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Counting the Cells Based on Their Size Using Fuzzy Logic and the Image J System

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Abstract

Medical diagnosis is the process of identifying the presence of a specific disease. Medical images play an important and major role in helping the doctor and specialist in the process of diagnosing the patient's condition. As a result of the technological development taking place in the fields of artificial intelligence, which processes data quickly and efficiently by responding to various conditions and situations, providing a great deal of flexibility and accuracy, it is widely used in helping to diagnose diseases. Fuzzy logic was used to find the number of cells in medical images, where the increase or decrease in the number of cells beyond the normal limit indicates the presence of a specific disorder or disease.

This paper aims to use fuzzy logic and the Image J system to determine the appropriate mechanism and method for counting cells according to their size in the medical image, where a set of basic steps are implemented on the image by using the Image J system. This includes converting to mask, outlining, filling holes, watershedding, setting measurements, and analyzing particles. After that, using fuzzy logic to determine the best appropriate methods for counting cells in medical images based on size, enter a single random value for size, and cells smaller than that value are ignored. The area and perimeter of cells are accounted for.

Keywords: Artificial Intelligence, Fuzzy Logic, Rules in Fuzzy Logic, Image J System, Red Blood Cells, White Blood Cells, Size of Cells.

عد الخلايا بناءً على حجمها باستعمال المنطق الضبابي ونظام الصورة J

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الخلاصة

التشخيص الطبي هو عملية التعرف على وجود مرض معين، والصور الطبية تلعب دورا مهما ورئيسي في مساعدة الطبيب والمختص في عملية تشخيص حالة المريض. ونتيجة للتطور التكنولوجي الحاصل في مجالات الذكاء الاصطناعي ومعالجته للبيانات والمعلومات بسرعة وكفاءة عالية وكيفية استجابته للظروف المختلفة والمواقف التي تتسم بقدر كبير من المرونة والدقة واستخدامه في المساعدة في تشخيص الامراض. تم استعمال المنطق الضبابي لإيجاد عدد الخلايا في الصور الطبية، حيث تشير الزيادة أو النقص في عدد الخلايا عن الحد الطبيعي الى وجود اضطراب أو حالة مرضية معينة.

يهدف البحث إلى استعمال المنطق الضبابي ونظام الصورة J لتحديد الآلية والأسلوب المناسبين لعد الخلايا حسب حجم الخلايا في الصورة، حيث تنفذ مجموعة من الخطوات الأساسية على الصورة باستعمال نظام الصورة

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ل، والتي تشمل (التحويل إلى قناع، المخطط التفصيلي ، ملء الخلايا ، فصل الخلايا المتداخلة، تعيين القياسات ، تحليل الجزيئات) وبعد ذلك يستعمل المنطق الضبابي لتحديد أفضل الطرق المناسبة لعد الخلايا في الصور الطبية بناءً على الحجم وادخال قيمة عشوائية واحدة تمثل الحجم ويتم تجاهل الخلايا الأصغر من تلك القيمة، كما تم حساب مساحة الخلايا ومحيطها.

1. Introduction

Manual counting of blood cell images becomes difficult when there are a large number of cells in the image. Through this research, we have solved this problem. The process of dealing with blood cells is one of the most important topics because of its great impact on human life. Laboratory tests for disease detection can be done in laboratories, but some of these tests require high costs and a long time to obtain results [1–2]. In this research, image J is used, and it's a modern system for processing various images as it has many capabilities dedicated to solving various problems related to image processing and analysis. Through this system, it is possible to perform many measurements and calculations and to perform cell counting operations easily and with high accuracy [3].

Many studies have appeared about finding the number of cells in medical images, the most important of which are:

- In 2015, Rishi and Narinder Rana [3] presented a study on finding the size and shape of particles using the image j system, and the study was used to separate adjoining cells from each other.
- In 2016, Asmitaba, Gohil, and Dhaval Pipalia [4] presented a study on finding the number of small cells using the MATLAB language.
- In 2019, Davuluri, Suneetha, and D. Rathna Kishore [5] presented a study to find the number of cells to detect cancer.
- In 2020, Tarhini, Ghinwa M., and Reda Shbib [6] presented a study to separate the abnormal cells from the image using MATLAB language.

Through this research, a new algorithm was proposed that linked fuzzy logic and the image j system and was used to find the number of cells in medical images depending on the cell size. Whenever the number of cells increases or decreases from the normal limit, this indicates disease, and some of these diseases are serious [4].

Several processes were performed on the original image that was inserted, and the overlapping and interconnected cells were separated to obtain more accurate results. Using fuzzy logic, the required method was determined, and the cell counting process was executed easily and automatically in several ways, depending on the size of the cells.

