Basic Biology Course

1 Light Microscopy

BASIC BIOLOGY COURSE

UNIT 1

MICROSCOPY AND ITS APPLICATION TO BIOLOGY

BOOK 1

Light Microscopy

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Foreword

This book is part of a Basic Biology Course for undergraduates written by the Inter University Biology Teaching Project team at Sussex.

The main aim of the book is to teach you how to use the light microscope (including phase-contrast) and the stereomicroscope for examining and identifying biological material. Since, in our experience, the principles of microscopy are rarely taught either at school, college or university, we feel that there is an urgent need for a book which explains:

(i) the components of a light microscope;
(ii) how to set up the microscope correctly for use; and
(iii) some of the theoretical reasons why a microscope should be set up correctly.

We have included the theoretical aspects because most of the students whom we consulted during the trials of this course preferred to have a basic theoretical understanding of the resolving power of the light microscope rather than simply accept it as an instrument which magnified small biological objects.

The objectives that we have asked you to achieve (page 3) therefore have these points very much in mind. In addition, we have emphasized the quantitative aspects of biology and there is an important section on measurement of small objects, particularly cells, to show the variety and range of size of these fundamental biological units. To test your progress in achieving the objectives, you will find a short self-assessment test at the end of the book. You are strongly advised to attempt to answer these questions.

Finally, we should add that one of our major problems in writing this book was the great diversity of microscopes that are now used in laboratories throughout the country. This diversity in fact is not as great as it would seem, because the components and optical principles involved are virtually identical. The diversity arises in the layout of the controls and the movement of parts relative to one another. As a result of a small survey which we undertook, we have selected five different makes of microscope which are in common use. Each has been photographed with the components and controls labelled on a separate card so that it will facilitate your working through the book. These cards are to be found at the back of the book.

This book can be used quite independently of any other book in the series, but for those who wish to know more about the fine structure of cells and their inclusions, we would like to direct your attention to Book 2 on electron microscopy.

Sussex, 1974

M. A. Tribe
M. R. Eraut
R. K. Snook

Acknowledgements

This book was developed under the auspices of the Inter University Biology Teaching Project and is the responsibility of the Sussex University project team. However, it owes a great deal to the students who studied and criticized our earlier versions and to many colleagues both at Sussex and elsewhere who made constructive suggestions for its improvement.

In particular we would like to thank the following:

the Nuffield Foundation for financially supporting the project from 1969 to 1972;

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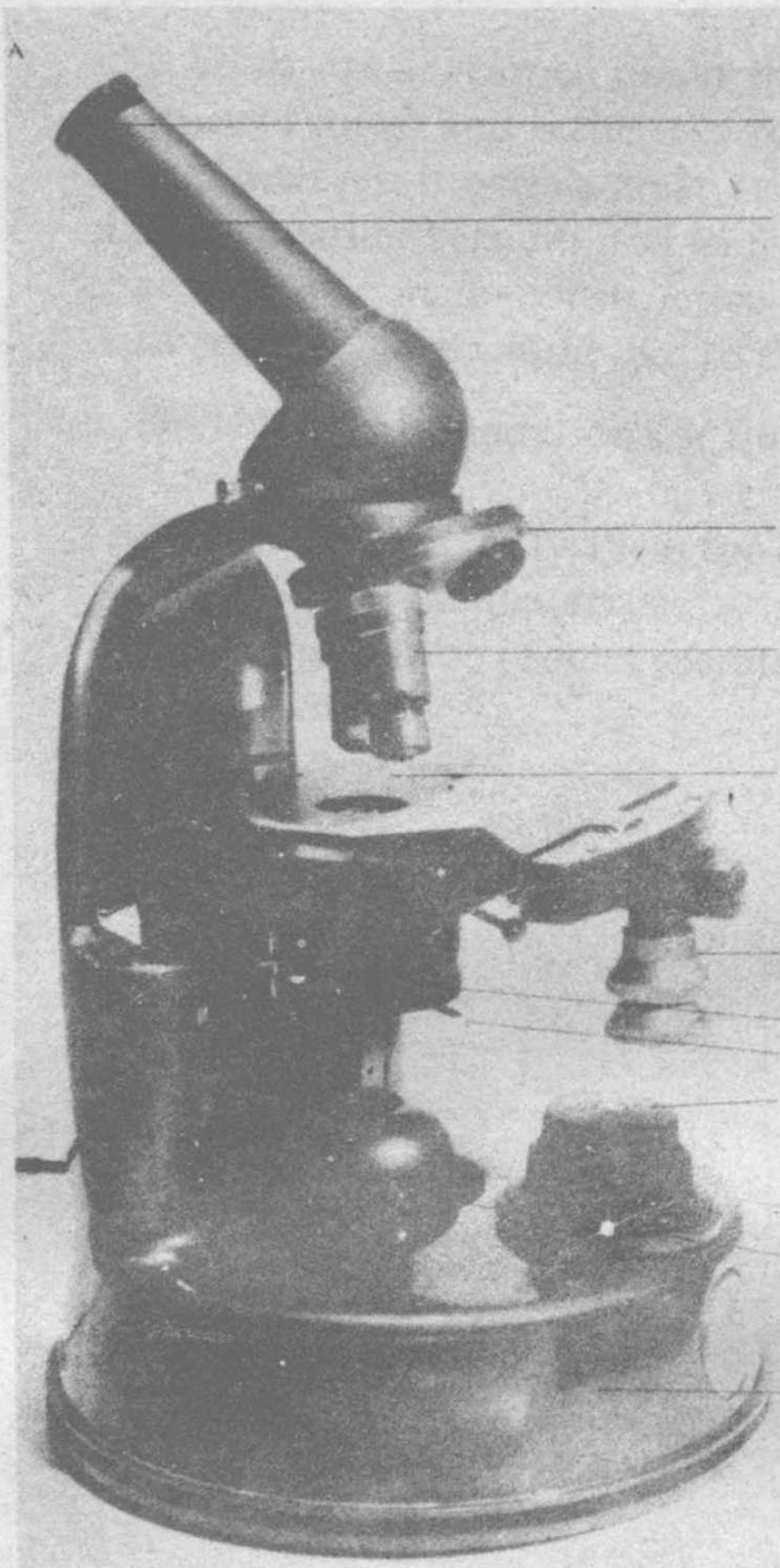
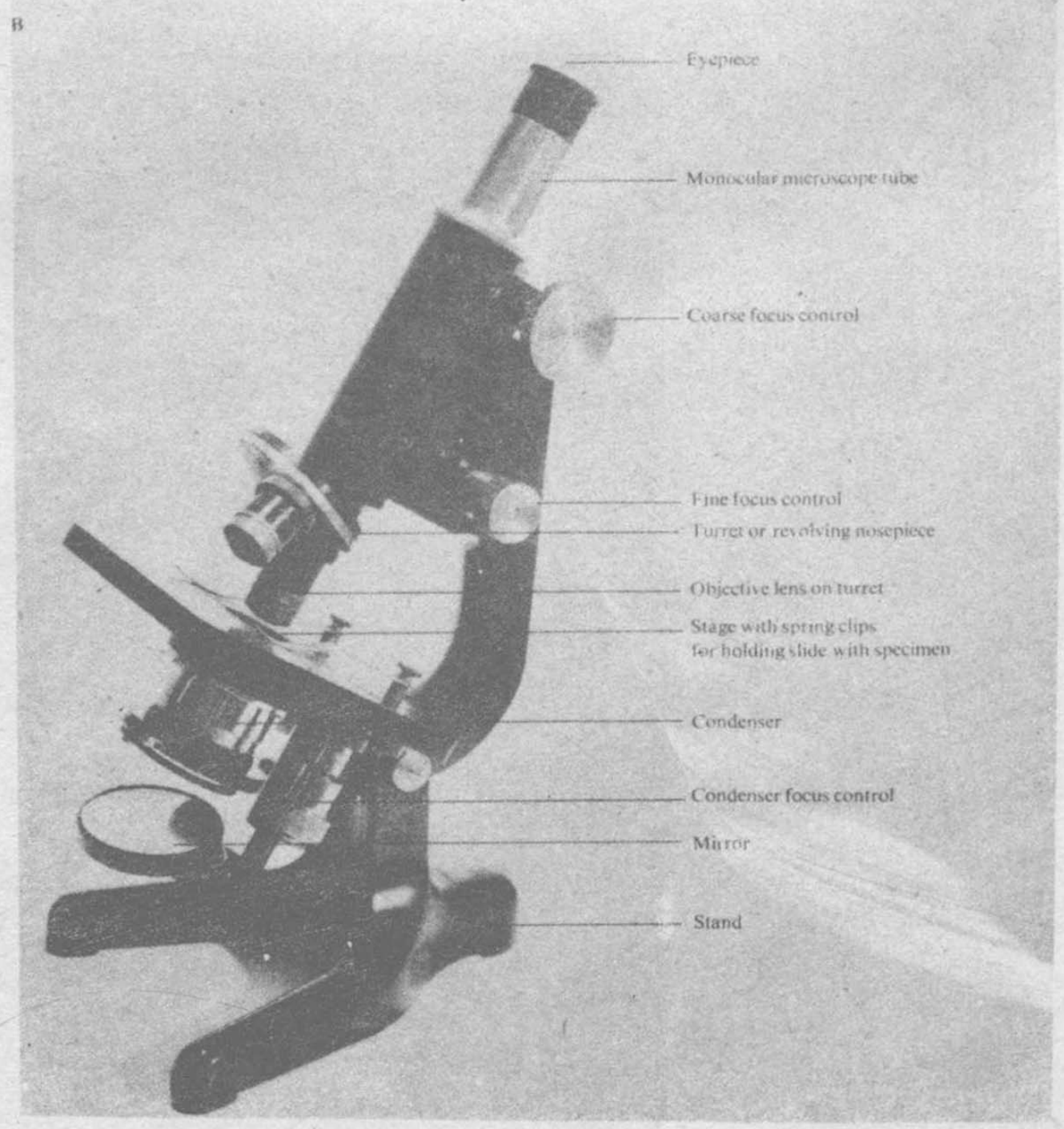
Mrs P. Smith, the project secretary; and Mr C. Atherton for photographic assistance.

