Morphological classification and grading of anemia by using Dielectrophoretic technique

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Abstract— Anemia is a condition in which the number of red blood cells or the concentration of hemoglobin in blood is lower than normal concerning age and gender. According to WHO, 40% of pregnant women and 42% of children are anemic. Anemia is a common blood condition affecting nearly 30% of the global population. India is one of the nations with the highest prevalence of iron deficiency in women. Based on its morphology anemia is classified into three types, Macrocytic, Normocytic, and Microcytic Anemia based on its morphology. Different types of anemia require distinct supplements, indicating the importance of diagnosing the type of anemia. A complete blood count (CBC) performed using a hemolytic analyzer is the most common diagnostic method used in identifying the type of anemia. This standard method requires a laboratory setup with trained technicians. As an alternative method to the existing system, the article focuses on designing a microfluidic channel to screen anemia in an outpatient setup. The previous research article has reported the separation of tumor cells from the whole blood, but only very few attempts were made for the separation of RBC based on size. The implication of the study is to differentiate the separation of microcytic and macrocytic cells with the principle of dielectrophoresis.

Index Terms— complete blood count, Laboratory setup, macrocytic, microcytic, microfluidic channel

I. INTRODUCTION

Anemia is a medical condition in which the number of red blood cells or hemoglobin concentration in the blood is lower than normal for the age and gender. It troubles around 2 billion of the global population which mainly encompasses women and children [1]. The etiology of anemia includes iron deficiency, hemoglobin disorder, unrestricted bleeding, improper supplements, and bone marrow destruction [1]. It is further classified as microcytic, normocytic, and macrocytic anemia based on size. Microcytic anemia (<7μm) is brought on by a diet, low in iron supplements as well as by inflammatory conditions. In Normocytic anemia, the size of RBC remains the same, whereas macrocytic anemia (> 8μm) is brought on by the deficiency in Vitamin B12 and folic acid [3]. For the synthesis of hemoglobin to occur, a sufficient amount of Folic acid and vitamin B12 are also necessary. Vitamin B12 also governs the hematological process and the nervous system's function. [2].

Diagnosing and screening for anemia is essential for the patient suffering from chronic inflammation, hookworm infection, cancer, kidney failure, and cardiac failure [2]. The gold standard method in diagnosing anemia is complete blood count (CBC) performed by the hemolytic analyzer and other prevailing methods include high-performance liquid chromatography, flow cytometer, levels of transferrin and serum ferritin, mean co count (CBC) performed by the hemolytic analyzer and other prevailing methods include high-performance liquid chromatography, flow cytometer, levels of transferrin and serum ferritin, mean corpuscular hemoglobin (Hb) level [1]. This procedure is expensive, labor intensive, requires a large number of samples, and normally produces results slowly [1].

Recent developments in microfluidic technology have increased interest in fluid manipulation and cell sorting [4]. These advancements include durability, mobility, cost-effectiveness, and precise fluid flow control [5]. Microfluidic separation is of two types active and passive. Active separation depends on an external field while passive separation solely depends on channel geometry and hydrodynamic force. The former is further classified into Dielectrophoresis (DEP), optical phoresis, magnetophoresis, and acoustophoresis. The latter is classified as inertia and dean flow fractionation, deterministic lateral displacement (DLD), and hydrodynamic flow focusing given its simplicity of use and low cost of fabrication, active separation is preferable to passive for the separation of blood plasma.

One of the most prominent active methods is DEP since it operates a single cell or a group of cells with high precision and efficiency. Highly pure segregation of the cells with similar sizes is possible because of the fact that DEP separation depends on the size and dielectric characteristics. It is widely utilized in a variety of fields due to its benefits of being label-free, low-cost, simple to control, and non-contact [6]. DEP is separated into positive and negative types; positive DEP is defined as when a particle's dielectric power is directed toward an area with a comparatively high electric field and the particle moves in that direction. Negative DEP is used if it is completely opposite [7].

