wjpmr, 2021, 7(12), 165-169

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

MANUAL CALIBRATIONS OF LENGTH OF COMET TAIL BY USING MICROMETRY

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Article Received on 21/09/2021 **Article Revised on 11/10/2021** **Article Accepted on 01/11/2021**

ABSTRACT

This study was aimed to calibrate one division of the ocular meter by using stage meter with 4X, 10X and 40X objectives of microscope and also to find out actual size of comet tail in manual mode. In 4X, the 1000 micrometer ruler of stage meter coincide with 40 divisions ruler of the ocular meter in which 25µm length was one division of oculometer. The comet tail length in 4X objective lens was 25 micrometer. Similarly, for 10 X objective eyepiece lens, 1000 µm ruler of stage micrometer coincide with entire 10 divisions ruler of ocular meter for which 10 µm was one ocular division. The extension of comet tail in 10X objective was 50 µm. In 40X objective lens 100 µm of stage micrometer coincide with 40 divisions of ocular meter, with distance of 2.5 micron in one ocular division. The tail length in 40X objective was 125 µm.

KEYWORDS: Compound Microscope (CM), Calibration, Stage meter (SM), Ocular meter (OM).

INTRODUCTION

Micrometry is the measurement of actual size of microscopic objects by using micrometers. Micrometers have microscopic graduations/rulers etched on their surface. The Ocular micrometer (OM) is a circular glass disc ruled with 10 equal divisions marked 0 to 10. During calibration it fits into the circular shelf inside the eye piece[1&2]. However the, scale on the oculo micrometer does not have any standard value, etching total length is 1cm (Fig.1). Since the value of one division of OM is not known, this could be calibrated with Stage meter(SM). The Stage Micrometer (SM) is a special microscopic glass slide which has small ruler with 1mm length engrafted at its centre. It consists of vertical lines called divisions markers which are three different vertical lines and the space between the lines are same. The divisions are marked from left to right. There are ten divisions between any two longest division markers. So there are total of 100 divisions, the large division markers are multiple of ten. Like wise mid size division markers are five, helps us to find the multiples of five. So it starts from 0 to 5,10,15 all the way to 100. Therefore there are 100 divisions in this tiny ruler. The stage micrometer has been calculated, that one division is 0.01mm in length which is converted to 10 micrometer in length. So the space between any two division is 0.01mm (10µm). If the micrometer is 100 divisions, each division is 10 µm, then multiply it into the total length of the stage meter is 100 division into 10 µm is equal to 1000 µm long (Fig. 2). To use these two pieces of information, now assign each division of the ocular micrometer determined based on the use of the objective lenses at that time.[3-6] To the best of my knowledge there was no literature available for manual calibrations of comet tail length. Therefore we explored the manual method for calibrating comet tail length. Single gel electrophoresis assay was carried out based on the guidelines of Singh et al.[7] Extension of length of comet tail was depends upon the severity of asphyxia.[8]

MATERILS AND METHODS

The current study was conducted at Department of Anatomy JIPMER Pondicherry during the Doctoral Research of the Principal author in the year 2008 to 2011 in collaborations with department of Paediatrics and Biochemistry. The study was approved by Institutional Ethics and Research committee. The materials required for manual method were Light Compound Microscope/ Fluorescent microscope, Stage micrometer (SM), Ocular micrometer (OM) and specimen /objects required for determination of size. The manual method of calibration of the objects was done as per procedures of Waelsch et al.[3] Comet assay was carried out based on guidelines of Singh et al.[7]

Procedure

- Turn the nose piece of the microscope to bring the desired objective to position and place the micrometers under it.
- The eyepiece is removed from the microscope, and its top lid is unscrewed. The lid is removed

carefully, the eye lens is removed. The ocular micrometer is placed carefully into the eyepiece. The eye lens is placed back and the top lid is screwed to its original conditions. The eye piece is placed back in the microscope.

