AIM

Measurements using the Ocular Micrometer (Micrometry Technique).

OBJECTIVES

1. Calibrate the Ocular Micrometer Scale.
2. Measure samples using the Ocular Micrometer Scale.

INTRODUCTION

Micrometer scales within the ocular of a microscope are employed to determine sample sizes. These specialized oculars possess a transparent scale graticule superimposed onto the observed image. By replacing the stage micrometer with the sample slide, you can measure cell sizes. Micrometry is a crucial technique in biology, especially in microscopy, using ocular and stage micrometers to measure biological structures.

1. OCULAR MICROMETER

Descri-ption:	Transparent ruler-like device without units, mounted on eyepiece.
Design:	Glass disc featuring 10 mm scale divided into 100 sub-divisions.
Visibility:	Scale visible through microscope eyepiece.

2. STAGE MICROMETER

Placement:	Positioned on microscope stage.
Constr-uction:	Slide with 2 mm scale divided into 0.01 mm (10 µm) sub-divisions.
Function:	Used to calculate ocular micrometer divisions at specific magnifications.

REQUIREMENTS

- ☐ Compound light microscope with bjectives (4X, 10X, 20X, 40X),
- ☐ Ocular micrometer scale
- ☐ Stage micrometer (0.01 mm scale).
- ☐ Microscope Glass slide
- ☐ Coverslip

PROTOCOL

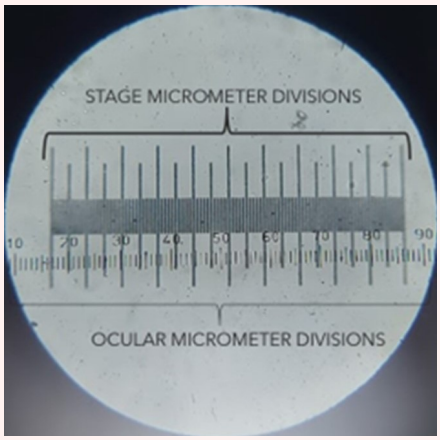
Part I: Calibration of Ocular Micrometer

1. Place the ocular micrometer in the microscope eyepiece.
2. Adjust the lighting for optimal Kohler illumination.
3. Focus on the stage micrometer using the lowest magnification.
4. Rotate to the objective designated for calibration.

PROTOCOL (cont)

5.Adjust the focus of the ocular micrometer to visualize both scales clearly.
6. Align the stage and ocular scales with a slight offset for ease of reading.
7. Choose two positions on each scale, preferably on opposite sides.
8. Count the divisions on the stage scale (ssd) between the chosen positions.
9. Count the divisions on the ocular scale (osd), which equals ssd.
10. Record the number of ocular spaces (y) and stage spaces (x).
11.Calculate the calibration factor: 1 ocular space = (x/y) × 10 µm.
12.Repeat the process for the second assigned objective.

Figure1:



Microscope Field of View Showing Ocular Micrometer and Stage Micrometer Superimposed each other.

Observation Table 1: Calibration of Objectives

Objective Power	Observation No.	Ocular Divisions (Y)	Stage Divisions (X)	Calibration Factor (X/Y × 10 µm)	Mean Calibr-ation factor
10X	1				
	2				
	3				
40X	1				
	2				
	3				

Part II: Protocol for Using Micrometers

1. Place biological sample slide (e.g. Pollen Grain), focus on area.
2. Locate ocular micrometer through eyepiece.
3. Align structure with ocular micrometer.
4. Count ocular divisions needed to span the structure.
5. Repeat for other objectives and other biological specimens.
6. Record divisions for each measurement.
7. Calculate structure size (e.g. pollen grain): Ocular divisions × calibration factor.
8. This protocol accurately measures biological structures.

Observation Table2 :Measurement of Plant Samples

Plant Samples	Microscope Objective Power	Calibration Factor (CF)	Observation No.	Ocular Divisions (OSD)	Size of Sample (OSD × CF)	Mean Size (µm)
Pollen Grain	10X		1			
			2			
			3			
	40X		1			
			2			
			3			

RESULTS

Size of Pollen Grain under 10X = _____ µm

Size of Pollen Grain under 40X = _____ µm

CONCLUSION

In conclusion, micrometer scales within microscope oculars are pivotal for accurate sample size determination in biology. By replacing the stage micrometer with a sample slide, cell sizes can be measured