Maria E. P. Emmerich et al suggested employing DEP to separate leucocyte types, bacterial cells, DNA, and unusual cells. Because DEP isolation requires no labeling or preparation [8]. But there was no evidence for the differentiation of distinct RBC sizes. Yanjuan wang et al devised a technique for cell separation that combines DEP and DLD. The two stages are distinct from one another because DEP demands a low flow rate that does not correspond to the DLD separation [6]. S Hussain et al state that microscopic examination of smears is vital as the cell analyzer cannot identify morphological parameters. Only RBCs with a volume between 36 and 360fl are recognized as RBCs by the analyzer RBCs with a volume between 24 and 6fl are still not counted as RBCs and are not taken into account by the counter [14].

Nur Tantiyani and Lee suggested, in DEP, a consistent electric field was used to separate platelets, which may be done with a small sample size and at a low cost. The DEP force was deemed optimal for the evaluated values at d=2.0 m and E=5 V/mm but as the electric field, which correlates to particle diameter, increased, so did the velocity. Additionally, there are errors that range from 3 to 40% for specific particle sizes [9]. According to Shailee Mitra et al studied that drag, Brownian, and DEP forces all affect DEP. In their study, RBC diameters of 3, 5, and 8 μm have been employed along with three different voltages of 3, 5, and 7V. The computational findings demonstrate that the optimum separation efficiency occurs when RBCs with a diameter of 5μm are used. However, the DEP forces led the blood to be

lodged in the channel when the particle size was raised to 8μm, making it a challenge to extract the RBC that is larger than typical[10].

Yanfang Guan et al designed zig-zag micro-channels that are preferable to the straight channel, and efficiency at 20V was about 99.4%. Due to the completeness and smoothness of the unique zigzag-shaped structure, which prevented potential loss of particles entrapped in the electrode slot. The main benefits of zigzag microchannels include better separations caused by larger corner angles and the reduction of space [11]. Yaolong Zhang et al carried out the separation of RBCs, spheroid WBCs, and platelets, the channel's whole width is covered by electrodes, and no force is applied to the particles. It is necessary for the conductivity of the fluid medium to be at least 5 ms/m or larger. Varying the voltages allowed the single shell sphere models to separate the three particles, but there was no conclusive evidence to support the separation of the types of RBCs and WBCs [7].

M Rahmanian et al designed an array of micropillars for isolating tumor cells in the blood and giving prognostic data and tracking the effectiveness of their treatments. Despite the fact that numerous geometries have been employed in research for CTC capture, there is no thorough comparison of micropillar geometry [12]. S C Saha et al proposed that using fluid dynamics computation, spiral geometries with various cross-sections were tested to separate two particles, such as RBC and WBC. The microchannel of 135-micrometer height was employed. When the inlet velocity was high (0.5-0.55m/s) particle concentration was successful. However, when the inlet velocity was dropped from 0.25 to 0.35m/s, it produced a spaced-out balance and made particle separation problematic [13]. R G mannino suggested that the anemia diagnosis has firm accuracy, where this ailment is screened rather than diagnosed, and less concern in setting up for smartphone detection [14].

There were very few studies stating the separation of different sizes of RBC, which plays a major role in identifying the type of Anemia. The commonly used channels employ a single geometrical structure either in a straight or zigzag channel, but geometry obtained by incorporating different structures (a hybrid model) gives results with better accuracy in the separation of microcytic and macrocytic RBCs. This separation of cells helps us in determining the type of anemia after reaction with 3,3',5,5' tetra methyl benzidine [16]. The aim and Objective of the proposed method are given below:

- To determine the types of anemia based on their size by using dielectrophoresis.
- To analyze the severity of anemia by using color scale grading
- To perform early diagnosis and screening of anemia in rural populations as it can be performed in an outpatient setup.

