WORKING *with the* MICROSCOPE

JULIAN D. CORRINGTON

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by

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Preface

MOST books written for the purpose of providing instructions in microtechnique fall into one of two classes. Either they are rankly juvenile and unscientific, invoking the sensational in an attempt to manufacture an interest in the subject, or they are highly professional and technical, designed for the upper collegiate level, and presupposing some classroom supervision.

There seems to be decided need for a manual aimed at that large body of serious workers who fall in between these two extremes. First we may list the amateur, a sadly abused designation too frequently giving an impression of some bungling tyro. Correctly used, the word amateur means one who takes up a study as a hobby or avocation, be this person a beginner or a skilled technician, often the equal and sometimes even the superior of any professional in knowledge and ability.

Such an amateur lacks one important aid. No matter how elaborate his equipment, he has no instructor at his elbow to demonstrate just how a given slide is to be prepared. The written word is never the equal of the demonstration; once we have seen someone else do an act correctly, it is easy to imitate and to understand. However, the next best thing is a book of instructions, and our aim in the present case has been to write a graded series of exercises in the mounting of materials for observation under the microscope. Beginning with the simplest and proceeding by easy stages to advanced operations, the explanations have been made as detailed and nontechnical as possible. The plan is to learn as you go, with the intent of training the reader from scratch or from any point he may previously have reached.

It must be emphasized that this volume does not pretend to include directions for the making of every known sort of slide; indeed, such an encyclopedia is neither possible nor desirable. Having once learned the construction of a given type of preparation, the reader may be expected to apply the same manipulations in the making of many others that fall in the same category. Nor does this book attempt to teach either the facts or the principles of the many sciences to which the microscope is handmaiden; that is the province of the numerous and splendid texts, widely available, and covering such fields as biology, bacteriology, criminology, and textiles.

In addition to appealing to the amateur working alone, it is hoped that this manual will serve the needs of classes and extracurricular clubs in the high school, junior college, teachers colleges, and lower levels of senior colleges and universities, as well as organizations such as microscope clubs, Boy Scouts, and similar groups unaffiliated with schools. The reading matter is designed for adults, from high school age onward, and the various chapters have been made progressively advanced, stimulating the user toward constantly higher attainments in both an understanding and an application of the materials and methods with which he is working.

The reader is advised to use the Index freely and refer to Chapter 17 for formulas. Consult the Appendixes and the List of Abbreviations. Above all, master each chapter as it is taken up and do not go on to the next until you are satisfied with your results. When in doubt as to the degree of proficiency attained, ask for a criticism of your slides by a college or high school teacher of biology, or buy a professionally made slide of the same subject from a supply house, for comparison.

Little of the material in these pages is original; what merit the volume possesses depends primarily on organization and presentation, together with illustrations that often go far toward overcoming the handicap of the written explanation of some performance. Most of the methods are time-honored and to be met with in numerous manuals. In particular cases, acknowledged at the appropriate place, we have borrowed extensively from a number of standard works.

Among those to whom we feel especially indebted for cooperation and for permission to include copyright material are the University of Chicago Press, for techniques described in Guyer's *Animal Micrology* and Chamberlain's *Methods in Plant Histology;* the Collegiate Press, Ames, Iowa, for excerpts from Becker and Roudabush, *Brief Directions in Histological Technique;* Bausch & Lomb's *Educational Focus* for illustrations; the General Biological Supply House, Inc., Chicago, for various formulas; and Ward's Natural Science Establishment, Inc., Rochester, New York, for loan of the slides from which photomicrographs were made. Every volume of this nature depends a great deal also on Lee's *Microtomist's Vade-Mecum,* now edited by Gatenby and Painter and published by The Blakiston Company, Philadelphia, and on the journal, *Stain Technology,* edited by H. J. Conn. Our thanks to all of these authors and firms.

Professor Robert T. Hance of Duquesne University, Pittsburgh, gave generous permission to reproduce a number of his new procedures. The RCA Manufacturing Company, Camden, New Jersey, provided an illustration of their famous electron microscope, while leading optical companies furnished illustrations of instruments, including Bausch & Lomb, Spencer, Zeiss, Leitz and Reichert (Pfaltz & Bauer). Owing to conditions in Europe at the time of publication we were unable to extend this representation.