2. The Research Methodology

The process of counting blood cells based on their size is one of the important and main topics that helps the doctor and the person concerned diagnose the patient’s condition without making any effort or taking any extra time. This research introduces new methods for counting the cells based on their size using fuzzy logic and the image J system.

2.1 Fuzzy Logic and Image J system

Fuzzy logic is one of the theories by which such systems can be built [7], as it simulates the method that a person follows in decision-making, which depends on taking into account all possible possibilities [8]. It is a technique for defining complex models with simple and understandable fuzzy variables and rules and provides a mathematically accurate method to

manipulate the available information. It uses fact-based rules to determine which action should occur in response to a set of input values. Application of rules is called fuzzy inference. Evaluate each base for its generator input from the fuzzification process [9–10].
There are many modern computer systems used to process medical images, and the Image J system is one of the most important of these systems due to its flexibility in dealing [11], its ability to solve complex problems, and its ability to deal with different types of images and perform analysis and processing on them [12].

2.1.1 Rules in Fuzzy Logic

In this research, the following rules of fuzzy logic are used to determine the cells that can be counted:
Method 1: Count the number of cells, the area, and the perimeter of each cell in the image.
Method 2: If the size of each cell in all images is greater than random number 1, then calculate the number of cells, area, and perimeter.
Method 3: Calculate the number of cells, area, and perimeter of all cells in the image that are not at the edge.
Method4: If the size of each cell in all images without cells in the edge is greater than the random number 1, then calculate (Number of Cells, Area, Perimeter).

2.2 Cells in the Blood

Blood is a type of connective tissue that performs important functions in the human body [13]. It is a fluid that transports essential substances such as nutrients and oxygen to cells and transports metabolic wastes such as carbon dioxide away from those cells themselves. Blood constitutes 8% of the body mass and consists of red blood cells, white blood cells, platelets, and plasma [14]. In this research, the number of cells was found, as the increase or decrease in the number of cells indicates the presence of a specific pathological condition. When the size of the cells increased, this indicated a vitamin B12 deficiency, hypothyroidism, or cancer. When the size of red blood cells decreases, this indicates anemia. An increase in the number of red blood cells beyond the normal limit is a type of leukemia, but in the case of a decrease in the number of red blood cells below the normal limit, it may indicate low rates of iron in the body or the presence of problems with vital organs such as lung, heart, and kidney diseases [1]. An increase in the number of white blood cells beyond the normal limit indicates a problem in the immune system, bone marrow, or a bacterial infection. A decrease in the number of white blood cells below the normal limit indicates the presence of autoimmune disorders or infections with cancer, tuberculosis, or malaria. In these cases, patients should visit their doctor to get a medical examination to diagnose their case and treat them [15].

2.3 Proposed Method

The following steps are used to execute the proposed algorithm:

- Convert to Mask: Creating a binary mask converts an image to black and white [4].
- Outline: Generate a one-pixel-wide outline of foreground cells in a binary image. The line is drawn inside the cell, i.e., on the previous foreground pixels [12].
- Fill holes: This command is used to fill holes (four connected background elements) in cells by filling the background [12].
- Watershed: This command is used when cells in binary images that are overlapping are separated using: Process-Binary-Watershed [6]
- Set measurements: To specify which measurements are recorded, select Analyze-Set Measurements and then Area and Perimeter.

  Area: Area of selection in square pixels [16]

  Perimeter: The length of the outside boundary of the selection.

- Analyze particles: This command counts and measures cells in binary or threshold images. It works by scanning the image or selection until it finds the edge of a cell. It then outlines the cell using the wand tool, measures it using the Measure command, fills it to make it invisible, and resumes scanning until it reaches the end of the image or selection [3]. Particles (cells) outside the range specified in the size field are ignored. Enter a single size value, and particles smaller than that value are ignored. Select outlines from the show popup menu, and Image J will open a window containing numbered outlines of the measured particles (cells) [5].
- Fuzzy logic: It is considered one of the best modern artificial intelligence methods used at the present time [17–19]. It was used in this research to determine the best appropriate methods for counting cells in images. The size of cells in the image is calculated, and you enter a single value in size (a random number), and cells (particles) smaller than that value are ignored in counting.

2.4 Proposed Algorithm

In this research, the following algorithm was proposed for counting cells based on size:

1- Input the original image (an image of blood cells).
2- Duplicate image.
3- Convert the image to binary, then convert to mask.
4- Set the outline of the image.
5- Set fill holes of image.
6- Set the watershed of the image.
7- Set measurements for area and perimeter.
8- Analyze particles.
9- Generate random number 1 (RN1).
10-Calculate R in equation 1, which equals:

$$R = \text{size of cell} / \text{random number1} \quad (1)$$

11-Use fuzzy logic to determine the methods that can be executed:

- Method 1: If R > 0, then calculate (number of cells, area, perimeter): go to 12
- Method 2: If R >= 1, then calculate (number of cells, area, perimeter): go to 12
- Method 3: If R (without cells in the edge of the image) > 0, then calculate (number of cells, area, perimeter): go to 12
- Method4: If R (without cells in the edge of the image) >= 1, then calculate (number of cells, area, perimeter).