- Bring into focus 1mm line of stage micrometer.
- Now when you observe under the microscope, you should be able to see two sets of lines, one of stage micrometer (SM) and other of oculometer (OM).
- Looking through the microscope adjust the ocular lens and stage micrometer appropriately so that the zero of the two scales coincide.
- The eye piece is rotated till the etchings on the both micrometers superimpose.
- Starting from zero position move your eyes towards the right look for and reads lines/ruler of coincidence across the rulers of stage meter.
- Calculate as follows and determine the value of one OM division.
- Value of one OM division (microns) = Graduations of the stage meter which will be appeared at the field, corresponds ocular division, will depends upon its image while changing the objectives divided by the divisions of Ocular meter correspond with stage meter ruler.
- The calibration factor for the objective used is calculated. Calibration formula / Micrometric unit of One Ocular division will be Total graduations stage micrometer divisions divided by corresponding number of ocular division.
- In similar way, calibration factors are calculated for the other objectives with 4X,10X and 40X .Record your results and calculate the value of one OM divisions.
- The stage micrometer has to be removed.
- The slide containing the objects/specimen to be observed is placed on the stage and focused.
- The number of ocular divisions corresponds by the objects/specimen is counted by viewing through the eye piece whose measurement has to be taken.
- The size of the objectives is determined by multiplying the number of ocular divisions corresponds by the objects/specimen with calibration factor of low and high magnifications.

Calculations

1. For One division of Ocular micrometer (C) = Number of divisions of stage micrometer (A) divided by Number of divisions of Ocular micrometer (B) = in 4X,10X and 40X Objectives could be 25 µm, 10 µm and 2.5 µm respectively.
2. Determine the size of the object/specimen = Size of specimen correspond with the oculometer division into one division of respective objectives (C).

Figure 1: Showing Ocular Micrometer (OM) with 10 non-standard divisions etched at centre.

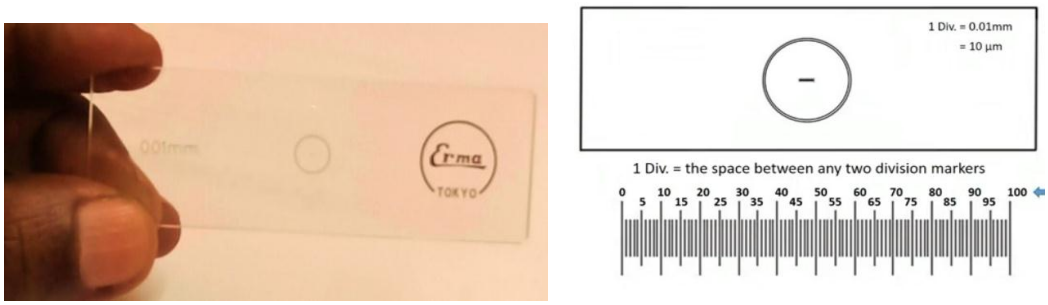

Figure 2: Exhibiting Stage Micrometer (SM) with 100 equal divisions have 10 µm (0.01mm) mark value for one division etched at centre of scale.

OBSERVATION AND RESULTS

The observations and results of calibrations of micrometres with 4X, 10X and 40X objective lenses and the measurement of the length of comet tail length were explored separately. The calibrations of one ocular division in 4X objective was found out, that one stage micrometer division was 10 µm, so for 100 division the total length of the stage micrometer was 1000 µm. The whole length of the stage meter ruler was coincide with 40 divisions of the ocular meter. Therefore 1000 µm was equal to 40 ocular divisions (40 ocular division =1000 µm) (Fig. 3A). It was answered mathematically by doing

cross multiplication 1 ocular division into 1000 µm was equal to 40 ocular division.

1 OD = 1 OD x 1000 µm /40 OD = 40 OD x ? µm / 40 OD
Total magnification of 4X objective = 1000 µm /40 OD =25 µm (Table 1).

