A SIMPLE IMAGING METHOD FOR DEMONSTRATING RED CELL SIZES TO LIFE SCIENCES STUDENTS.

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ABSTRACT

INTRODUCTION:

The red cell sizes are described in the scale of micrometers. For a novice it is difficult to imagine the cell size. Measuring cell size requires the use of advanced instruments which mandates use of trained personnel.

Objective:

To measure the red cell size using a custom built microscope camera and computer based image analyzing software.

Methods:

Sixty peripheral blood smears were photographed using a microscope camera. The images were stored in a computer. The retrieved images were measured offline using the software. Two independent observers recorded the red cell sizes on both randomly selected and tagged red cells of each slide.

Results:

The mean red cell width of the tagged red cells as measured by the first and second observer was 7.70-7.82 (±0.61µ) and 7.71-7.76 (±0.61µ) respectively. The measured red cell width ranges between 7.49-7.67 (±0.54µ) and 7.34-7.44 (±0.60µ) in untagged red cells for both the observers. The intraclass correlation coefficient for inter rater & intra rater reliability is 0.956 (observer 1) and 0.944 (observer 2) for tagged red cell width and 0.723 (observer 1) and 0.730 (observer 2) for untagged red cell width.

Conclusions:

Computer based image analysis method to determine red cell size, provides an accurate and reliable measurement, which is simple and cost effective. This method can be used to demonstrate measurement of red cell width to life-sciences students in the setting of hematology practical.

Keywords: Red cell width, Image analysis, Microscopic measurement, Red blood cell.

INTRODUCTION:

The need for an accurate measurement of cell size is tremendous. A direct relationship between cell size and metabolic rate of the cells and tissues as a whole is known to exist[1]. Measuring cell size helps in determining the differences between cell capabilities of different species. And also the functional capabilities of different cells and tissues within the same organism[2].

Various techniques have been described to determine the cell size of which planimetry, flexible microprocessor system and spectral analysis are important[3]. All these methods have certain drawbacks. It needs extensive tissue processing and use of sophisticated equipment[4,5]. The methods are also time consuming and need trained personnel[6,7]. In the present study our objective was to describe a simple method for capturing a red cell image and a computer based manual analysis to measure the size of red blood cells has been described. This method can be used to measure any other structure that needs quantification. The image capture and analysis method described can be used for classroom teaching as well, especially within the medical colleges.

Materials and Methods:

Sixty first year MBBS students of the St John's medical college, Bangalore, Karnataka, India, were asked to prepare three peripheral smears using their own blood under standard conditions and closed observation. These smears were fixed and stained using Leishman's stain. One slide out of three that had the best monolayer, as determined by observing under the microscope by the observer was picked and tagged for identification. The tagged slides were focused to an area with monolayer of RBC's under oil immersion magnification and images were captured using a digital camera attached to the eye piece of a compound microscope with an image

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resolution of 1.3 mega pixel. The images were stored in the computer online. These images were modified using windows photoshop 7.0 to obtain uniformity in size and resolution. All the images were scaled to a size of 800X600 dpi and saved as a .gif image before actual determination of red cell size. The stored images were then analyzed using java image analyzer software offline (figure 1). The software has an option of drawing a line from one point to another point and then it measures the distance between two points and gives the value in pixels. The most spherical RBC was taken for measurement. Two sets of RBC measurement were done. The first set included measuring the same tagged RBC image by two different observers. In the second set of measurements the observers made measurements on randomly chosen RBC separately. However while doing a random selection, the observer had to match the red cell against standard criteria to ensure optimal selection. Both the sets of measurements were repeated by each observer on three different days. Over all three readings of the tagged red cell and three readings of the untagged red cell was taken by both observers at different times and on different days. Standard criteria were used to tag a red cell and subsequently measure the red cell diameter (see Table 1).

Calibration and Scale:

In order to establish a scale for the image analysis we captured an image of the smallest square grid seen on the RBC counting field on a modified Neubauer's chamber was captured. This image was captured at a high power magnification. However in view of the blood smear images being taken under oil immersion magnification, a necessary manual correction factor was calculated before we devised the scale for red cell size measurement was devised. The correction factor was given by a magnification factor of 2.5 times. (high power is 400X and oil immersion is 1000X). All the above images were again captured and processed at similar settings for resolution and formatting.

RBC cell size Calculation:

The RBC cell width was calculated by:

RBC diameter in the image is A pixels (oil Immersion)

Width of the RBC counting chamber is B Pixels (high power)

Therefore diameter of the RBC counting chamber is B*2.5 (oil Immersion)

We know that the width of RBC counting chamber is 50 µ (microns)

B*2.5----------50 µ

A----------?

Width of RBC is = $\frac{A*50}{(B*2.5)}$ µ

Statistical Analyses

Data was analyzed using SPSS version 17. All the summary measures are reported as mean ± SD. Intraclass correlation coefficient (ICC) was used to analyze the inter rater & intra rater reliability of tagged & untagged red cell width measurement. An Intraclass Correlation Coefficient of >0.7 was considered to indicate good reliability. With this analysis an attempt was made to establish reliability and reproducibility.

