VIVEKANANDA COLLEGE

THAKURPUKUR

NAAC ACCREDITED GRADE—'A'

Subject: Botany

Topic: Micrometry – Measurement of the size of microorganisms under microscope

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Micrometry: Measurement of the size of microorganisms under microscope

Micrometry (micro: microscopic, metry: measurement) refers to the measurement of the dimensions of microscopic objects under light microscope in terms of length, breadth, diameter and thickness. Size is one of the most important physical features employed in the identification and characterization of an organism. For measuring the exact size of the cell or some specialized structure of the microbes like bacteria, algae, fungi and protozoa micrometry is used.

It is achieved by the use of:

- **Stage Micrometer**
- **Ocular Micrometer**

Stage Micrometer (Fig.1) is of a slide's shape and size containing a very finely graduated scale. The scale measures only 1 mm and has a least count of 0.01 mm, i.e. 1 mm region is divided into 100 divisions. As 1 mm has 1000µ, one division of stage micrometer is equivalent to 10µ.

Ocular micrometer (Fig. 2**)** is a glass disc on which a series of uniformly spaced lines has been inscribed. This scale is arbitrary and do not indicate any measurement of length. It usually contains 100 equally spaced divisions, marked 0 to 10 equally. The ocular micrometer is placed in one of the eyepieces of the microscope; however, the distance between the etched lines depends upon the objective lens used to view the specimen. In order to determine the precise distance between the lines of an ocular micrometer, it must be calibrated with a stage micrometer. The exact size of a microorganism can only be determined by utilizing a calibrated ocular micrometer.

OBJECTIVE: To measure the dimensions of common microorganisms by calibration and standardization of microscope using stage micrometer and ocular micrometer.

Requirements:

Microscope, stage micrometer, ocular micrometer, slide of the microorganism to be measured.

Calibration of ocular micrometer (Fig 3):

The scale of ocular micrometer is calibrated in comparison with stage micrometer, under different objectives. In order to calibrate the ocular micrometer for a particular objective lens, the ocular and stage micrometers are superimposed, and the number of ocular graduations per stage micrometer graduation is determined.

Procedure:

- Place the stage micrometer on the microscope stage and centre the scale in the field with the ruled area directly over the center of the condenser lens**.**

- Insert the ocular micrometer into eye- piece and focus the eye- piece scale. Look through the eyepiece with the low power objective in place and identify the ocular micrometer, check by turning the eyepiece. Note the number of gradations between numbers.
- With the objective (10X or 45X) to be used for micrometry, focus the scale of the stage micrometer and adjust in such a way that the stage scale and ocular scale lie in parallel, side by side.
- Align an ocular micrometer line with a stage micrometer line at the left side of the field. Look for another ocular micrometer line toward the right side of the field which is aligned similarly with a stage micrometer line.
- Determine the number of ocular micrometer spaces that fall within a given number of stage micrometer spaces.
- Calculate the distance between each ocular graduation by using the following formula:

1 ocular micrometer space (µm) =(y spaces on the stage micrometer ÷ x spaces on the ocular micrometer) x 10 µm

∴ **The value of one ocular micrometer =** $\frac{y}{x} \times 10$ **µm**

- Repeat the procedure for the 10X or 45X objectives and record results.
- Then return the stage micrometer to its case carefully after measurement.

Microscope No. ----------
Objective: ----------
Eye piece: ----------

Table for Standardization (calibration) of ocular micrometer using stage micrometer

No. of observation	No. of ocular divisions(x)	No. of corresponding stage divisions(y)	Value of one ocular division $\frac{y}{x} \times 10$ **µm**	Mean value of one ocular division (Calibration factor) **µm**
1. 2. 3 4. 5.				

Measurement of the size of microorganisms under microscope

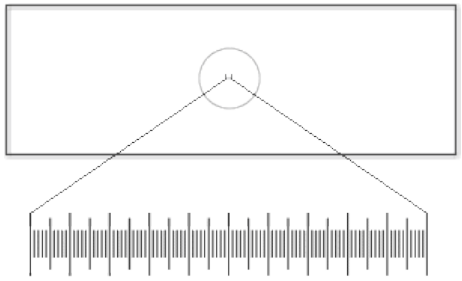

Figure 1: Stage micrometer

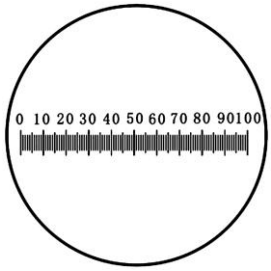

Figure 2: Ocular micrometer

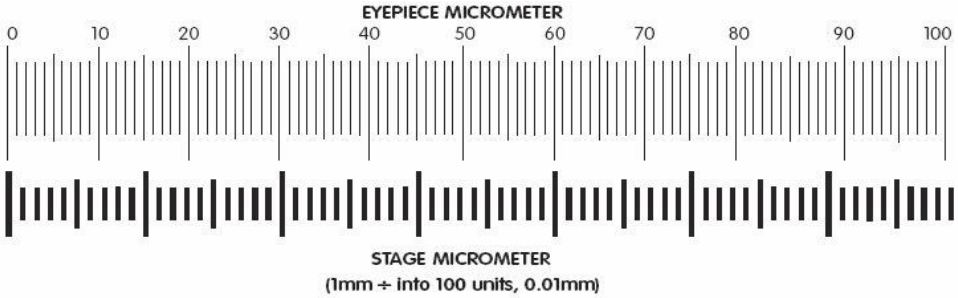

Figure 3: Alignment of Ocular micrometer scale on Stage micrometer

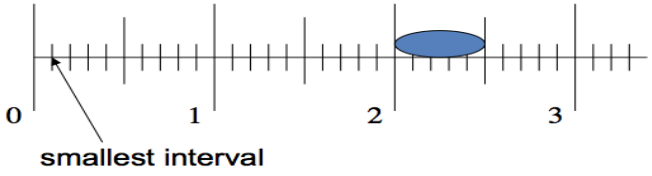

Figure 4: Measurement of fungal spore in ocular micrometer

Measurement: When the microscope is calibrated, then the object or organism to be measured is kept on the sage of the microscope and is observed through the eyepiece with ocular (**Fig 4**). The object is measured in the particular magnification by ocular divisions and then is changed into microns by multiplying ocular divisions with calibrated value of one ocular division in that particular magnification. At least five such readings are recorded for this purpose.

Table: Measurement of the size of Microbial Cell/ Reproductive Structure

Sl. No.	**Diameter/ length/ breadth in ocular division**	**Ocular divisions X calibration factor** **µm**	**Range of Diameter/ length/ breadth** **µm**
1 2 3 4 5			

Result: The size of the microbial structure was recorded to be…… µm.

Further Reading

Dubey R C amd Maheswari (2002) *Practical Microbiology***.** New Delhi: S.Chand and Company Ltd.

Reddy Rajeshwar K. (2009) *General Microbiology*. New Delhi: New Age International (P) Limited, publishers.

Reddy, Eswara N P.and Surendra V. (2015) *An Introduction to Microbiology*. New Delhi: Kalyani Publishers.