A


Eyepiece
Monocular microscope tube
Revolving nosepiece or turret
One of the objective lenses attached to the turret
Stage on which specimen is mounted
Mechanical stage control
Condenser
Lever of aperture diaphragm
Coarse and fine focus control (This is in an unusual position on this microscope)
Built-in light source
Stand

B


Eyepiece
Monocular microscope tube
Coarse focus control
Fine focus control
Turret or revolving nosepiece
Objective lens on turret
Stage with spring clips for holding slide with specimen
Condenser
Condenser focus control
Mirror
Stand

Contents

1.0. Introduction

1.0.1. Discussion

Advances in any area of science either anticipate or follow advances in technology and instrumentation. The explosive expansion of our biological knowledge in recent years has its roots in the development of various techniques and instruments. Certainly, one of the most crucial of these has been microscopy and the microscope.

The microscope enables the biologist to see order and organization within very small living things. This information can be correlated with physico-chemical information acquired using other techniques. Thus the microscope is an important tool in building up a composite picture of the structure and function of living material.

There are two main types of microscope - the light microscope and the electron microscope.

1.0.2. Overview

This book is intended to teach you, amongst other things, the use of the light microscope, and you will need a microscope for sections 1.1, 1.2, 1.3 and 1.5. We have given specific instructions for the Gillett & Sibert 'Lynx' or 'University Lynx' microscopes. There are however, many other microscopes on the market, which on a cursory glance may appear vastly different from each other. In general, however, the differences are only in details because invariably all microscopes consist of the following components (see also the illustrations opposite):

1. A microscope tube with eyepiece.
2. A strong, stable stand with a stage supporting the specimen: on most modern microscopes the stage is moved relative to the microscope tube (see A opposite); on other microscopes the microscope tube may move relative to the stage (see B opposite).
3. A revolving nosepiece or turret with a range of objective lenses attached to it. As a result any lens can be selected and swivelled into the light path.
4. A condenser for focusing the beam of light at the point of the object and/or an aperture diaphragm which controls the numerical aperture of the objective lens.
5. An illuminating device, which may be external to the microscope (as in B opposite) or built into the microscope as in A opposite.