Comet Tail Calibration for 4X Objective lens
Replaced the Stage micrometer with slide contains Comet. The comet tail was corresponds with 1 divisions of the Ocular ruler. So, remember the calculation of one OD in 4X objective was 25 µm. Tail length was 1 divisions of OM. One OD for 4X objective lens was 25 µm. So 1 OD x 25 µm = 25 µm was the tail length of comet (Table 2) (Fig. 4A).

The calibration of 10 X objective lens was done by replacing the stage micrometer. We could not changed the Ocular lens and Oculometer and they were stayed as such. One division of stage micrometer was 10 µm. So the entire length etched on stage micrometer for 100 divisions was 1000 µm. Here in 10X objective lens, the stage micrometer and ocular meter rulers were coincide equally, that was 1000 µm superimposed with entire 100 divisions of the oculometer (1000 µm = 100 divisions) (Fig. 3B).
1 OD =? µm, it was obtained by cross multiplication, as used in 4X objective lens.
1 OD = 1 OD x 1000 µm /100 OD = 100 OD x ? µm / 100 OD
Total magnification of 4X objective was 1000 µm /100 OD =10 µm (Table 1).

Comet Tail calibration for 10 X Objective lens
The comet tail was coincide with 5 divisions of the Ocular ruler. So the remember the calculation of one OD in 10X objective was 10 µm length. Tail length was 5 divisions of OM. One OD for 10X objective lens was 10 µm. So 5 OD x 10 µm = 50 µm, was the tail length of comet (Table 2) (Fig. 4B).

Inorder to calibrate one division of oculometer in 40X magnification, replaced the stage micrometer. Here the stage micrometer ruler was so big, therefore the entire ruler was not fit in the field. Remember the stage micrometer ruler was 1000 µm in length, so each stage division was equal to 10 µm. Therefore 10 stage division was 100 µm (10 stage division x 10 µm = 100 µm). 40 divisions of oculometer was coincide with 10 divisions of stage meter (40 OD = 100 µm) (Fig. 3C).
Calculate, one ocular division in 40X objective lens.
1 OD = ? µm, it was obtained by cross multiplication, as used in 10X objective lens.
1 OD = 1 OD x 100 µm /40 OD = 40 OD x ? µm / 40 OD
Total magnification of 40X objective was 100 µm /40 OD =2.5 µm (Table 1).
So One OD in 40X magnification was 2.5 µm.

Comet Tail calibration for 40 X Objective lens
The comet tail was coincide with 50 divisions of the Ocular ruler. So remember the calculation of one OD in 40X objective was 2.5 µm length. Tail length was 50 divisions of OM. One OD for 40X objective lens was 2.5 µm. So 50 OD x 2.5 µm = 125 µm, was the tail length of comet (Table 2) (Fig. 4C).

Table 1: Showing length of one division of Oculometer in 4X, 10X and 40X objectives.

Objective lens	Stage Micrometer Ruler (A)	Oculo meter Ruler (B)	One division of Oculometer (C) in µm
4X	1000	40	25 µm
10X	1000	100	10 µm
40X	100	40	2.5 µm

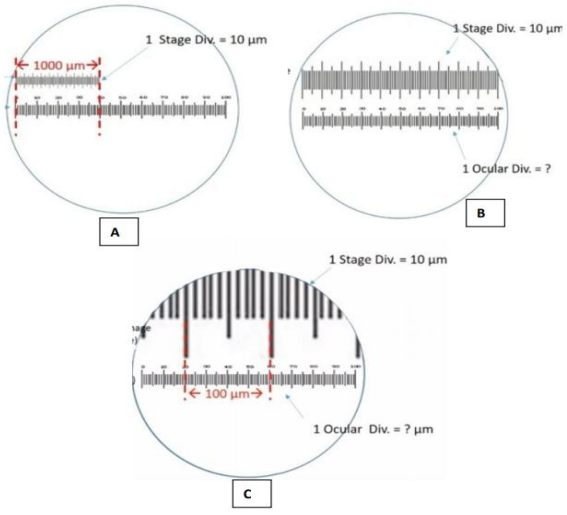

Figure 3: Delineating Micrometry Calibrations of Oculometer ruler coincide with Stage meter graduations in A-4X, B-10X and C-40X Objective lenses.

