ± 0.0033	172 ± 18
	Granulocyte	0.0018 ± 0.0099	0.0095 ± 0.0543	0.0034 ± 0.0209	0.0096 ± 0.0163	175 ± 19
	Monocyte	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0054 ± 0.0025	205 ± 25
G	Mino	0.0346 ± 0.0308	0.0757 ± 0.0713	0.0067 ± 0.0063	0.0427 ± 0.0059	...
	Jurkat	0.0524 ± 0.0238	0.2485 ± 0.1553	0.0567 ± 0.0731	0.0950 ± 0.0372	...
	B-L	0.0065 ± 0.0138	0.0138 ± 0.0281	0.0011 ± 0.0025	0.0289 ± 0.0054	...
	T-L	0.0631 ± 0.0309	0.3233 ± 0.1727	0.1611 ± 0.2082	0.1089 ± 0.0516	...
	Granulocyte	0.0142 ± 0.0356	0.0305 ± 0.0773	0.0034 ± 0.0094	0.0265 ± 0.0107	...
	Monocyte	0.0001 ± 0.0004	0.0004 ± 0.0011	0.0000 ± 0.0000	0.0258 ± 0.0058	...
B	Mino	0.0446 ± 0.0105	0.7478 ± 0.0955	1.1653 ± 0.2633	0.4444 ± 0.0447	...
	Jurkat	0.0579 ± 0.0128	0.7669 ± 0.0802	0.7115 ± 0.2357	0.3387 ± 0.0591	...
	B-L	0.0738 ± 0.0292	0.8336 ± 0.0863	1.6023 ± 0.6183	0.3387 ± 0.0881	...
	T-L	0.0689 ± 0.0257	0.8421 ± 0.0876	1.4367 ± 0.5849	0.3288 ± 0.0792	...
	Granulocyte	0.0646 ± 0.0254	0.8892 ± 0.0905	1.3054 ± 0.5463	0.3117 ± 0.0876	...
	Monocyte	0.0755 ± 0.0214	0.8795 ± 0.0350	1.0215 ± 0.5491	0.2921 ± 0.0913	...

cell area. The relative degree of each cell in each characteristic parameter was compared (Fig. 4). Combined with the characteristic parameters of the cell, R-entropy variance, G-color mean, and cell area were selected to identify hematological lymphoma cells. The following procedure was performed to achieve cell classification. First, we performed the R-channel analysis of cell entropy variance to separate $CD19^+$ cells from negative cells. Second, we classified normal B-lymphocytes and B-lymphoma cells according to the cell area. Based on the G-channel color mean parameters, T-lymphocytes and T-lymphoma cells were apart from granulocytes and monocytes, and then further classified by cell area, and gradually classified into various types of cells (Fig. 5). Finally, the identification of T-lymphocytes, B-lymphocytes, T-lymphoma cells, and B-lymphoma cells was achieved.

F. Analysis of clinical pathological lymphoma cell subtypes in patients

To analyze the effect of microfluidic chip technology on lymphoma cells in patients with lymphoma, the previously set analysis conditions were used to determine leukocytes and lymphoma cells in the blood of volunteers and lymphoma patients. The SSPU was used to classify and count lymphocytes and lymphomas cells and then combined with anti-CD3 and anti-CD19 immune markers to calculate the number of T-lymphocytes and B-lymphocytes in the blood of lymphoma patients as an experimental control group. At the same time, the microarray test conditions were set according to the above experiments to determine the number of normal lymphocytes and lymphoma cells in lymphoma patients, and the detection rate of T-lymphoma cells and/or B-lymphoma cells was calculated. At R-entropy variance, the detection rate of lymphoma cells reached 62.50% and that of B-lymphocytes, T-lymphocyte, granulocytes, and monocytes reached, respectively, 100.00%, 100.00%, 99.18%, and 100.00% in lymphoma patients (Table III). A low detection rate of lymphoma cells in lymphoma patients may be due to large differences in the expression of immune antigens in clinical specimens. At G-color mean, the detection rate of lymphoma cells reached 100.00% and that of B-lymphocytes, T-lymphocyte, granulocytes, and monocytes reached, respectively, 95.00%, 82.29%, 97.54%, and 100.00% in lymphoma patients. At area, the detection rate of lymphoma

FIG. 4. Comparison of energy variance, entropy variance, moment of inertia variance, and color mean in the three channels of RGB for normal leukocytes and lymphoma cells. (a) Using RGB three channels to analyze four characteristic parameters of various types of normal white blood cells and lymphoma cells. (b) Calculate the area of various types of leukocytes and lymphoma cells. p-B-L: pathologic B lymphoma cells (Mino cells); p-T-L: pathologic T lymphoma cells (Jurkat cells); B-L: B lymphocyte; T-L: T lymphocyte; N: neutrophils; M: monocytes.