Most of the photomicrographs appearing in these pages are the work of John V. Butterfield, while the pen drawings were designed by Veronica E. Corrington.

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Abbreviations Used in This Book

The abbreviations indicating measurements include both singular and plural, as hr. is either hour or hours, ft. is foot or feet.

A —alcohol, percentage of. Throughout the book such phrases as 70 per cent ethyl alcohol, or 70% alcohol, are rendered as 70A.
C. —centigrade.
cc. —cubic centimeter.
c.p. —chemically pure.
E.F. —equivalent focus.
F. —Fahrenheit.
Fig. —figure (illustration).
ft. or ′ —foot.
g. —gram.
hr. —hour.
in. or ″ —inch.
min. —minute (of time).
ml. —milliliter.
mm. —millimeter.
mu or μ—micron.
N.A. —numerical aperture.
oz. —ounce.
p. —page.
R.I. —refractive index.
sec. —second (of time).
U.S.P. —United States Pharmacopeia.
wk. —week.
$\times$ —magnification, as 10$\times$ means an enlargement of ten times a linear dimension.

Contents

WORKING WITH THE MICROSCOPE

CHAPTER I

THE MICROSCOPE

In This Chapter: parts of the compound microscope and their use; setting up the microscope for work, focusing, proper illumination; care of the instrument and of the eyes; definition and kinds of microscopes; the science of microscopy.

MICROTECHNIQUE deals with the preparation of material of one sort or another so that it may be studied under the microscope to the best possible advantage. The operations are many and varied and will claim our attention throughout the following chapters; just at this point, however, we are mindful of certain readers for whom the microscope and its employment will constitute a new experience. These will need first of all an introduction to the microscope itself, together with some indication as to kinds of magnifying instruments and the many purposes for which they may be used.

Before going into the matter of defining a microscope, let us analyze the make-up of the variety that most people have in mind when the word is mentioned—the regulation biological compound microscope. Certain details that follow will apply only to particular models, but the general description will fit all ordinary types.

The instrument as a whole (Fig. 1) may be divided into two parts—mechanical and optical. The *mechanical parts* are secondary, but necessary for the ease of operation and the exactness of results. There must be a *stand*, the framework for all other parts, defined farther on. This rests on a *base*, the favorite design for which is a horseshoe, having three points of contact with the table top and using the familiar principle of support of the tripod. Rising from the base is the *pillar;* at the top of this, in the more expensive types, is an *inclination joint* which permits tilting the remainder of the instrument at any desired angle, often useful as a matter of comfort but having no effect otherwise, hence not an essential feature

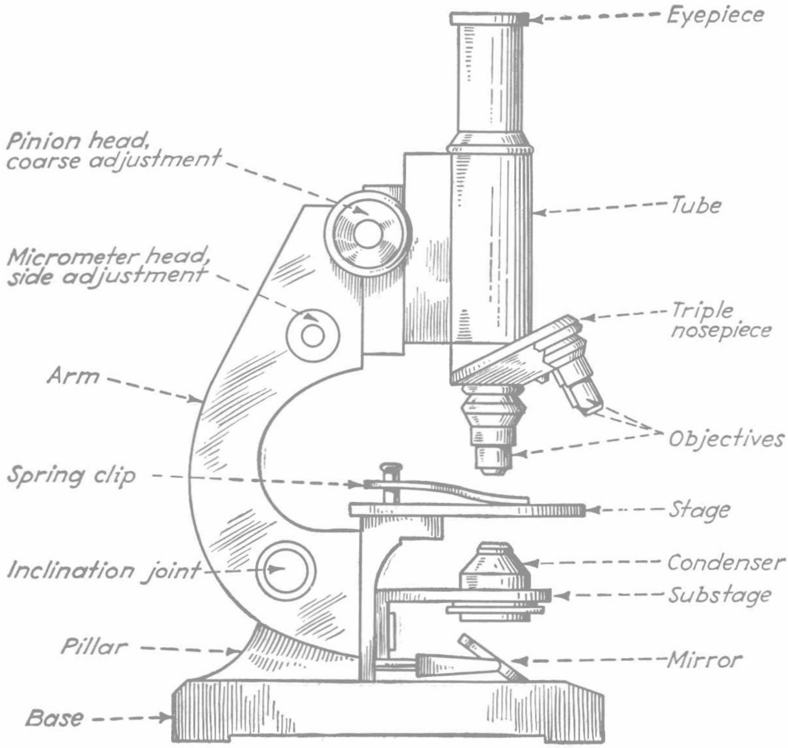

Fig. 1.—The compound microscope.