II. MATERIALS AND METHOD

The simulation model is analyzed using the COMSOL Multiphysics 5.5 platform, which provides a user interface to segregate cells. The principle of Dielectrophoresis is employed where the cells are subjected to a non-uniform electric field and the separation of two different sizes of particles (5 and 10 micrometers) is achieved. The optimal channel design for microcytic and macrocyte RBC separation is examined and the comparison was made between the channel with and without DEP strength. The setup of the electrode is crucial in carrying out the separation process, here the electrodes are arranged above and below the channel which helps in achieving the result with better accuracy.

A. Algorithm

1. Open the application and add the required physics for the separation of cells, like electric current (for separation of cells),

creeping flow (for the flow of the liquid), particle tracing for fluid flow (fpt) for specifying the properties of the particle which is under separation)

2. The graphical window opens and the desired channel geometry with the required inlet and outlet, is designed using the model builder.
3. The suitable material for the channel is added from the material library.
4. In creeping flow physics, the entire channel is selected as wall 1 except the inlet and outlet as they are chosen individually.
5. In electric current physics, alternating potential is assigned for the electrodes and the rest of the channel is electrically insulated. The potential value is user-defined.
6. In fpt physics, the properties of the particle and the forces acting upon it are defined. The inlet where the particle is to be driven is defined by the user.
7. The element size for the mesh is selected from extremely coarse to fine.
8. The results are computed based on three different conditions.
   i. The potential along the channel is computed.
   ii. The movement of the particle when no DEP force acts on the channel.
   iii. The movement of the particle when DEP force acts on the channel.
9. The output can be represented in animated form and can be plotted in the graph.

B. Geometry

The whole channel is designed with a width of 560 μm, where seven electrodes of positive and negative are placed, ensuring the uneven distribution of the electric field. The electrode is positioned over and under the channel. The dimensions of the inlets are 40 μm in width. The outlets are the mirror of the inlets. Fillet points are selected within the inner boundary of the channel, to eliminate the sharp edges so that the cells do not get disrupted.

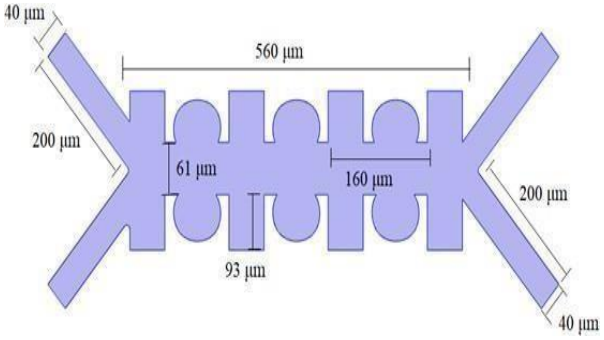


Figure 1: Geomentry of the Channel

Table 1: description of channel design

Design description	**Width in Micrometer**	**Height in micrometer**
Channel	560	61
Inlet	40	200
Outlet	40	200
Electrode (rectangle)	56.5	93

C. Flowchart

Blood is allowed to pass through one of the inlets and Saline is given to the other inlet of the channel to ensure proper flow of blood. The array of electrodes supplies the required potential along the channel resulting in the appropriate cell separation. According to the size, the cells are collected in different outlets.

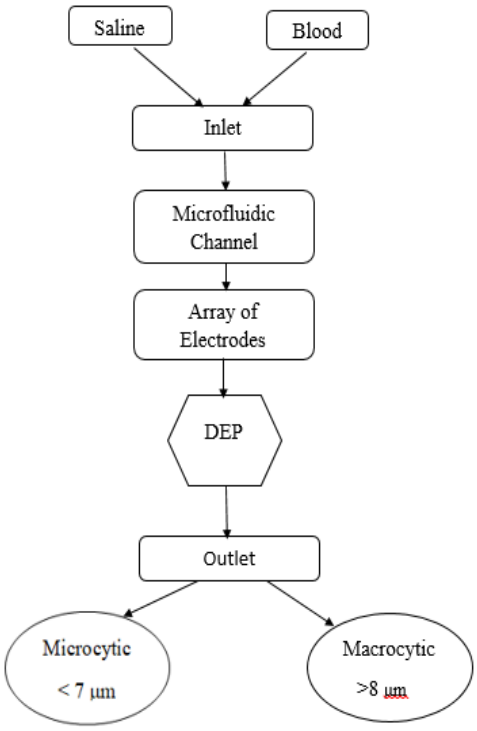


Figure 2: Flow chart

The electrodes of the channel have potential as +5V and -5V, the diameter of particle 1 and particle 2 has values as 5 and 10 µm, and their density is about 1080 kg/m^3.