12-Find size of cell in equation 2, which equals:

$$\text{Size of cell} = R * \text{random number1} \quad (2)$$

13-Show result images that contain cells counter
14-End

3. Result

In this research, three medical images were selected that contain cells. A set of basic steps are performed on the images to separate the overlapping cells. Four methods were applied to counting cells based on size by using fuzzy logic, as shown in Figures 1, 3, and 5. The values of cells to be counted, area, and perimeter for the four methods that were calculated are shown in Tables 1–3. The histogram of the number of cells to be counted is shown in Figures 2, 4, and 6.

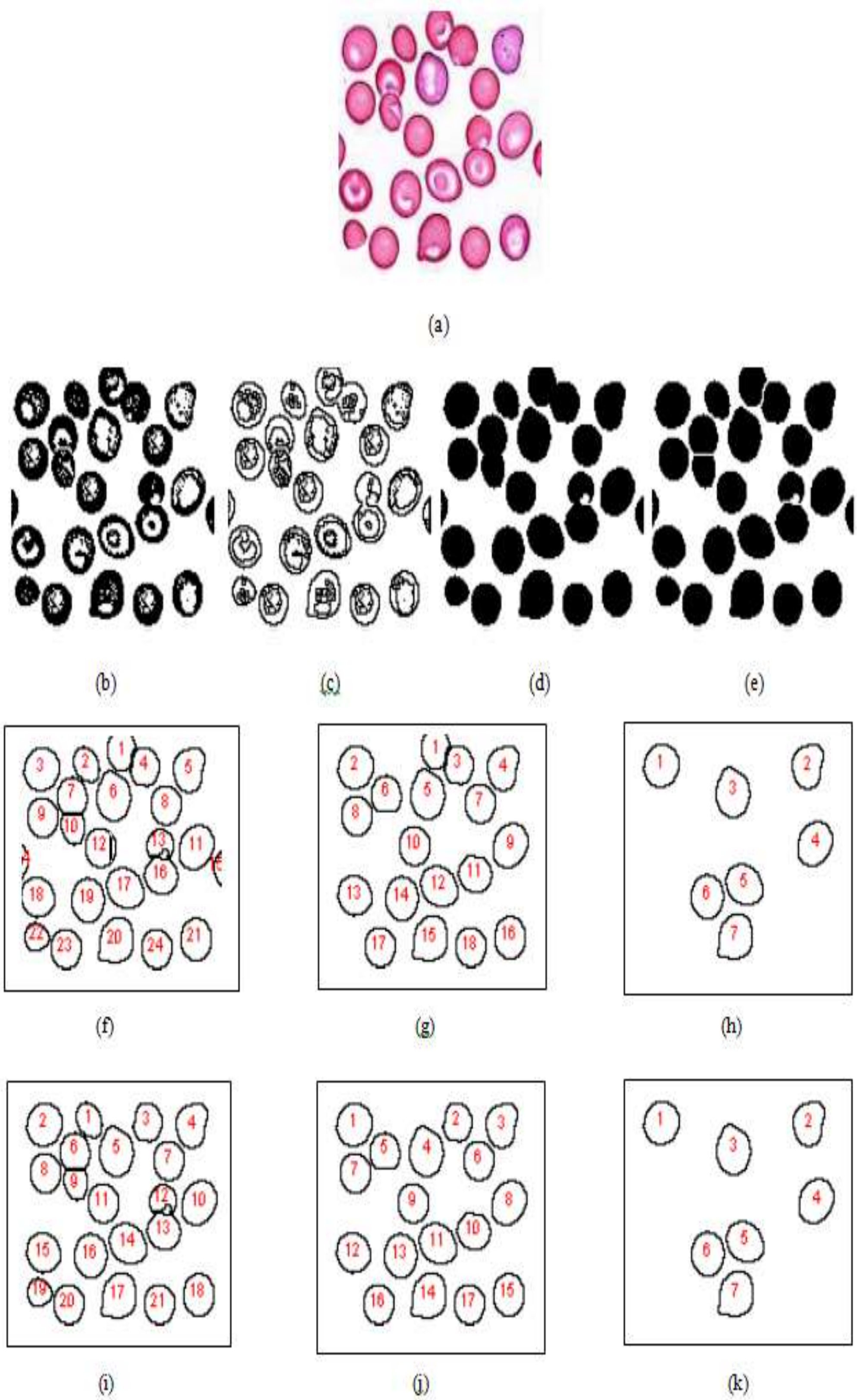

Figure 1: This figure represents (a) Image1 of red blood cells (b) Convert to mask (c) Outline (d) Fill holes (e) Watershed (f) Method 1 No. of Cells=24 (g) Method 2 (If random number1=250) No. of Cells=18 (h) Method 2 (If random number1=330) No. of Cells=7 (i) Method 3 No. of Cells=21 (j) Method 4 (If random number1=250) No. of Cells=17 (k) Method4 (If random number1=330) No. of Cells=7