cells reached 93.75% and that of B-lymphocytes, T-lymphocyte, granulocytes, and monocytes reached, respectively, 85.00%, 68.75%, 74.59%, and 44.44% in lymphoma patients. A low detection rate of T-lymphocyte in lymphoma patients may be due to strong expression of cellular antigens to increase cell area. There is a cross between the granulocyte and monocyte areas, resulting in a low detection rate of both cells. Currently, the FDA-approved hematology analyzer can only differentiate lymphocytes from granulocytes and monocytes, but cannot further identify T-lymphocytes and B-lymphocytes.^{37,38} The experimental results of this study suggest that the microfluidic chip analysis system can classify normal T-lymphocytes, normal B-lymphocytes, pathological T-lymphoma cells, pathological B-lymphoma cells, normal granulocytes, and normal monocyte cells. Thus, the chip can be used to identify subtypes of blood cells in patients with lymphoma, which would be helpful for clinical laboratory tests of lymphoma diseases.

FIG. 5. Detection procedure of blood cell characteristic parameters. Three characteristic parameters of R-entropy variance, G-color mean, and area were selected to identify cells. First, the cells were analyzed by the R-entropy variance in the detection procedure. The cells were divided into two groups. One group identified the p-B-L and B-L with the area parameter, and the other group identified T-L, p-T-L, N and M with G-color mean parameter. Second, T-L and p-T-L are distinguished by area parameter, and M and N are distinguished by area parameter. N: Neutrophils; B-L: B lymphocytes; T-L: T lymphocytes; p-B-L: pathological B lymphoma cells (Mino cells); p-T-L: pathologic T lymphoma cells (Jurkat cells); M: monocytes.

TABLE III. Detection rate of lymphoma cells and normal white blood cells based on the microfluidic chip system.

Characteristic parameters	Cell type	Threshold	Healthy volunteers		Lymphomas	
			Measured value	Detection rate (%)	Measured value	Detection rate (%)
R-entropy variance	Mino	>0.1	0.3062 ± 0.1433	91.30	...	...
	Jurkat	<0.1	0.0505 ± 0.0581	80.80	...	...
	B-lymphoma cells	>0.1	...	...	0.1730 ± 0.1470	62.50
	B-lymphocyte	>0.1	0.4910 ± 0.1728	100.00	0.5790 ± 0.2160	100.00
	T-lymphocyte	<0.1	0.0000 ± 0.0000	100.00	0.0000 ± 0.0000	100.00
	Granulocyte	<0.1	0.0095 ± 0.0543	97.14	0.0011 ± 0.0107	99.18
	Monocyte	<0.1	0.0000 ± 0.0000	100	0.0000 ± 0.0000	100.00
G-color mean	Mino	<0.05	0.0427 ± 0.0059	89.9	...	...
	Jurkat	>0.05	0.0950 ± 0.0372	95.2	...	...
	B-lymphoma cells	<0.05	...	...	0.0270 ± 0.0049	100.00
	B-lymphocyte	<0.05	0.0289 ± 0.0054	100	0.0335 ± 0.0062	95.00
	T-lymphocyte	>0.05	0.1089 ± 0.0516	96.1	0.0994 ± 0.0381	82.29
	Granulocyte	<0.05	0.0265 ± 0.0107	97.14	0.0279 ± 0.0064	97.54
	Monocyte	<0.05	0.0258 ± 0.0058	100	0.0278 ± 0.0059	100.00
Area	Mino	>200	450 ± 103	100	...	...
	Jurkat	>200	435 ± 117	99.2	...	...
	B-lymphoma cells	>200	...	...	264 ± 48	93.75
	B-lymphocyte	<200	174 ± 18	91.9	178 ± 15	85.00
	T-lymphocyte	<200	172 ± 18	92.2	192 ± 28	68.75
	Granulocyte	<190	175 ± 19	80	179 ± 22	74.59
	Monocyte	>190	205 ± 25	81.8	187 ± 17	44.44

IV. CONCLUSIONS

This paper is the first to report a microfluidic chip system that allows for one-step examination of lymphoma cell morphology and cell surface antigens to identify lymphocytes, pathological lymphomas, and lymphoma subtypes. When the flow rate of the microfluid is 300 $\mu\text{l/h}$, fewer than 2 red blood cells are in contact with the surface of the leukocyte membrane. Thus, the approach separates the red blood cells from white blood cells effectively and reduces their influence on the recognition of the white blood cells. In this study, the rate of detection of lymphoma cells in clinically diagnosed lymphoma patients reached 62.50%.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the effect of DAPI staining of blood cells.

ACKNOWLEDGEMENTS

This work was totally supported by the National Natural Science Foundation of China (NNSCF, Grant No. 61703304).

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