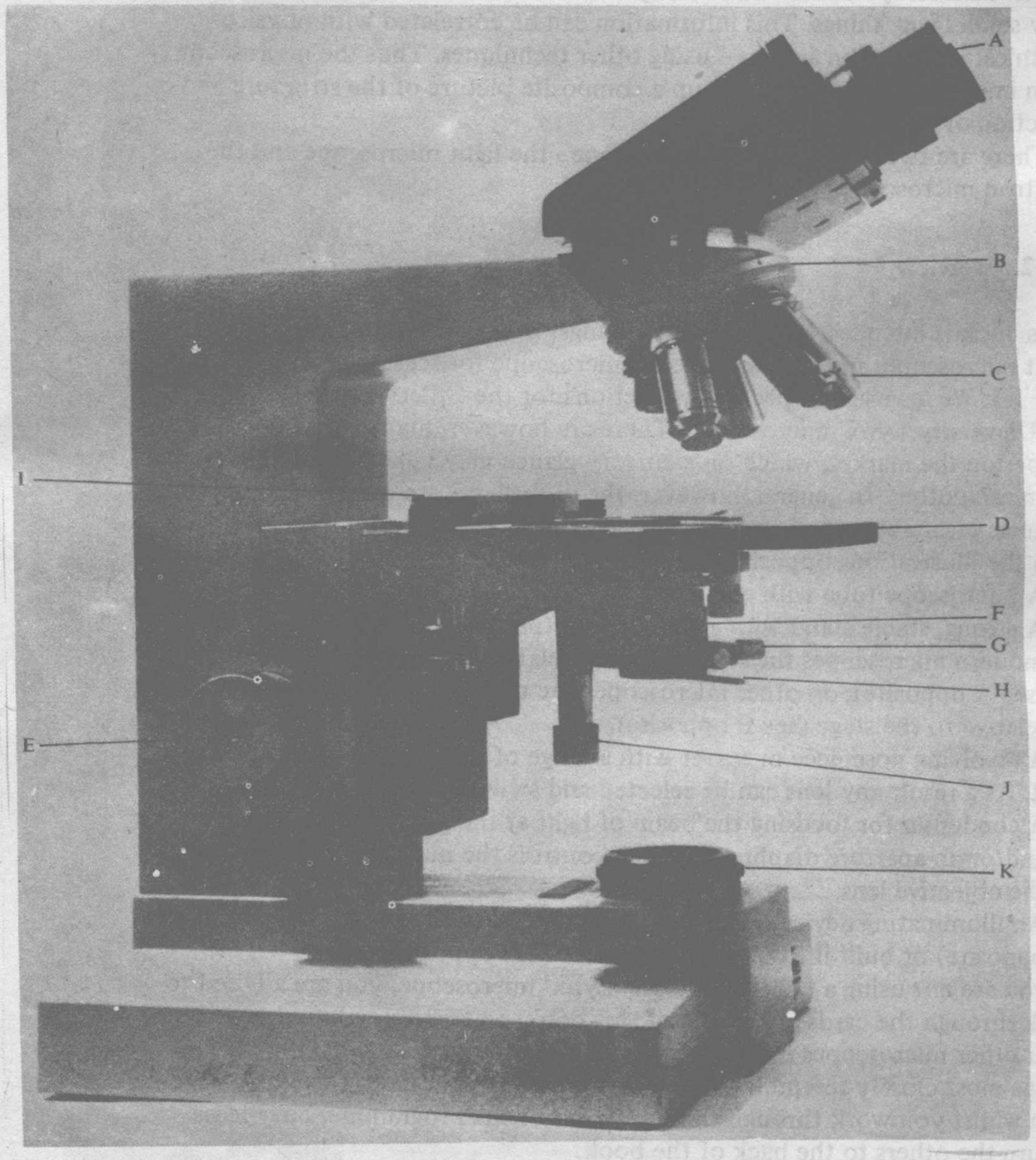
If you are *not* using a Gillett & Sibert 'Lynx' microscope, you are advised to look through the cards at the end of this book, where you will find details of four other microscopes in common use. Select the card showing the microscope most closely resembling the one in front of you. Keep this in front of you whilst you work through the remainder of the programme, but please return the others to the back of the book.

LIGHT MICROSCOPY

The photograph below shows a modern microscope (Leitz Microscopes Ltd). The important parts are lettered A to J.

H is the lever of the aperture (iris) diaphragm; G is one of the centring screws for the condenser; I is the mechanical stage; J the substage condenser focusing control and K the light source.

Can you name the parts A to F?



A. Eyepiece (binocular head)
B. Turret
C. Objective lenses
D. Stage
E. Coarse and fine focus control
F. Condenser

1.0.3. Preknowledge requirements

Very little knowledge is assumed in this programme except for an elementary understanding of optics.

1.0.4. Objectives

The objectives of this book are that you should be able to:

(*a*) Operate the microscope without instructions - including oil-immersion and phase-contrast techniques. Operate the stereomicroscope.
(*b*) Follow simple instructions for the preparation of objects for microscopic examination - including staining techniques.
(*c*) Use the microscope to measure the size of a small object.
(*d*) Interpret microscopic observations.
(*e*) Describe the structure of some plant and animal cells.
(*f*) Recognize some intracellular structures that are visible under the light microscope.
(*g*) Give examples to illustrate the range of size and appearance of living cells.
(*h*) Explain in outline both the theoretical and practical limitations on the resolution of the light microscope.
(*i*) Describe the methods useful in overcoming some of the limitations on resolution.

1.0.5. Instructions on working through programmed sections

In the programmed sections, questions and answers are arranged sequentially down the page. You are provided with a masking card and a student response sheet. When you come to a programmed section you will be given warning of it. Cover each page in turn, and move the masking card down to reveal two thin lines

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This marks the end of the first question on that page. Record your answer to the question under the appropriate section heading in the response booklet provided. Then *check* your answer with the answer given. If your answer is correct, move the masking card down the page to the next two thin lines and so on. If any of your answers are incorrect retrace your steps and try to find out why you answered incorrectly. If you are still unable to understand the point of a given question, make a note of it and consult your tutor. The single thick line

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is a demarcation between one frame and the next.

1.1. Structure

1.1.1. Introduction

Take out the card on the Gillett & Sibert 'Lynx' microscope at the back of this book and keep this in front of you whilst working through the programme.

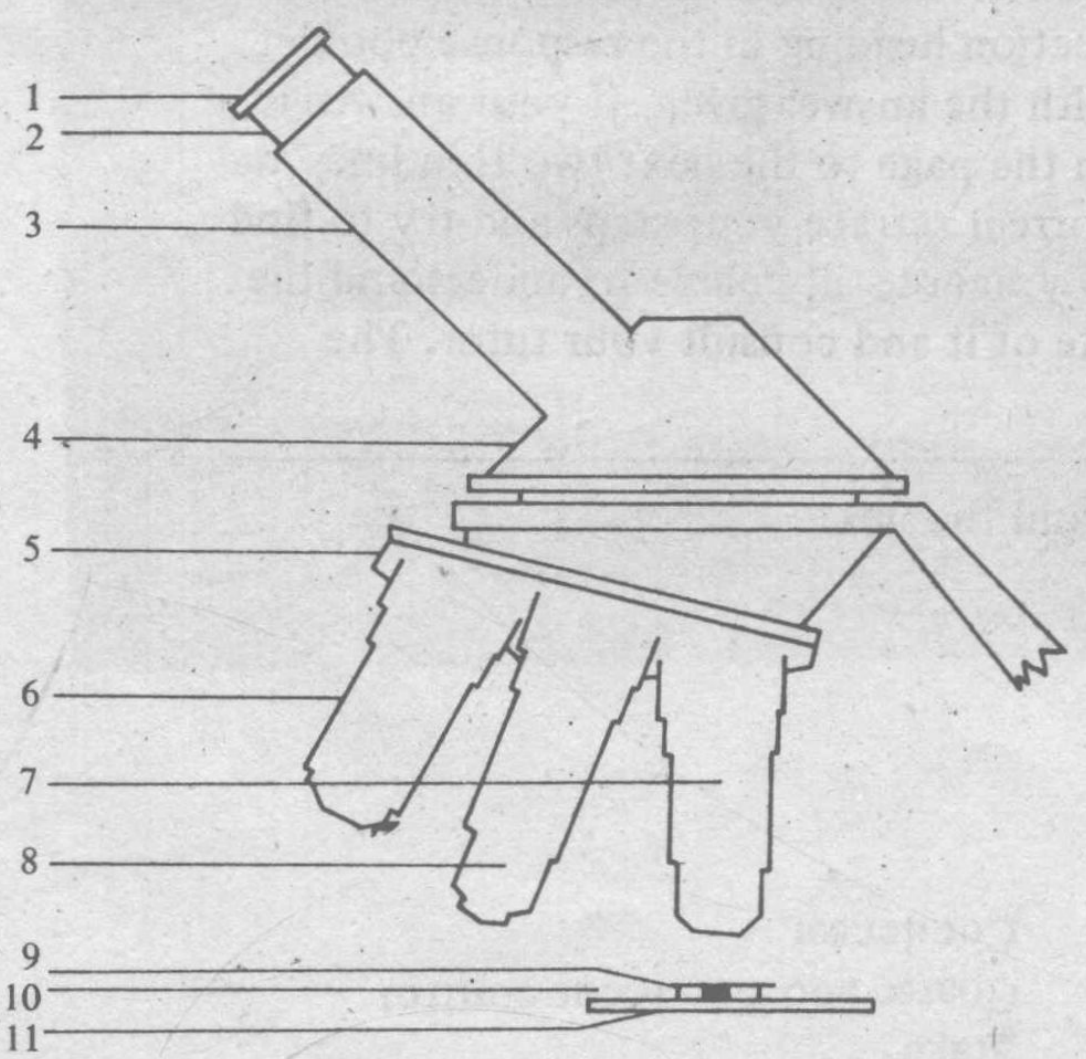
In this section you examine the structure and function of your microscope and learn how to adjust the moving parts. The next section covers operational instructions. All parts marked 'M' are movable and should be moved in the manner indicated. All parts marked 'R' are removable and should be carefully removed and examined.