Table 1: Results of Execution Proposed Algorithm to Image1

Method1			Method 2 If random number1=250			Method 2 If random number1=330			Methods3			Method 4 If random number1=250			Method4 If random number1= 330		
No. of cells	Area	Perimeter	No. of cells	Area	Perimeter	No. of cells	Area	Perimeter	No. of cells	Area	Perimeter	No. of cells	Area	Perimeter	No. of cells	Area	Perimeter
1	258	60.28	1	258	60.284	1	374	71.012	1	216	55.355	1	374	71.012	1	374	71.012
2	216	55.35	2	374	71.012	2	342	69.355	2	374	71.012	2	273	61.355	2	342	69.355
3	374	71.01	3	273	61.355	3	410	75.841	3	273	61.355	3	342	69.355	3	410	75.841
4	273	61.35	4	342	69.355	4	377	71.012	4	342	69.355	4	410	75.841	4	377	71.012
5	342	69.35	5	410	75.841	5	368	71.598	5	410	75.841	5	276	61.113	5	368	71.598
6	410	75.84	6	276	61.113	6	354	69.012	6	276	61.113	6	295	61.941	6	354	69.012
7	276	61.11	7	295	61.941	7	396	74.527	7	295	61.941	7	299	61.941	7	396	74.527
8	295	61.94	8	299	61.941				8	299	61.941	8	377	71.012			
9	299	61.94	9	377	71.012				9	183	51.213	9	297	61.941			
10	183	51.21	10	297	61.941				10	377	71.012	10	301	63.598			
11	377	71.01	11	301	63.598				11	297	61.941	11	368	71.598			
12	297	61.94	12	368	71.598				12	176	56.77	12	328	67.012			
13	176	56.77	13	328	67.012				13	301	63.598	13	354	69.012			
14	61	39.79	14	354	69.012				14	368	71.598	14	396	74.527			
15	61	38.14	15	396	74.527				15	328	67.012	15	325	65.355			
16	301	63.59	16	325	65.355				16	354	69.012	16	295	61.941			
17	368	71.59	17	295	61.941				17	396	74.527	17	295	61.941			
18	328	67.01	18	295	61.941				18	325	65.355						
19	354	69.01							19	164	47.113						
20	396	74.52							20	295	61.941						
21	325	65.35							21	295	61.941						
22	164	47.11															
23	295	61.94															
24	295	61.94															