Jutting forward from the top of the pillar is the *stage*, which is truly named, since it is here that the many actors, living or inanimate, will be placed to perform for your magnified vision. A central opening or *stage aperture* allows for the transmission of light rays from below, and two *spring clips* are customarily provided in order to hold a slide securely in position. In the more costly instruments there is a *substage* beneath, carrying a *condenser*, which is actually a secondary microscope for the purpose of focusing light rays upon the specimen. In all cases there will be a *mirror*, usually with one surface plane and the other concave. In laboratory models this reflector is supported by a *mirror arm* or *bar*, swinging like a pendulum, and having at its end a *mirror fork*, rotating vertically, while the mirror itself turns within the fork on a horizontal axis. Thus, with three different motions available, the mirror may be set quickly in any desired position or at any angle.

Immediately beneath the condenser and mounted as one of its parts is a very important accessory, the *iris diaphragm*. Like the same appliance on a camera shutter, this consists of a number of very thin pieces of curved metal, operated by a lever so as to squeeze down the opening almost to the vanishing point. In microscopes of standard size that have no condenser, either an iris or a disk diaphragm is generally mounted immediately beneath the stage aperture and serves to regulate the amount of light admitted to the specimen. The *disk diaphragm* is a revolving wheel containing a series of holes of regularly decreasing diameter, any of which may be aligned with the stage aperture. It gives the same effects as an iris but is somewhat less flexible. Miniature microscopes have no diaphragm; to make up for this omission, we can endorse the ingenious idea sent us by several correspondents, who in one way or another have contrived to fasten an old camera iris to the underside of the stage.

Rising above the inclination joint is the *arm*, which is that part of the stand carrying the *tube*, also known as the *body* or *body tube*, the carrier of the optical parts. In order to focus the lenses, this tube must be capable of vertical motion, for which purpose there are generally two wheels. The *coarse adjustment*, sometimes the only one present on cheap models, operates by a rack-and-pinion gear and changes the elevation of the tube rapidly; the knurled wheel grasped by the operator for this action, generally one on each side, is the *pinion head*.

If a *fine adjustment* is also present, it is actuated by a smaller knob, the *micrometer head*. There may be only one of these heads, located in older instruments at the top of the arm and revolving horizontally, when the arrangement is termed a "top fine adjustment." If situated at the side of the arm, below the pinion head, and revolving vertically, the style is a "side fine adjustment," with either single or double micrometer heads. In many of the better models the right-hand one of these heads bears a micrometer scale so that the exact amount of vertical motion may be determined. Further, the fine adjustment may have definite upper and lower limits, reaching either of which it refuses to turn farther, or it may operate on an endless, reversing gear. In the high-priced instruments, movement of this adjustment ceases as soon as the objective touches the cover glass over the specimen, thus preventing damage to either object or lens. The fine adjustment moves the tube up and down very gradually and so permits accurate focusing, but it is necessarily one of the most expensive parts of the stand and is dispensed with in miniature or other of the less expensive forms of microscope. In some amateur instruments, the same service is performed by a button that moves the stage instead of the tube, effecting a marked saving in cost of construction.

The tube is a hollow cylinder bearing the objective at the lower end and the eyepiece at the upper. In some models, one objective must be unscrewed and removed before another can be put in position; in others higher power is gained by adding one objective lens directly upon the first one; in still others, there is a *revolving nosepiece* carrying two or more objectives so that lenses of different powers may be swung into line as required, and all of them protected from dust whether in use or not.

In some instruments there is a *drawtube* at the top of the body tube, pulling out like the sections of a small telescope. This act increases the magnification.