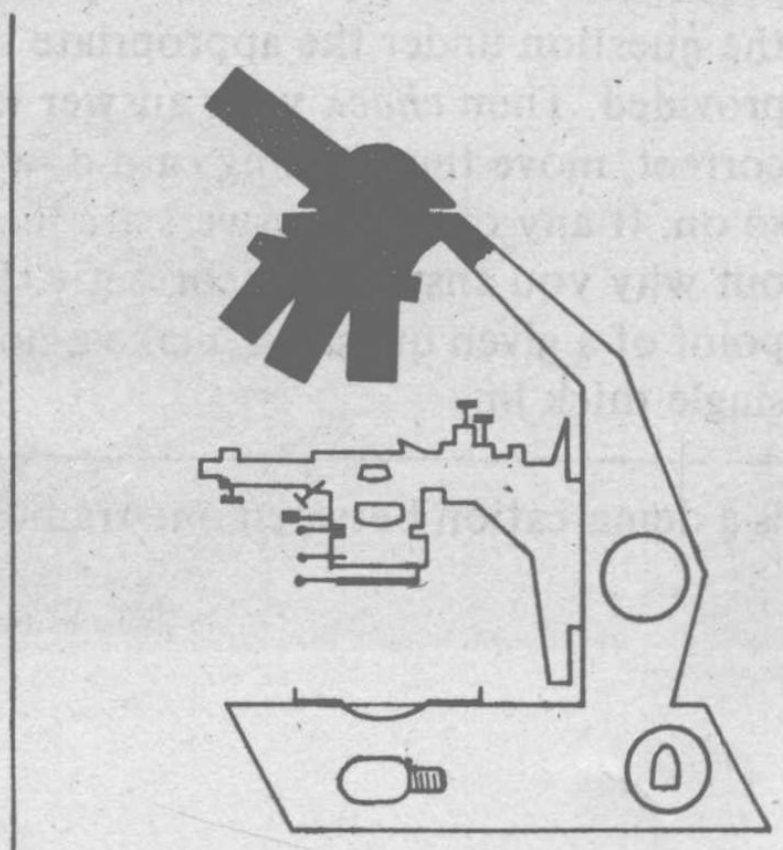
For convenience this section has been divided into three, each subsection dealing with a different aspect of the microscope's structure.

1.1.2. The magnification system

Examine your microscope with the aid of the information and diagrams below. *Do not allow dust or moisture to enter the microscope.*

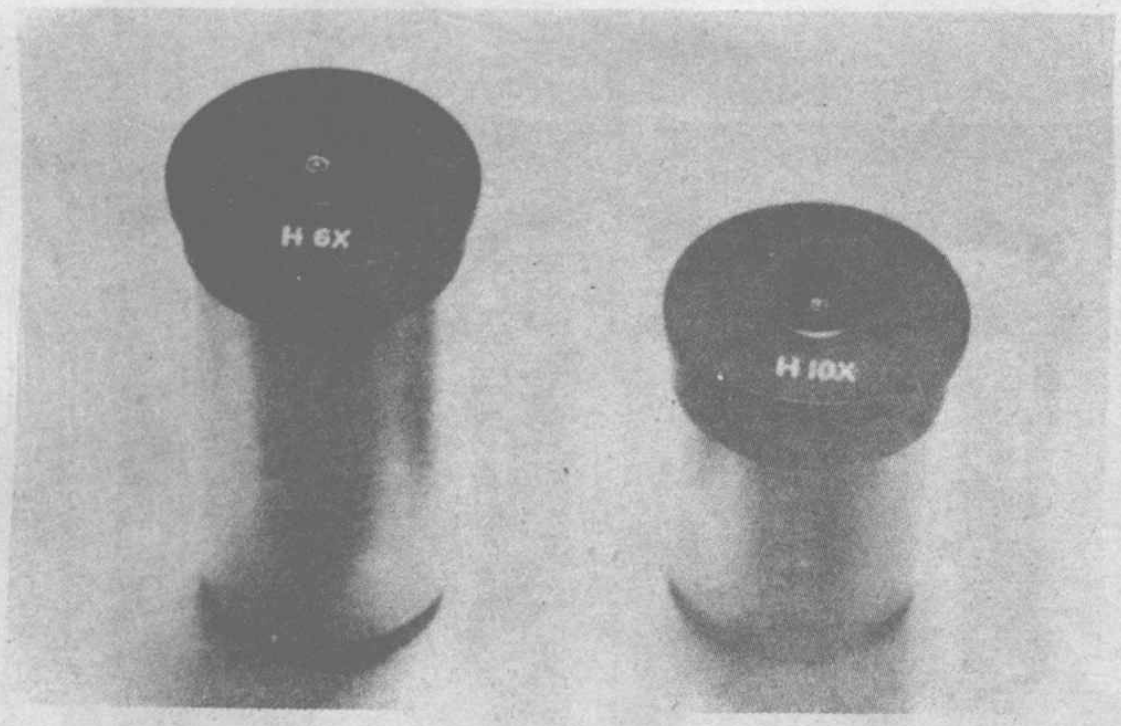
R 1. Eyepiece - can be withdrawn from the ocular tube. See opposite for detailed structure.
R 2. Collar - supports eyepiece on the ocular tube.
3. Ocular tube - sloping for ease of viewing.
M 4. Rotating turret or monocular body - for demonstration purposes.
M 5. Rotating nosepiece - carries the objectives. Moved by rotating until the desired objective clicks into position in the light path, i.e. right over the object.
R 6. Low-power objective - can be unscrewed. See page 6 for detailed structure.
R 7. High-power objective - ditto.
R 8. Oil-immersion objective - ditto.
9. Coverslip - a thin square of glass covering the object.
10. Object - mounted in air, water, resin, etc.
R 11. Microscope slide - oblong glass sheet supporting the object.