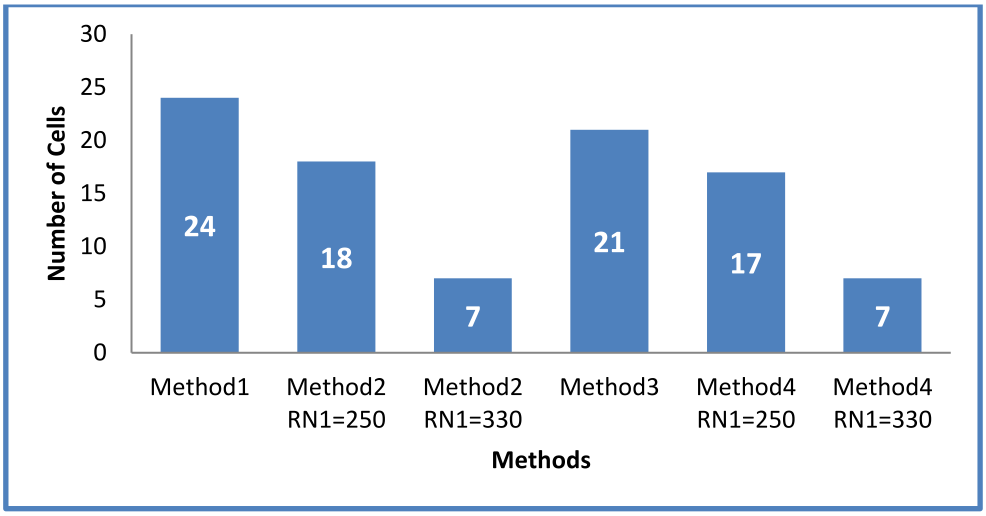

Figure 2: Histogram of the Number of Cells Calculated in Image 1

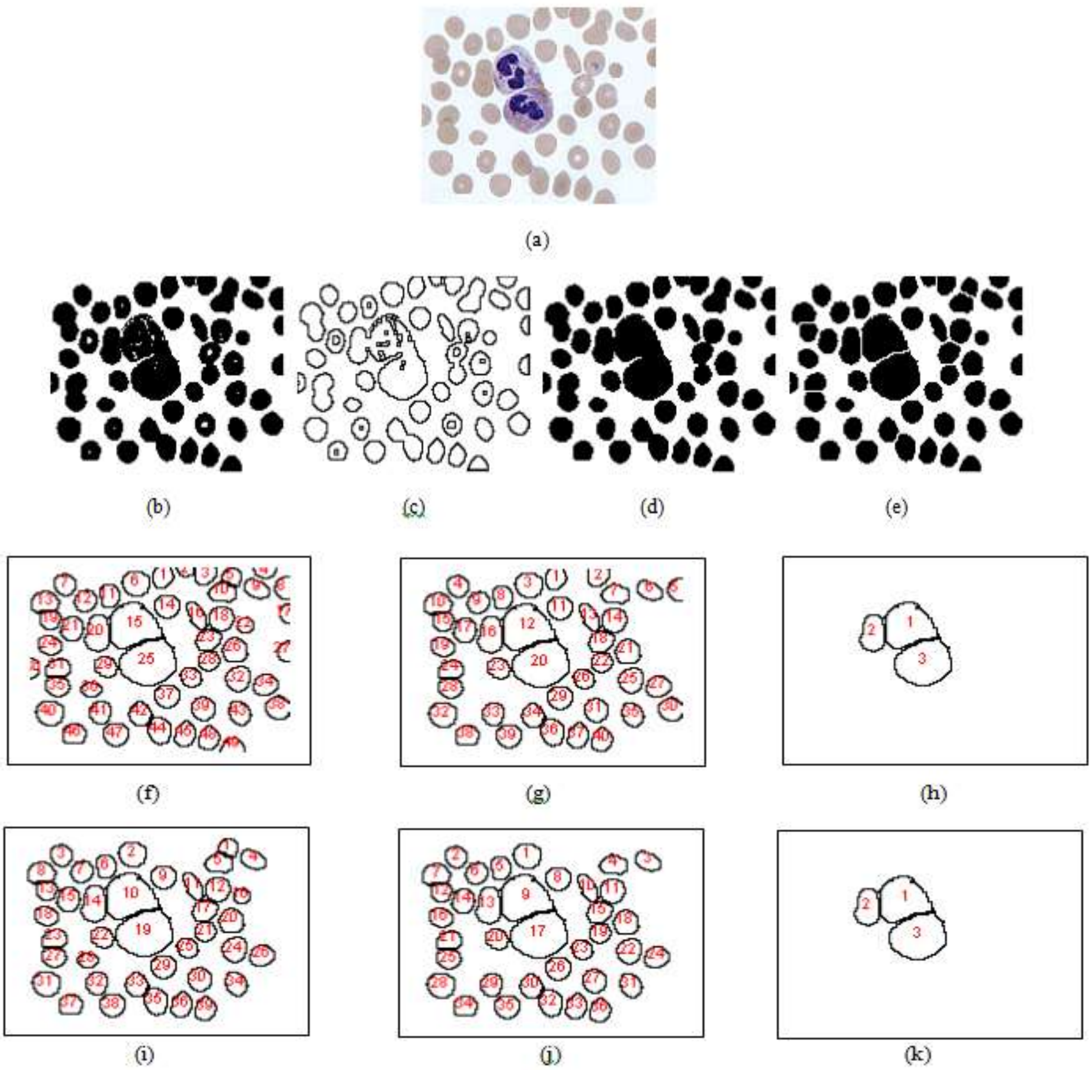

Figure 3: This figure represents (a) Image2 of white blood cells (Neutrophil) (b) Convert to mask (c) Outline (d) Fill holes (e) Watershed (f) Method 1 No. of Cells=49 (g) Method 2 (If random number1=100) No. of Cells=40 (h) Method 2 (If random number1=250) No. of Cells=3 (i) Method 3 No. of Cells=39 (j) Method 4 (If random number1=100) No. of Cells=36 (k) Method 4 (If random number1=250) No. of Cells=3

