2.6 Micrometry

caliberation of microscope

This lesson forms part of Unit II in paper I Research Methodology.

You have already learnt the basic principles of microscopy and the working of microscope in the previous unit. In the present unit, after having learnt the principles involved and the methods of culture, you have to learn micrometry as a part of general microbiological technique.

Objectives of this lesson

After studying this lesson, you would have learnt the following:

- Principles of micrometry
- Use of Stage and Ocular micrometers
- Making measurements of microscopic objects (Micro measurements)
- The need and method of calibrating microscopes

2.6.1 Principles of micrometry

Micrometry is the measurement of microscopic objects. The unit of measurement in micrometry is

micron (μ), which is one thousandth of a millime
Microscopic objects are seen with the help
microscopes. Therefore, the scale that is used f
micrometry is to be somewhere in the microscope. F
this purpose, a micrometric scale is lodged in the ocul
lens system of the microscope. Hence, this scale
called Ocular scale or Ocular Micrometer. Using ocul
micrometer, dimensions of the object under observatio
(cells, microbes etc.,) are measured only as number
ocular divisions covered along the length and breadt
of the object, as the value of the divisions in the ocul
scale is not known. Moreover, the object mounted o
the stage for observation, is magnified variously unde
different ocular and objective power combinations
Therefore, the ocular scale is to be calibrated for eac
power combination, using a known scale. This is don
with the help of Stage Micrometer.

Each microscope is to be calibrated, as the exac
degree of magnification differs from microscope t
microscope. This is called calibration of microscopes.

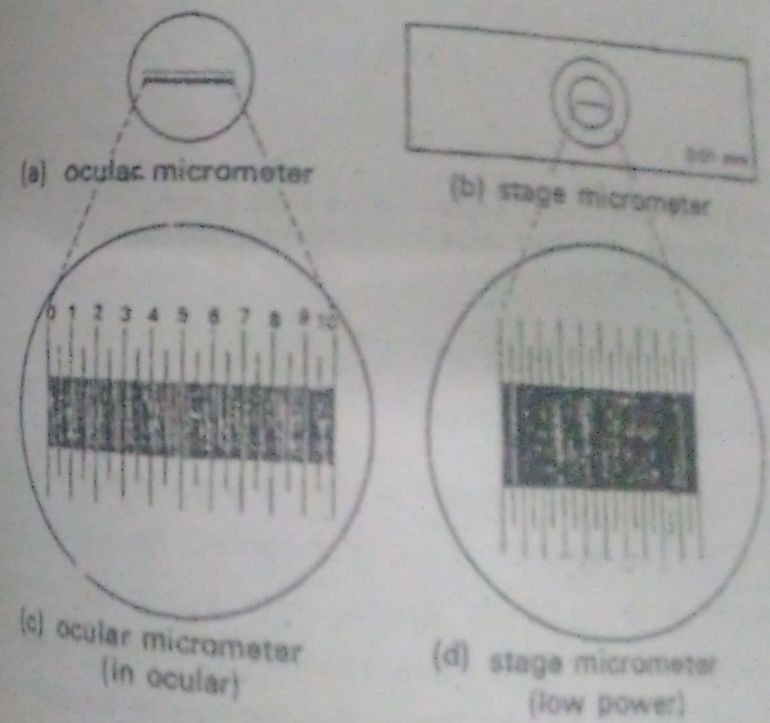
2.6.2 Ocular micrometer

An ocular micrometer serves as a scale or rule
is simply a disc of glass upon which the ocular scal
with 100 equally spaced divisions is etched. The scal
has 10 larger divisions marked from 0 to 10, each having
10 smaller divisions. When placed in the ocular len
system (eye piece), the ocular scale appears
superimposed on the filed of vision.

The divisions in the ocular do not have a specific value. It can be found out by calibrating it with a known scale, namely the stage micrometer.

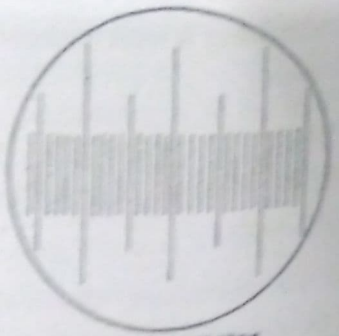
The ocular scale is fixed, in the sense that its magnification does not change with change of objective lens systems from low power to high.

Ocular scale is used to measure the cell dimensions. The number of ocular divisions covered by the object (cell or organism) along the length and the breadth under the power combination in use (i.e. ocular and objective powers) is noted. Once, the microscope is calibrated, this value can be converted into microns, once, the microscope is calibrated.