STRUCTURE

Eyepieces



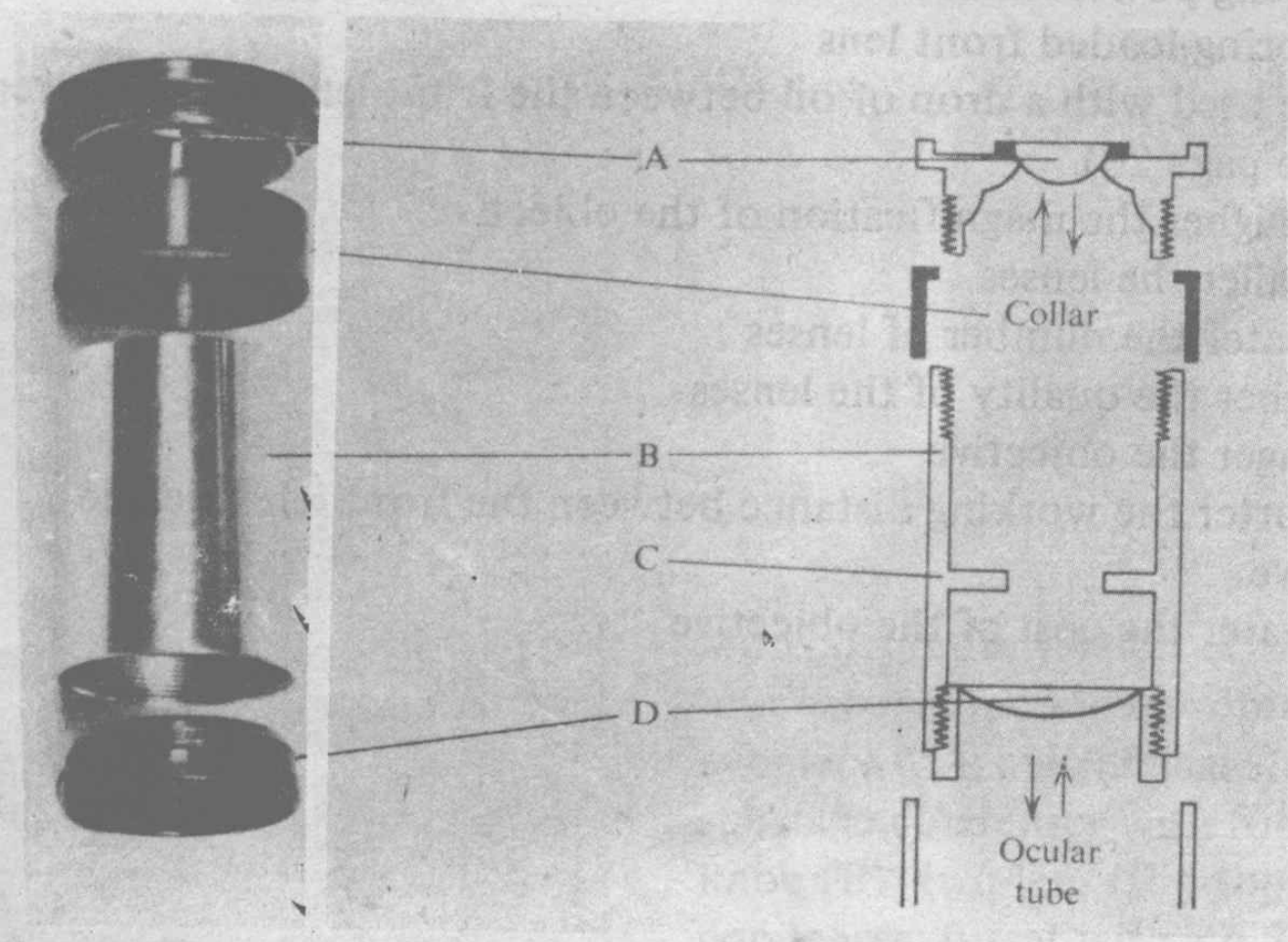
R 1. *Eyepiece*
Withdraw the eyepiece from the ocular tube and examine it with the aid of the information and diagram below. *Do not allow any dust, grease or moisture to settle on the inner surfaces of the eyepiece.*

R A. Eye lens - supported in a unit which may be unscrewed from the eyepiece tube.

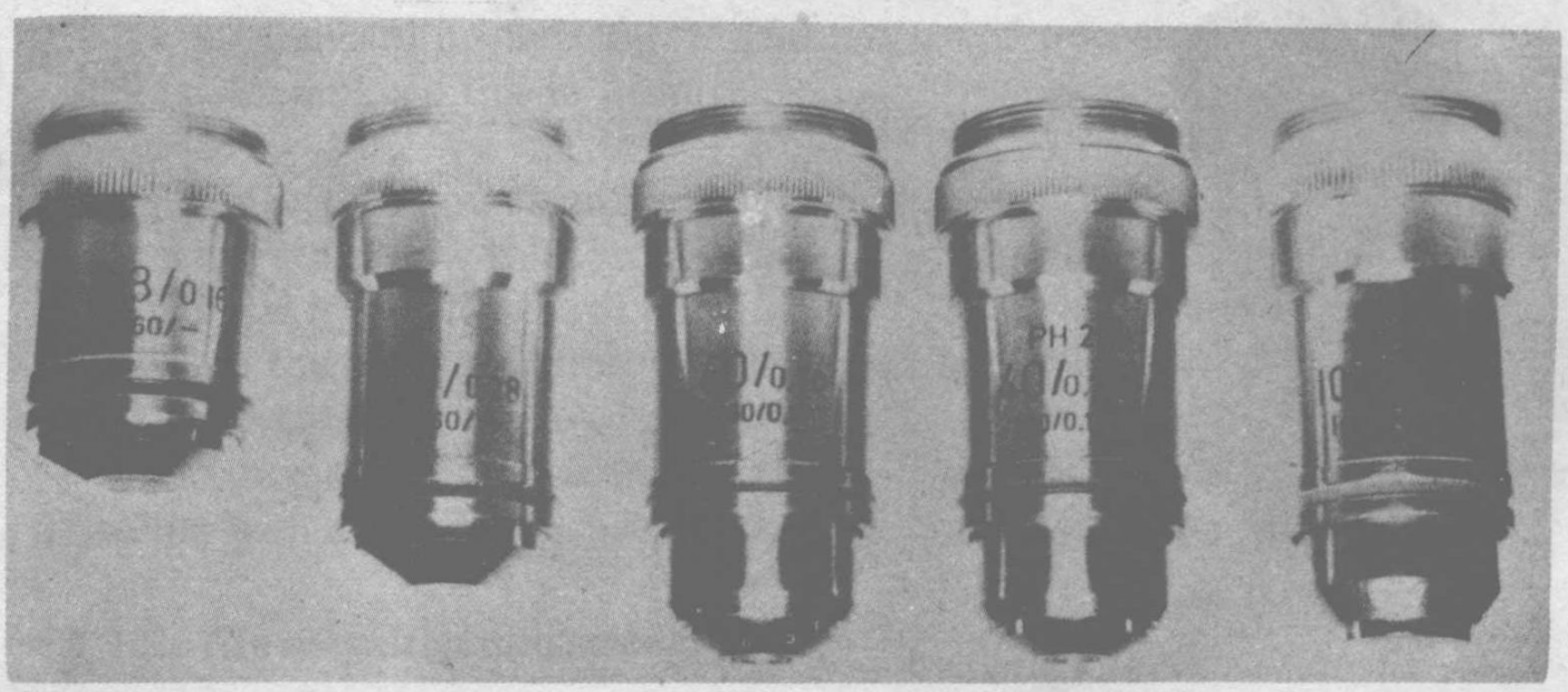
B. Eyepiece tube.