Table 2: Results of Execution of the Proposed Algorithm on Image 2

Method1			Method2 If random number1=100			Method2 If random number1=250			Methods3			Method4 If random number1=100			Method4 If random number1=250		
No. of cells	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**
1	132	42.62	1	132	42.62	1	606	101.98	1	93	36.04	1	190	50.28	1	606	101.98
2	57	29.31	2	126	41.55	2	252	62.87	2	190	50.28	2	128	40.87	2	252	62.87
3	126	41.55	3	190	50.28	3	701	101.98	3	128	40.87	3	127	42.28	3	701	101.98
4	85	36.14	4	128	40.87				4	127	42.28	4	143	45.69			
5	93	36.04	5	102	38.97				5	143	45.69	5	130	41.79			
6	190	50.28	6	127	42.28				6	130	41.79	6	132	42.87			
7	128	40.87	7	143	45.69				7	132	42.87	7	160	48.28			
8	102	38.97	8	130	41.79				8	160	48.28	8	160	45.45			
9	127	42.28	9	132	42.87				9	160	45.45	9	606	101.9			
10	143	45.69	10	160	48.28				10	606	101.9	10	124	46.52			
11	130	41.79	11	160	45.45				11	124	46.52	11	169	47.45			
12	132	42.87	12	606	101.9				12	169	47.45	12	109	38.38			
13	160	48.28	13	124	46.52				13	109	38.38	13	252	62.87			
14	160	45.45	14	169	47.45				14	252	62.87	14	153	46.28			
15	606	101.9	15	109	38.38				15	153	46.28	15	146	44.87			
16	124	46.52	16	252	62.87				16	69	28.97	16	124	40.04			
17	58	30.14	17	153	46.28				17	146	44.87	17	701	101.9			
18	169	47.45	18	146	44.87				18	124	40.04	18	168	47.69			
19	109	38.38	19	124	40.04				19	701	101.9	19	114	39.21			
20	252	62.87	20	701	101.9				20	168	47.69	20	128	41.45			
21	153	46.28	21	168	47.69				21	114	39.21	21	127	41.79			
22	69	28.97	22	114	39.21				22	128	41.45	22	170	47.69			
23	146	44.87	23	128	41.45				23	127	41.79	23	113	39.45			
24	124	40.04	24	127	41.79				24	170	47.69	24	143	43.45			
25	701	101.9	25	170	47.69				25	113	39.45	25	135	42.62			
26	168	47.69	26	113	39.45				26	143	43.45	26	160	45.45			
27	73	34.14	27	143	43.45				27	135	42.62	27	153	44.87			
28	114	39.21	28	135	42.62				28	82	32.04	28	190	50.28			

29	128	41.45	29	160	45.45				29	160	45.45	29	137	42.62			
30	65	32.97	30	157	45.11				30	153	44.87	30	144	44.52			
31	127	41.79	31	153	44.87				31	190	50.28	31	140	43.69			
32	170	47.69	32	190	50.28				32	137	42.62	32	171	48.28			
33	113	39.45	33	137	42.62				33	144	44.52	33	140	44.28			
34	143	43.45	34	144	44.52				34	140	43.69	34	134	41.79			
35	153	42.62	35	140	43.69				35	171	48.28	35	160	45.45			
36	82	32.04	36	171	48.28				36	140	44.28	36	131	42.28			
37	160	45.45	37	140	44.28				37	134	41.79						
38	157	45.11	38	134	41.79				38	160	45.45						
39	153	44.87	39	160	45.45				39	131	42.28						
40	190	50.28	40	131	42.28												
41	137	42.62															
42	144	44.52															
43	140	43.69															
44	171	48.28															
45	140	44.28															
46	134	41.79															
47	160	45.45															
48	131	42.28															
49	97	38.38															