Table 2: Depicting Comet tail length in 4X,10X and 40X objectives.

Objective lens	Tail of Comet coincide with Oculometer Divisions	One division of Oculometer (C) in µm	Comet Tail Length (µm)
4X	1.00	25	25 µm
10X	5.00	10	50 µm
40X	50.00	2.5	125 µm

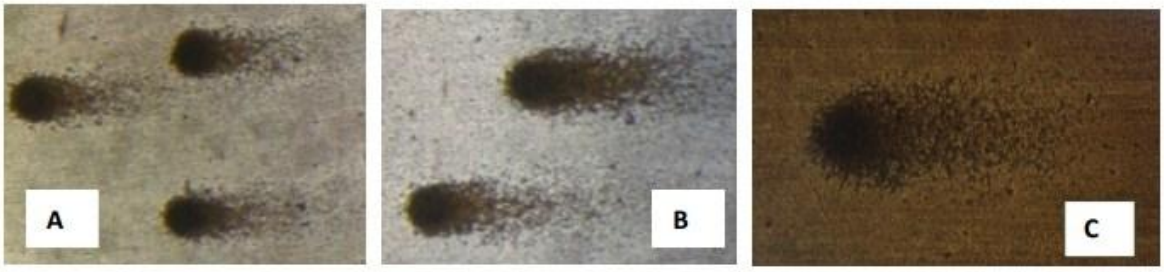

Figure 4: Depicting Comet Images in 4X, 10X and 40X Objective Lens.

DISCUSSION

The image size of any specimen viewed under microscope has magnified by twice, first magnified by objective lens and secondly by the ocular lens.[1-2] The micrometers such as oculo micrometer and stage micrometer were used in the current study to calibrate one ocular division in 4X,10X and 40X objectives. The divisions etched on stage micrometer was standard graduations. However the oculometer divisions were arbitrary. Since the divisions of the oculometer was not standard, so the stage micrometer has been using to find out the distance of one division of oculo meter. Therefore the current study explored to find out one ocular division correspond with stage meter in 4X, 10X and 40X objectives. In this study we found the amount of one ocular division in different objectives of the microscope. In 4X objective (Fig3A), the stage micrometer corresponds only 40 divisions of the oculometer and the readings etched on stage meter was 40 times magnified. Therefore one division of oculometer was obtained by dividing the total length of stage micromerter with corresponding divisions of oculometer, which was 150 µm. Similarly in 10X objective the magnifications was 100 times larger, so the two scales were correspond with each other (Fig.3B) which have 10 µm length distance for one division of the oculometer. In case of 40X objective, the image had 400 times magnification, so only one large division of the stage micrometer (Fig:3C) was appeared in the field which corresponds 40 divisions of the oculometer, calibrated 2.5 µm length in one division of oculometer. We have been observed that when magnification of objective lens increases the length of one division of oculometer was decreases in which 25 µm,10 µm and 2.5 µm for 4X,10X and 40X respectively. To the best of my knowledge there was no data available for manual calibrations of the comet tail length in single gel electrophoresis. Previous reports of the same author documented that the extension of comet tail length was significantly increases severe Perinatal asphyxia in automated method of comet scoring under 20X objective magnification [7&8]. In our study the length of comet tail length was significantly increases when magnification increases in which 25 µm, 50 µm and 125 µm for 4X, 10X and 40X objectives respectively.

CONCLUSION

Apart from measurement of comet tail, Manual method of Calibrations can be used for Evaluating the actual size of an organism or tissues in microscopic slides for clinical research.

ACKNOWLEDGEMENT

Sincere gratitude to my mentor and doctoral research guide Dr. Ramachandra Rao former Senior Professor and Head Department of Anatomy, JIPMER Pondicherry, India.

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