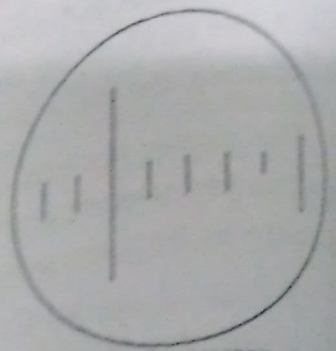


(c) ocular micrometer (in ocular)

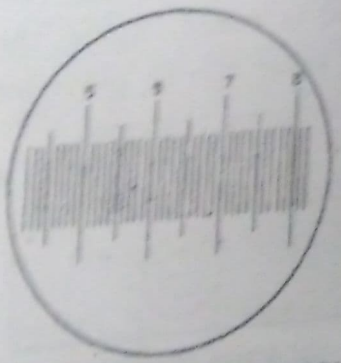
(d) stage micrometer (low power)



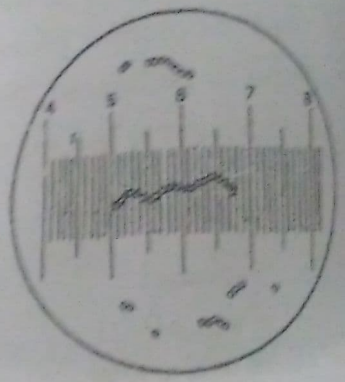
(e) stage micrometer (high power)



(f) stage micrometer (oil-immersion)



(g) ocular micrometer superimposed on the stage micrometer (oil-immersion)



(h) a slide having a microbe placed on the stage of microscope

2.6.3 Stage micrometer

The stage micrometer is simply a glass micro slide in which a micrometric scale is mounted, encircled and covered with a cover glass. The scale on stage micrometer has one mm divided into 100 equally spaced divisions. There are 10 large divisions each having 10 smaller divisions. Contrary to the divisions of ocular

scale, these divisions are not marked by any serial number.

Thus in the Stage Micrometer, 1000 µm are divided into 100 divisions. Therefore, each small division in the stage micrometer equals 10µm (0.01mm, which is marked at the right lower end of the micrometer as an index of the scale)

With changes in the objective lens systems from low to high power, the distance between the divisions in stage micrometer is enlarged correspondingly. Therefore, the ocular micrometer has to be calibrated using stage micrometer under each power combination (10x Eye piece/10x Objective; 10x Eye piece/45x objective; 10x Eye piece/100x oil immersion objective; 15x Eye piece/10 objective; and so on).

By determining how many ocular divisions exactly correspond to a known distance in the stage micrometer, the value of one ocular division under the power combination in use can be found out.

2.6.4 Calibrating the ocular scale and the microscope

* Unscrew the Eye Lens of the ocular. Place the Ocular micrometer disc on the circular shelf (metal diaphragm). Screw the eye lens back in position.

* Replace the ocular lens system in the body tube of the microscope. Adjust the light source and

view through the ocular system. The ocular scale is seen in the field of vision.

Mount the stage micrometer and bring its scale to the field of vision and focus it under 10x objective.

Bring the Stage micrometer scale parallel to the ocular scale in such a way, that the lines of the latter superimpose the former.

Move the Stage Micrometer Scale laterally until a particular division exactly coincides with the zero of the ocular(zero coincidence). It can be seen that the two scales again coincide after a few divisions farther from zero coincidence.

Now count the intervening divisions of ocular and stage scales between the zero coincidence and the next point of coincidence.

Thus we can find out how many divisions of ocular scale (unknown) equal how many divisions in the stage micrometer scale (Known).

E.g. if 10 divisions of Ocular equal 5 divisions of stage, then,

10 Ocular division = 50 µm. And,

1 Ocular division= 5 µm.

Repeat this with objectives of higher power including oil immersion.

Now transfer the ocular scale to 15x oculars and 4x or 6x oculars if available, and repeat the exercise for all the power combinations.

2.6.5 Micromeasurements

Measuring the cell dimensions using a calibrated ocular micrometer scale mounted in an ocular lens system is called Micro measurement.

Dimensions of cells or organisms mounted on a micro slide can be measured using a calibrated ocular scale.

The ocular scale can be rotated in its plane so that it can be conveniently oriented with reference to the length and breadth of the cells or the organisms mounted.

The length and/or breadth of the object are measured as the number of ocular divisions, which can be converted into µm from the calibration chart.

The calibration chart is permanent for a microscope, unless and until the eye pieces and oculars are changed.

2.7 Camera lucida

Camera Lucida (Latin. Light Chamber) is an optical instrument invented in the year 1807 by William Hyde