Table 2: Particle properties

Parameters	**Values**
Positive potential	5V
Negative potential	-5V
Particle 1 diameter	5µm
Particle 2 diameter	10µm
Particle density	1080 kg/m^3
Relative permittivity	50
Electrical conductivity	0.31 S/m
Shell thickness	9 Nm
Shell conductivity	1 e^{-6} S/m

II. RESULTS AND DISCUSSION

A. Study 1

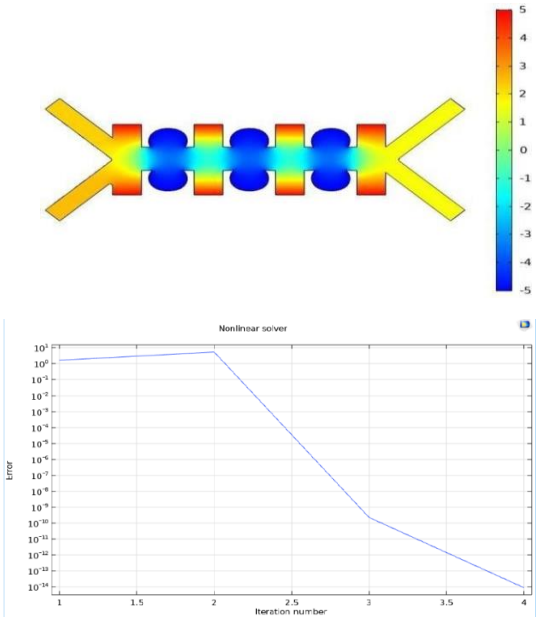


Figure 3: Potential distribution of the channel

The potential acting along the channel, inclusive of the inlet and outlet, is represented in figure 3, the rectangular electrode supplies the positive potential of 5V, and the semi-circle electrode that is alternatively placed between the rectangular electrode supplies the negative potential of - 5V, which is placed at a distance of 80 micrometers. This arrangement gives the required potential for the proper flow of the cells without getting trapped in the channel. The inlets and the outlets are at an angle of 45°, and a small range of positive potential resides here. The potential distribution is not uniform across the channel, and the given color scale represents the value of potential. The convergence plot shows the relation between the iteration number and the error.

B. Study 2

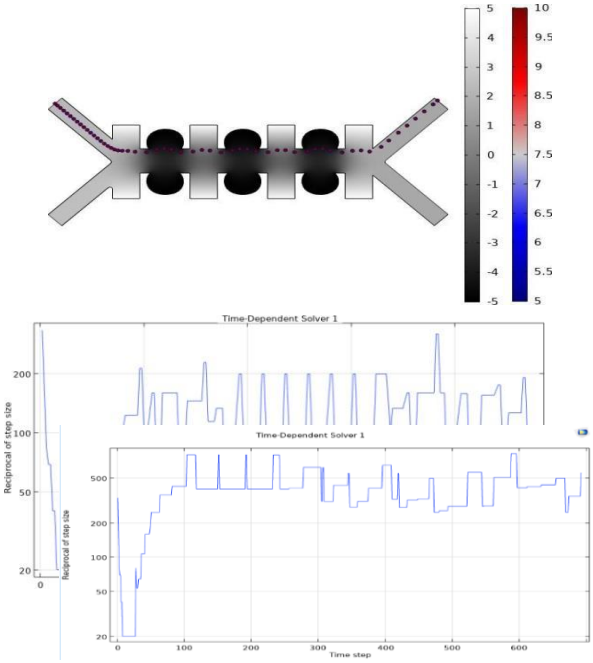
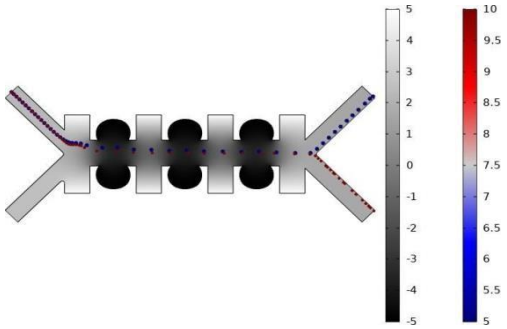