C. Field diaphragm.

R D. Field lens - supported in a unit which may be removed from the eyepiece tube.



Objectives



Magnification ×6.3 ×10 ×40 ×40 ×100

R 6. *Low-power objective*
- A. Abbreviation L.P.
- B. Is marked with a *BLUE band**
- C. Magnifying power ×10

R 7. *High-power objective*
- A. Abbreviation H.P.
- B. Is marked with a *GREEN band**
- C. Magnifying power ×40

M D. Has a spring-loaded front lens to reduce damage caused if the front lens is allowed to touch the object.

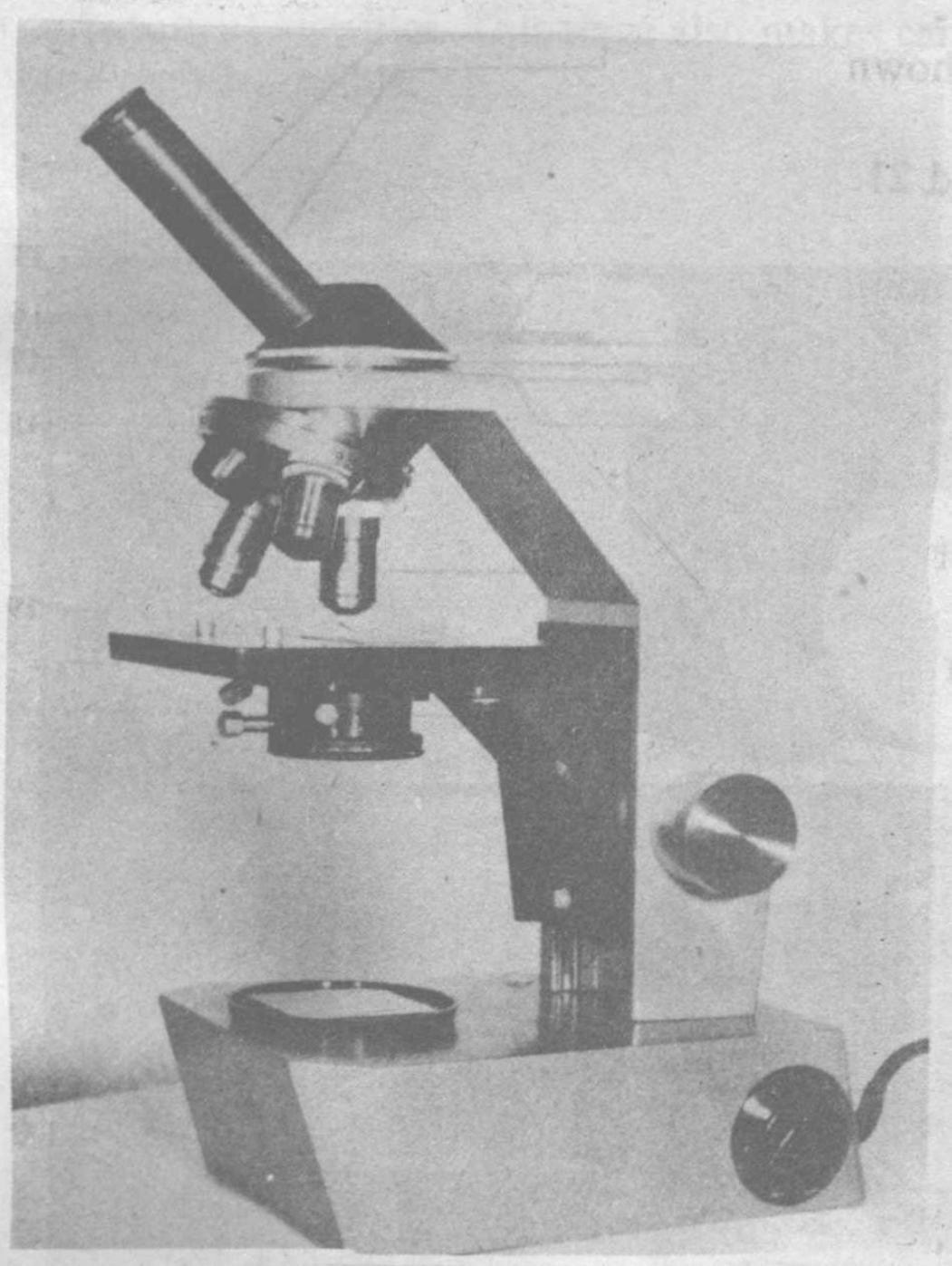
R 8. *Oil-immersion objective*
- A. Abbreviation O.I.
- B. Is marked with a *RED band**
- C. Magnifying power ×100

M D. Has a spring-loaded front lens
- E. Must be used with a drop of oil between the front lens and the cover slip (see page 26).