So much for the mechanical parts, all of which except the tube and its accessories are collectively referred to as the *stand*, previously mentioned. The *optical parts* include the

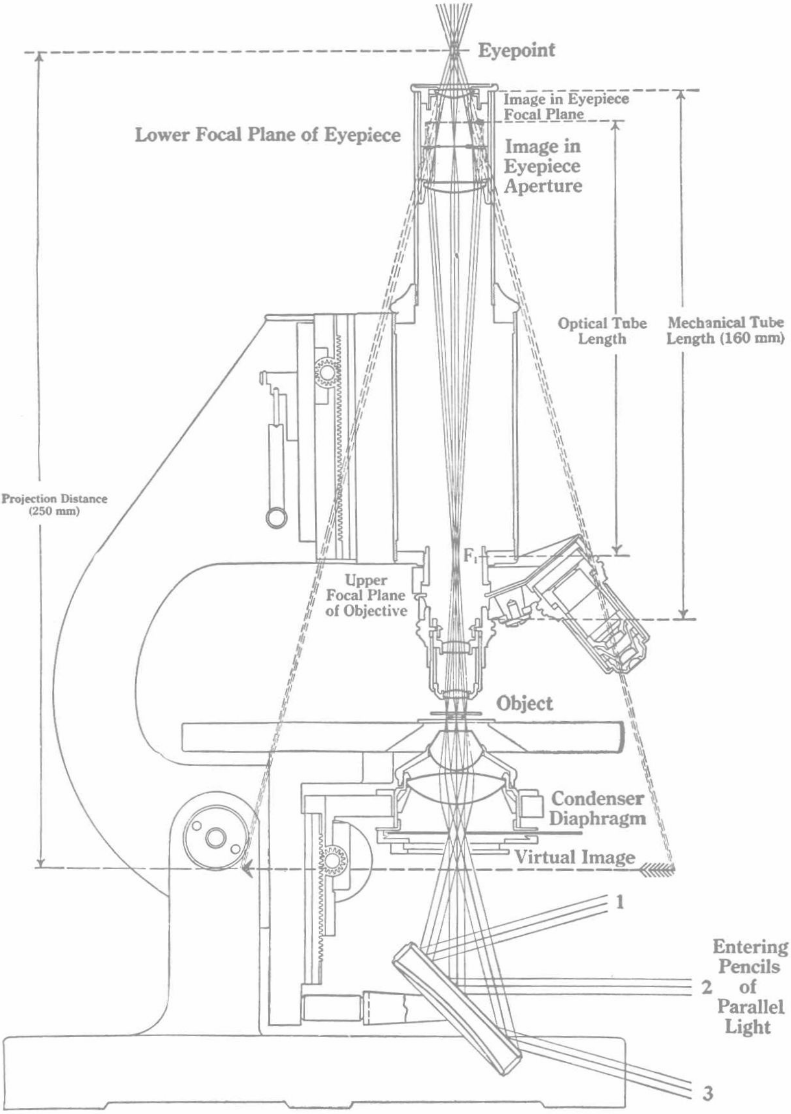

FIG. 2.—Path of light rays through a compound microscope. (*Courtesy of Bausch & Lomb.*)

mirror, condenser (when present), objective, and ocular, all of which must be in perfect alignment or, in other words, lie along the *optical axis* of the microscope. The aperture and specimen must also be placed on this axis, which is that of a beam of light in passing from the mirror surface through the aperture, specimen, and lenses to the eye (Fig. 2). The eye itself is an optical instrument, with its own focusing lens and sensitive recording plate or retina, and forms an integral part of the whole system whereby magnified images of objects are recorded in the brain.

The *objective* is so called because it is situated next to the *object*, the specimen being examined. The *eyepiece* or *ocular* is that part nearest the eye of the *observer*. Object, microscope, and observer are hence the triple alliance for all studies of the very little. In this book we shall devote the lion's share of our time to the most variable as well as the most easily altered of these three elements—the object.

The commonest type of laboratory microscope is equipped with two oculars and two objectives. The shortest objective in any given series is always the lowest in magnifying power; the reverse is true of oculars, the longest ones being the lowest in power. Since any eyepiece can be used in conjunction with any objective, if two of each are present, four combinations are possible. The more elaborate stands are equipped with a triple nosepiece carrying three objectives, the additional one being an *oil immersion* lens, designed for use only when connected to the cover glass over the specimen by a drop of especially purified cedar oil.

Most users of microscopes are interested in questions of magnification and will wish to know how the various powers are designated. For many years there was no universal standard for indicating the performance of an eyepiece or an objective, and even today not all manufacturers use the same schemes. The earliest markings were arbitrary symbols, as 1, 2, 3; I, II, III; or A, B, C, the first in each such series denoting the lowest power. When optical instruments came into general use, however, there