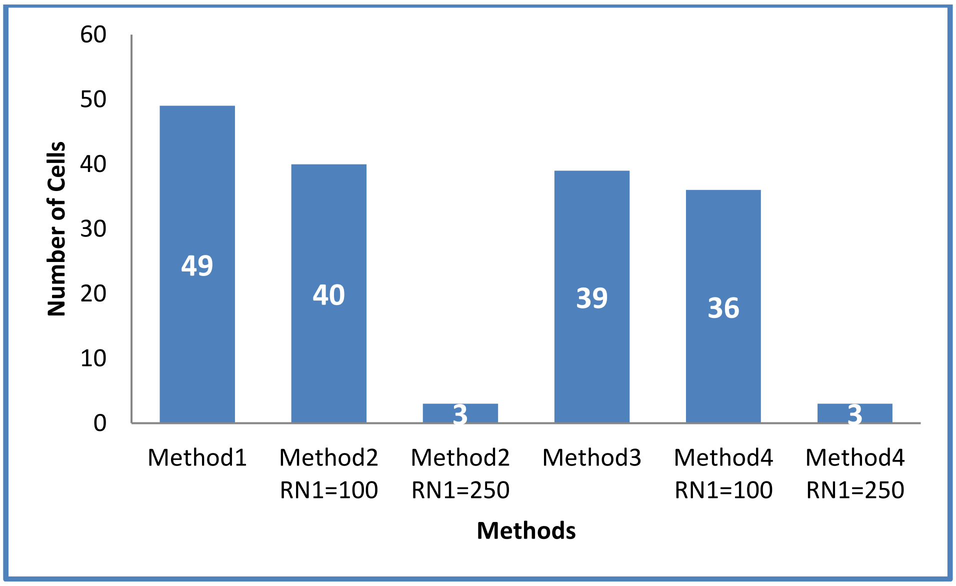

Figure 4: Histogram of the Number of Cells Calculated in Image 2

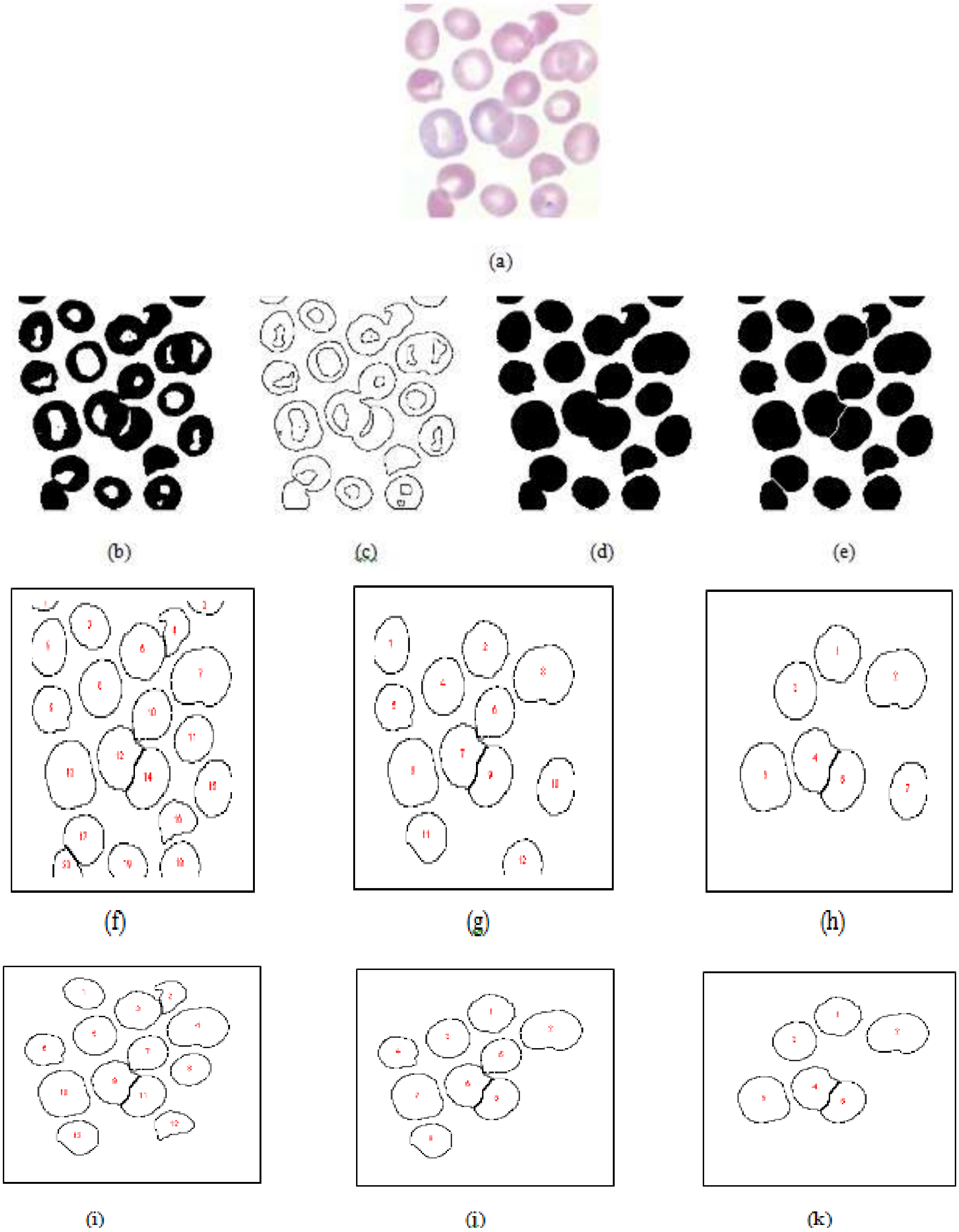

Figure 5: This figure represents (a) Image3 of red blood cells (b) Convert to mask (c) Outline (d) Fill holes (e) Watershed (f) Method 1 No. of Cells=20 (g) Method 2 (If random number1=1400) No. of Cells=12 (h) Method 2 (If random number1=1600) No. of Cells=7 (i) Method 3 No. of Cells=13 (j) Method 4 (If random number1=1400) No. of Cells=9 (k) Method4 (If random number1=1600) No. of Cells=6