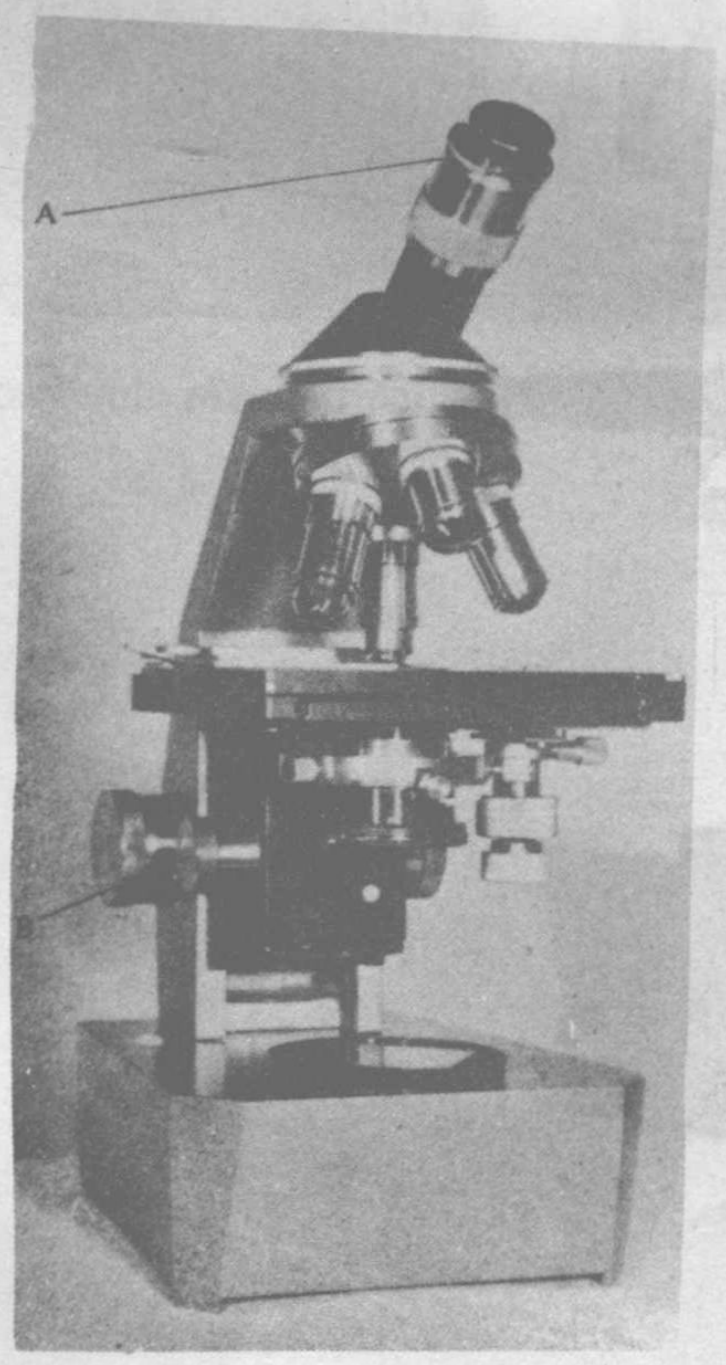
NOTE. The higher the magnification of the objective:
- A. The smaller the lenses
- B. The greater the number of lenses
- C. The higher the quality of the lenses
- D. The longer the objective
- E. The shorter the working distance between the front lens and the objective
- F. The greater the cost of the objective

*Colour codes are not used on many foreign microscopes; look carefully therefore at the magnification marked on the objectives.

Variations in the design of the Gillett & Silbert microscope



Basic monocular microscope with spring clips instead of mechanical stage; fixed, single lens condenser; turret with ×6.3, ×10 and ×40 objectives.



More elaborate version of the same microscope; monocular drawtube eyepiece (A); mechanical stage; substage condenser with focusing knob (B); two-lens (flip-top) condenser; turret with ×6.3, ×10, ×40 and ×100 objectives.

