Measuring with microscope scales

An eyepiece graticule has evenly spaced divisions but no fixed specimen-length value. Calibration gives those divisions a meaning in micrometres.

EYEPIECE GRATICULE

A scale inside the eyepiece, seen over the specimen image. Its divisions are arbitrary until calibrated. Use it to count how many divisions a cell spans.

STAGE MICROMETER

A slide with an accurately known scale. Place it on the microscope stage to find the real length represented by each graticule division.

1. CHECK THE KNOWN SCALE

Read the stage micrometer’s markings or instructions. A common example is 1 mm divided into 100 intervals: each interval is 0.01 mm = 10 µm. Do not assume every scale has this spacing.

2. ALIGN THE TWO SCALES

Choose an objective and focus the stage micrometer. Rotate or move the scales until they are parallel and one pair of marks coincides. Find another clear coincidence well away from the first.

3. COUNT INTERVALS, THEN CALIBRATE

Count the graticule divisions and the known stage distance between the same two aligned positions. Divide the known distance by the graticule count to find µm per division.

4. RECORD THE OBJECTIVE

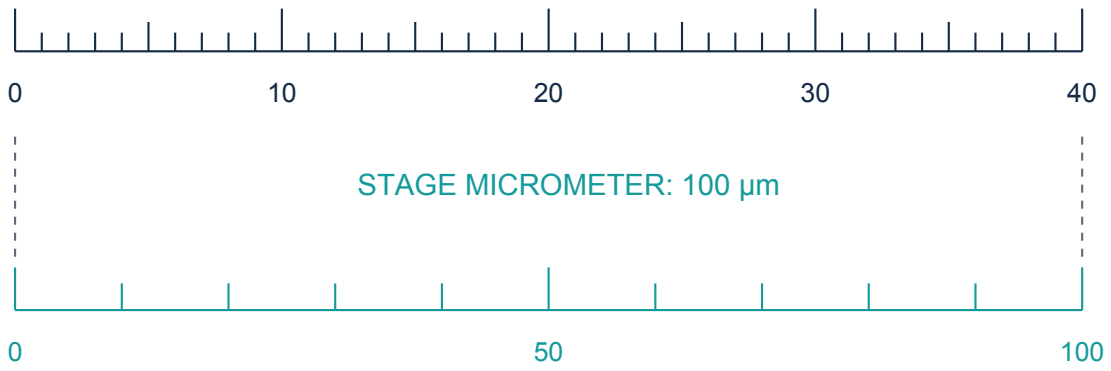
Write down the calibration with the objective lens and microscope used. Each objective needs its own calibration; changing the optical setup can change the value of a graticule division.

Cambridge Biology 9700 syllabus: section 1.1, learning outcome 4, p. 15.

Calibrate: compare equal lengths

Worked example at the ×40 objective. The stage micrometer has 10 µm intervals. Both scales below cover the same length between the dashed guides.

EYEPIECE GRATICULE: 40 DIVISIONS



One stage interval = 10 µm. There are 10 intervals, not 11.
Therefore, 40 graticule divisions represent 100 µm.

$$\text{Calibration} = \frac{100\ \mu\text{m}}{40\ \text{divisions}} = 2.5\ \mu\text{m/division}$$

1. WHAT THE RESULT MEANS

At this objective, each graticule division represents 2.5 µm of the specimen. This is a length per division, not a magnification. Keep the value with the objective used: ×40 → 2.5 µm/division.

2. SUBTRACT READINGS IF ZERO IS NOT ALIGNED

The starting marks do not have to be zero. For example, graticule readings 12 and 52 span 40 divisions; stage readings 20 µm and 120 µm span 100 µm. The calibration is still 2.5 µm/division.

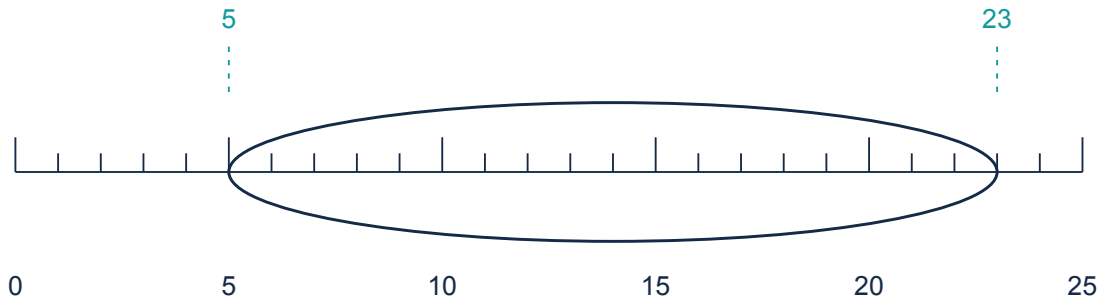
Use a long, clearly aligned span to reduce the relative effect of reading error. Count spaces between marks rather than counting the marks themselves.

Cambridge Biology 9700 syllabus: microscope measurement skills, p. 53. Example by Jimlaoshi.

Measure: apply the calibration

Replace the stage micrometer with the specimen slide. Keep the same objective and eyepiece setup, focus the specimen, and align the graticule along the length to measure.

EXAMPLE: A CELL SPANS 18 DIVISIONS



Readings: 23 - 5 = 18 divisions. Use 2.5 µm/division from page 2.

Actual length = 18 × 2.5 µm = 45 µm

WHY CHANGING OBJECTIVE MATTERS

At higher objective magnification, the same cell spans more graticule divisions. Each division therefore represents a smaller real length.

Objective	µm per division	Cell divisions	Actual size
×10	10	4.5	45 µm
×40	2.5	18	45 µm

Illustrative calibrations for the same optical setup. The cell's actual size stays the same; its image covers four times as many divisions at ×40.

MEASUREMENT CHECKLIST

Use the correct calibration for the current objective. Read both endpoints and subtract. Multiply by µm per division, state a sensible precision, and include the unit. Recalibrate if the objective or relevant optical setup changes.

Unit reminder: 1 mm = 1000 µm; 1 µm = 1000 nm. Never report an uncalibrated graticule reading as a length in µm.

Cambridge Biology 9700 syllabus: section 1.1.4 and microscope measurements, pp. 15, 53.