Figure 4: Result obtained when no DEPforce acts along the Channel

The study involves the movement of particles when no DEP acts upon the cells. The particles entering the inlet are ejected via a single outlet, as shown in Figure 4. When no DEP force comes into action, the flow is generally governed by forces like drag force, Brownian force, and buoyancy force. The time-dependent solver graph is plotted between the time step and the Reciprocal of the step size.



C. Study 3

Figure 5: Result obtained when DEP forces acts on the channel

In addition to the force mentioned in study 2, the DEP force acts on the cell and results in separation based on the size, as shown in figure 5. The blue and red colored particle has it ranges from 4 μm and 10 μm respectively. The time-dependent solver graph is plotted between the time step and the reciprocal of step size.

Figure 6: 3D printing of the designed channel using Polylactic Acid (PLA)

D. Color Reaction

In general, the hemoglobin level of males ranges from 13.2 to 16.6 grams per deciliter, and for females, it ranges from 11.6 to 15 grams per deciliter, According to the reduction of hemoglobin count, the severity of anemia varies from mild, moderate, and severe. The severity range of anemia in males and females is represented in Figures 6 and 7 respectively

Figure 7: Hbg range for men

Figure 8: Hbg range for women

After the separation of the microcytic and macrocytic cells through the channel, the cells reaching either of the outlets are allowed to react with the combination of hydrogen peroxide and 3,3', 5,5' – tetra methyl benzidine. This response helps us to identify

the severity of anemia based on the resulting color shift.

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Figure 9: Hgb levels

When the hemoglobin is in the range of 7- 10g/dl, it results in a bluish-green color, if it is in the range of 10-12g/dl, the resulting color is yellowish-orange and we obtain dark shades of red, the range of 10-12g/dl. When the hemoglobin is in the range of 10- 12g/dl the subject suffers from mild anemia and dietary changes can help in recovery, when it is below 10g/dl but not greater than 8g/dl then we term it as moderate anemia and right medications are given, in rare cases when the persons hb level is less than 8g/dl then the physicians suggest blood transfusions for those subjects. When fabricated as an instrument, the designed channel, combined with the chemical reaction, can be used in screening the types and severity of Anemia.

This instrument is user-friendly and provides a better Point of Care (POC). It is portable and can be used in places, where no sufficient laboratory setup is available. As this channel is designed on a micro-scale a minimum sample of blood is required, thereby reducing discomfort to the patients. This screening procedure is comparatively faster than CBC and is less expensive.

III. LIMITATIONS AND RECOMMENDATIONS

The idea is impactful and the fabrication of the design requires a skilled MEMS (Micro electro mechanical system) specialist and a laboratory setup with fine-tuned equipment. The main concept behind the separation is dielectrophoresis, where the size, shape, and number of electrodes play a crucial role, so they must be manufactured with great precision. The instrument will be recommended in rural regions where there are fewer lab facilities.

Mass screening can be carried out with the aid of the instrument. It is appropriate to lay down the sensitivity and specificity of the instrument.

V. CONCLUSION

This article provides a detailed idea on the This article details the morphological separation of RBC with the aid of the dielectrophoretic force. The microfluidic channel designed with the stained chemical reaction should be used as a first step in detecting anemia based on hemoglobin concentration. It also reduces patient discomfort with minimum blood collection for testing. At present, this is a rough method, which can be normalized by carrying out other research studies.