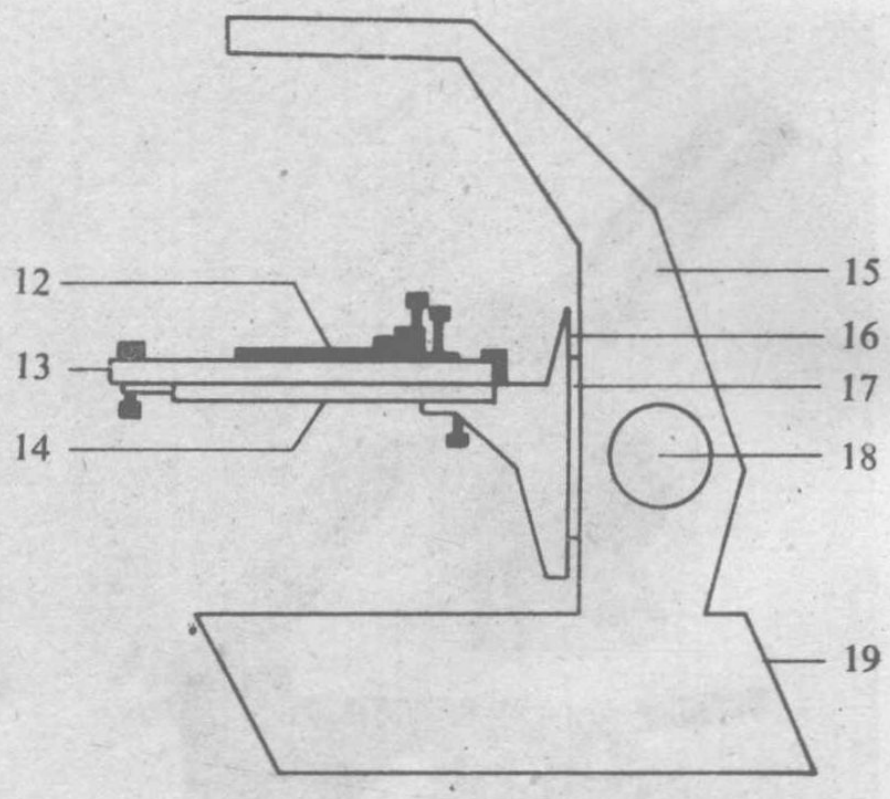
1.1.3. The mounting and movement system

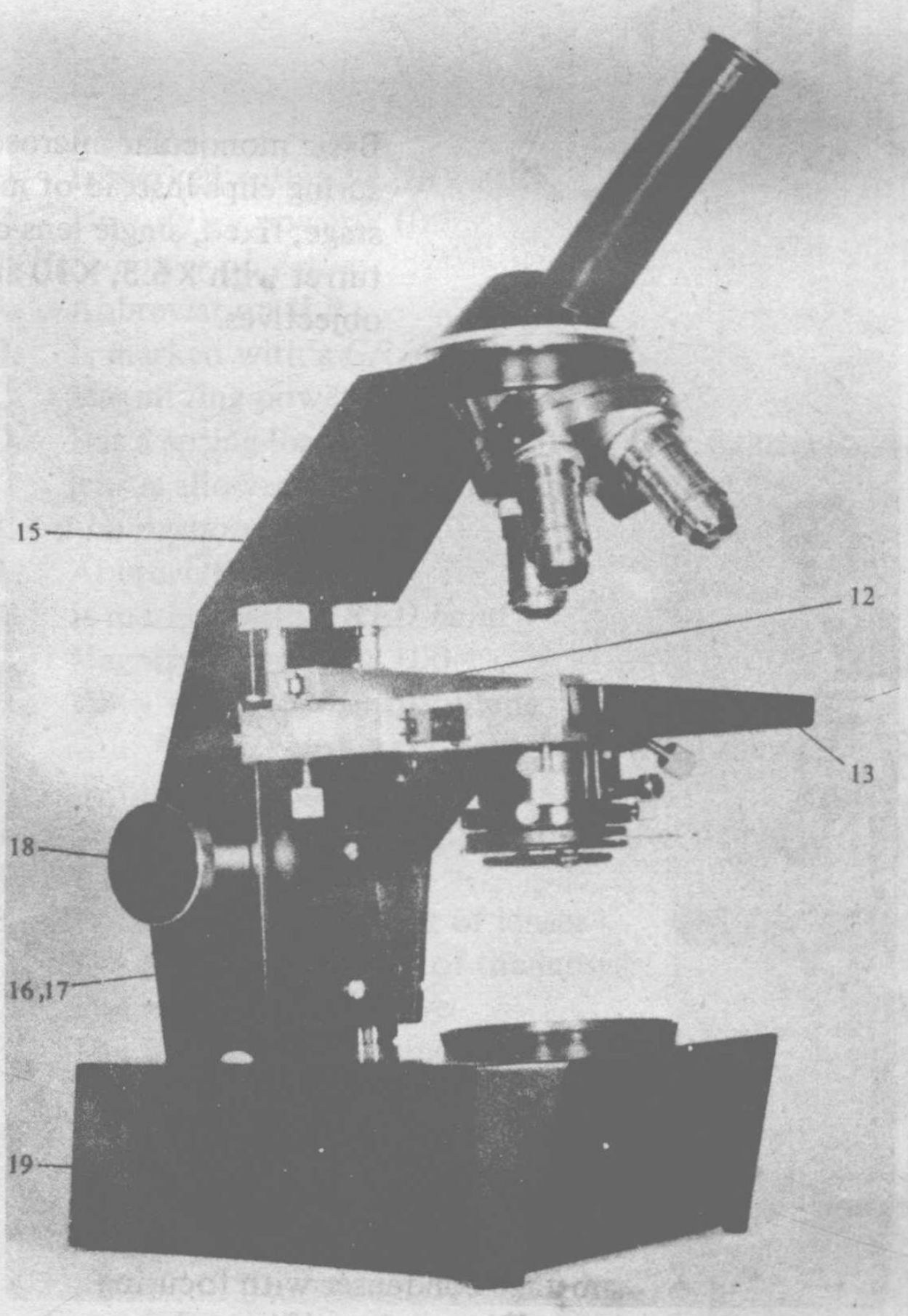
Examine this part of your microscope with the aid of the diagrams and information below.

12. Attached mechanical stage - blackened parts on right (shown enlarged on page 9).
13. Fixed stage. See also 20 and 21.
14. Stage support.
15. Arm, linking the magnification system, object and the illumination system.
16. Bracket supporting stage on arm.
17. Ratchet for moving bracket vertically.

M 18. Coarse and fine control for ratchet (17).

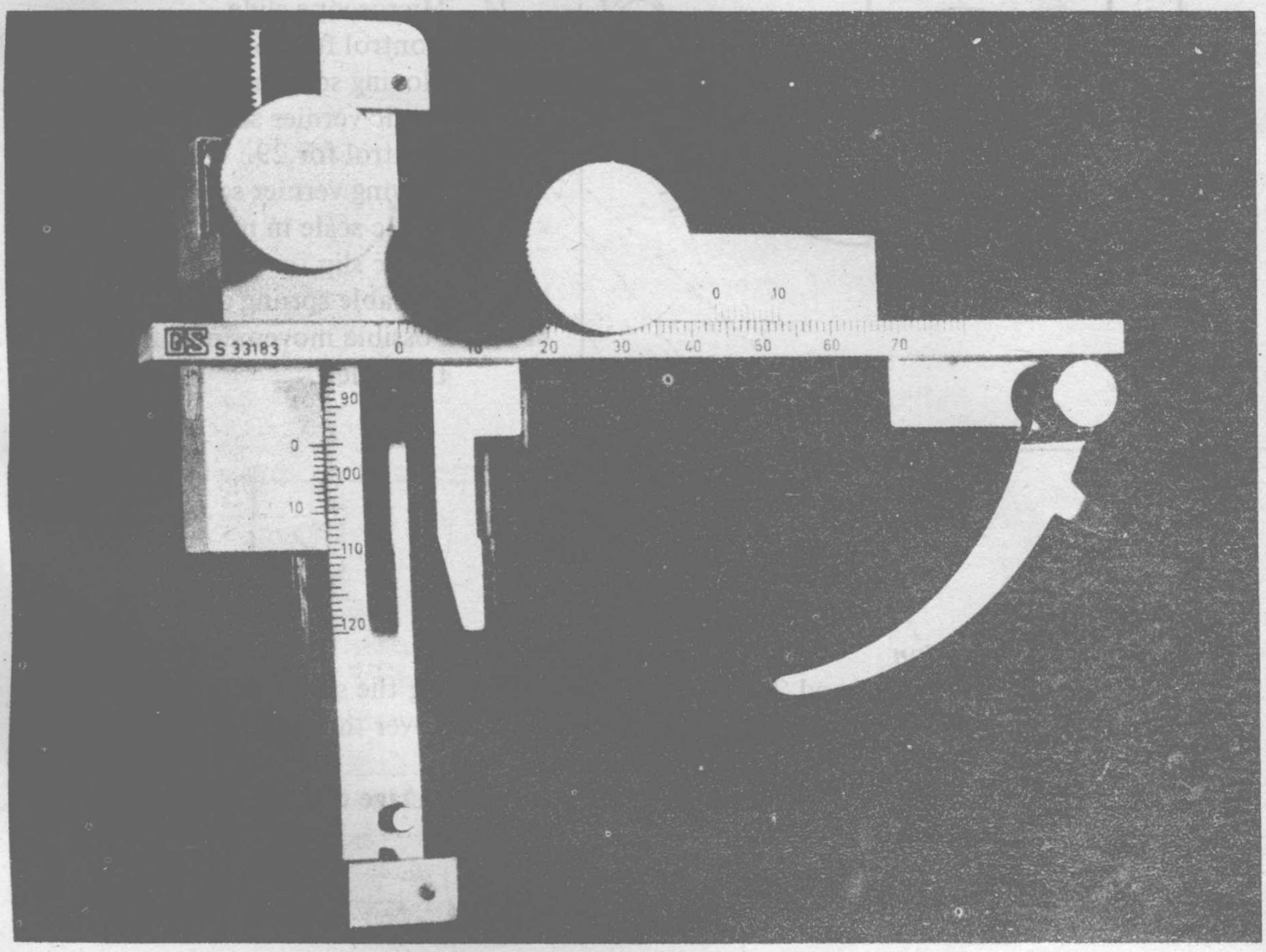
19. Base.





The mechanical stage

This stage is equipped with scales which permit accurate mechanical positioning of the slide - and any point on the object. A part of the object often has to be viewed on several separate occasions. If the slide position is recorded after the first viewing, the point can be mechanically centred for subsequent viewings. This stage also makes carefully controlled movement of the object possible.



Detachable mechanical stage