Table 3: Results of Execution of the Proposed Algorithm on Image 3

Method1			**Method2 If random number1=1400**			**Method 2 If random number1=1600**			**Methods3**			**Method 4 If random number1=1400**			**Method4 If random numaber1=1600**		
No. of cells	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**	**No. of cells**	**Area**	**Perimeter**
1	272	78.87	1	1571	153.19	1	1918	166.75	1	1338	137.19	1	1918	166.75	1	1918	166.75
2	448	102.18	2	1918	166.75	2	2754	203.82	2	899	129.29	2	2754	203.82	2	2754	203.82
3	1338	137.19	3	2754	203.82	3	1873	161.43	3	1918	166.75	3	1873	161.43	3	1873	161.43
4	899	129.29	4	1873	161.43	4	1987	175.23	4	2754	203.82	4	1410	143.88	4	1987	175.23
5	1571	153.19	5	1410	143.88	5	2653	194.75	5	1873	161.43	5	1578	153.78	5	2653	194.75
6	1918	166.75	6	1578	153.78	6	1805	165.33	6	1410	143.88	6	1987	175.23	6	1805	165.33
7	2754	203.82	7	1987	175.23	7	1646	151.53	7	1578	153.78	7	2653	194.75			
8	1873	161.43	8	2653	194.75				8	1386	138.95	8	1805	165.33			
9	1410	143.88	9	1805	165.33				9	1987	175.23	9	1501	144.02			
10	1578	153.78	10	1646	151.53				10	2653	194.75						
11	1386	139.95	11	144.02	144.02				11	1805	165.33						
12	1987	175.23	12	143.29	143.29				12	1067	132.71						
13	2653	194.75							13	1501	144.02						
14	1805	165.33															
15	1646	151.53															
16	1067	132.71															
17	1501	144.02															
18	1461	143.29															
19	1301	135.78															
20	880	114.32															

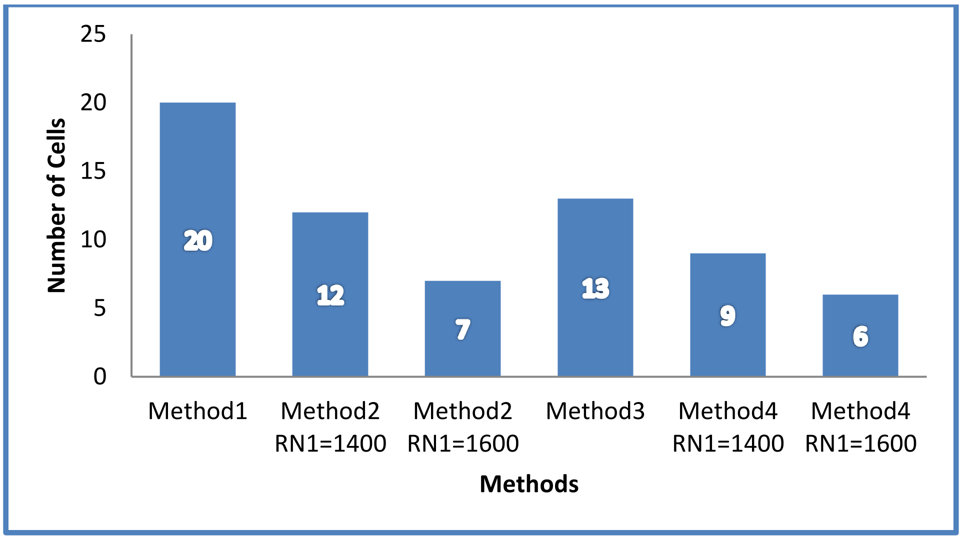

Figure 6: Histogram of the Number of Cells Calculated in Image 3

4. Conclusion and Discussion

Fuzzy logic and the Image J system are used to effectively find the number of cells according to the size of the cells in the image. A set of basic operations were executed using the Image J system to separate neighboring cells and obtain more accurate results. The proposed algorithm is fast in implementation, efficient, and flexible in dealing with various types of medical images. Large or small cells may indicate an underlying problem that requires evaluation and medical intervention. This research, if executed correctly and appropriately on any image of blood cells, helps the doctor concerned diagnose the patient's condition, follow up on the patient's condition, and determine the appropriate medications and doses.

This research is considered less expensive, faster in execution, and more repeatable in cell counting than using other manual cell counting methods.

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