REFERENCES

[1] An, R., Huang, Y., Man, Y., Valentine, R. W., Kucukal, E., Goreke, U., & Gurkan,U. A. (2021). Emerging point-of-care technologies for anemia detection. *Lab on a Chip*, *21*(10), 1843-1865

[2] Wiciński, M., Liczner, G., Cadelski, K., Kołnierzak, T., Nowaczewska, M., & Malinowski, B. (2020). Anemia of Chronic Diseases: Wider Diagnostics—Better Treatment?. *Nutrients*, *12*(6), 1784.

[3] Teixeira, A. M., Macedo, B., Fontes, C. P., & Manuel, M. (2022). Macrocytic Anaemia: Not Always a Straightforward Diagnosis. *Cureus*, *14*(3).

[4] Saha, S. C., Francis, I., & Nassir, T. (2022). Computational Inertial Microfluidics: Optimal Design for Particle Separation. *Fluids*, *7*(9), 308.

[5] Maurya, A., Murallidharan, J. S., Sharma, A., & Agarwal, A. (2022). Microfluidics geometries involved in effective blood plasma separation. Microfluidics and Nanofluidics, 26(10), 1-38.

[6] Wang, Y., Wang, C., Zhang, Y., Han, M., Li, Y., Jin, S., & He, H. (2021). A Two-Stage Separation of Circulating Tumor Cells Based

on Deterministic Lateral Displacement and Dielectrophoresis Techniques. IEEE Access, 9, 143847-143859.

[7] Zhang, Y., & Chen, X. (2020). Blood cells separation microfluidic chip based on dielectrophoretic force. Journal of the Brazilian Society of Mechanical Sciences and Engineering,42(4), 1-11.

[8] Emmerich, M. E., Sinnigen, A. S., Neubauer, P., & Birkholz, M. (2022). Dielectrophoretic separation of blood cells. Biomedical Microdevices, 24(3), 30.

[9] Mitra, S., Rahman, M. H., Prince, H. A., & Rozin, E. H. (2020, June). Numerical Investigation on Dielectrophoresis Blood Cell Separation for Different Applied Voltage and Red Blood Cell Size. In 2020 IEEE Region 10 Symposium (TENSYMP)(pp. 730-733). IEEE.

[10] Guan, Y., Liu, Y., Lei, H., Liu, S., Xu, F., Meng, X., ... & Yang, G. (2020).Dielectrophoresis separation of platelets using a novel zigzag microchannel. Micromachines, 11(10), 890.

[11] Rahmanian, M., Hematabad, O. S., Askari, E., Shokati, F., Bakhshi, A., Moghadam, S., ... & Majidzadeh-A, K. (2022). A micropillar array-based microfluidic chip for label-free separation of circulating tumor cells: The best micropillar geometry?. Journal of Advanced Research.

[12] Saha, S. C., Francis, I., & Nassir, T. (2022). Computational Inertial Microfluidics: Optimal Design for Particle Separation. Fluids, 7(9), 308.

[13] Mannino, R. G., Myers, D. R., Tyburski, E. A., Caruso, C., Boudreaux, J., Leong, T., ... &Lam,W. A. (2018). Smartphone app for non-invasive detection of anemia using only patient- sourced photos. Nature communications, 9(1), 1-10.

[14] Hussain, S., & Frayez, M. (2022). Correlation of Automated cell counters RBC Histogram and Peripheral smear in Anemias. Indian Journal of Public Health Research & Development, 13(4), 234-237.

[15] Tyburski, E. A., Gillespie, S. E., Stoy, W. A., Mannino, R. G., Weiss, A. J., Siu, A. F., ... & Lam, W. A. (2014). Disposable platform provides visual and color-based point-of-care anemia self-testing. The Journal of clinical investigation, 124(10), 4387-4394.