Results:

Tables 2 & Table 3 summarize the Mean ± SD width of the 60 tagged red cells and 60 untagged red cells by two observers for three different readings respectively. For the diameter of the red cell which was tagged (Table 4), ICC as a measure of inter rater agreement for Reading 1,2 and 3 respectively, which were highly reliable. Also Intra rater agreement for tagged red cell width the ICC between the reading 1, 2 & 3 by the first observer was 0.956 & second observer was 0.944 (Table 5). The width of the untagged red cell, ICC as a measure of inter-rater agreement ranged between (0.538-0.640) for Reading 1, Reading 2 & Reading 3 which was moderately reliable (Table 6). The Intra rater agreement (ICC) for untagged red cell width between the reading 1, 2 & 3 by observe 1 & 2 respectively shown (Table 7). The diameter of the untagged red cell, ICC was lower compared to width of the tagged red cell.

Discussion:

The above result shows that the measurements made on the tagged red cells by different observers and at different time, agreement scale is higher (0.956, 0.954 respectively). But with respect to untagged red cell the agreement was moderate (0.64, 0.53) (reading 2 and 3), but significant with respect to same observer readings on untagged red cells (0.72, 0.73 respectively). All other techniques of measuring red cells are tedious and require expensive equipments and maintenance. This technique is fairly simple and cost effective. It can be performed without any formal training [Reproducibility, 0.956 (CI 0.934,0.972), 0.954 (0.915,0.964)]. This technique is ideal for demonstration to students of life sciences about

the measurement of all blood cells, Pathological and Microbiological structures. These measurements will help in easy identification and diagnosis of various diseases. The small difference between measured values and the reference values may be because of human error in placing the pointer at the exact edge of the red cell (Random error), may be also because the image of the Neubauer's counting chamber is taken in low power and calibrated to oil immersion image and the images of red cell are taken in oil immersion (Systematic error).

The image analysis in pathology is viewed as an ancillary method meant to provide objective support in the resolution of difficult problems[8]. Empirical observations and recent researches showed that the basal metabolic rate of all organisms is allometrically scaled by 2/3 (Rubner Law), or 3/4 (Keibler law)[9]. This technique can be employed for classification of aneamias in rural set-up.

Figure 1: Red cell image with Java image analyzer software

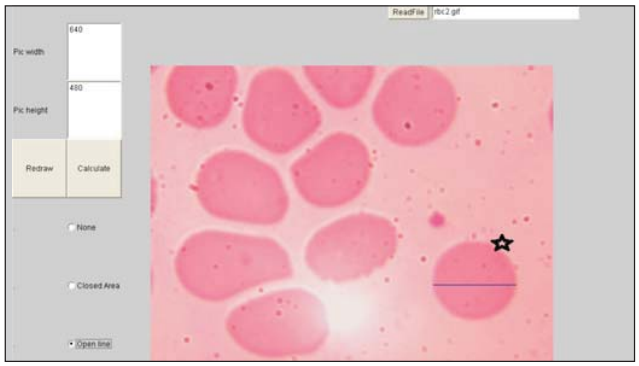

Table 1: Criteria for selection of Red cell and measuring technique

Parameter	Criteria
Selection of red cells for measurement	a) Smear should be evenly spread, with monolayer b) Average size of the red cell should be chosen c) Red cell should be perfectly spherical, borders should be seen clearly
Measuring Axis	Horizontal
Measuring distance	Shortest distance between opposite points

Table 2: Descriptive statistics of tagged red cell width (μ)

Tagged red cell width	Observer1 Mean ± SD	Observer2 Mean ± SD
Reading 1	7.701(±0.611)	7.76(±0.614)
Reading 2	7.81(±0.604)	7.68(±0.595)
Reading 3	7.82(±0.610)	7.711(±0.584)

Table 3: Descriptive statistics of untagged red cell width (μ)

Untagged red cell width	Observer1 Mean ± SD	Observer2 Mean ± SD
Reading 1	7.495(±0.542)	7.34(±0.607)
Reading 2	7.52(±0.594)	7.44(±0.614)
Reading 3	7.67 (±0.615)	7.352(±0.555)

Table 4: Inter rater reliability showing tagged red cell width reading by two observers

Tagged red cell width	Intraclass Correlation Coefficient Observer1 vs Observer2	Confidence interval (CI) (95%)
Reading 1	0.889	(0.953,0.983)
Reading 2	0.939	(0.90, 0.963)
Reading 3	0.971	(0.953,0.983)

Table 5: Intra rater reliability for tagged red cell width

Reading 1, 2, 3	Intraclass Correlation Coefficient(ICC)	
	Observer1	Observer2
Intra rater Reliability	0.956	0.944
CI (95%)	(0.934,0.922)	(0.925, 0.964)

Table 6: Inter rater reliability showing untagged red cell reading by two observers

Untagged red cell width	Intraclass Correlation Coefficient Observer1 vs Observer2	Confidence interval (CI) (95%)
Reading 1	0.606	(0.340,0.764)
Reading 2	0.640	(0.397,0.785)
Reading 3	0.538	(0.227,0.724)

Table 7: Intra rater reliability for untagged red cell width

Reading 1, 2, 3	Intraclass Correlation Coefficient(ICC)	
	Observer1	Observer2
Intra rater Reliability	0.723	0.730
CI (95%)	(0.575,0.826)	(0.585,0.830)

Conclusions:

Computer based image analysis method to determine red cell size provides an accurate and reliable measurement, which is simple and cost effective.

Limitations of the Study:

1. Observation variations by the examiner.
2. Instrumentation and Magnification errors.
3. Standard values for this type of technique for such studies are not available.

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