The Microtomist's Formulary and Guide

by

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Preface

The preface of a book affords the author an opportunity of speaking to his reader in a comparatively direct and personal manner, and of acquainting the prospective user of the book with the considerations which impelled the author to write it. (From *The Bookman's Glossary*. 3d ed. New York, Bowker [c. 1951]. Reprinted by permission of the R. R. Bowker Co.)

A few generations ago the English periodical *Punch* offered to its readers a "letter of advice to those about to be married": the applicants received the single word "Don't." The advice is pertinent for those about to write a source reference work

You may well, in reading this book, become incensed at what you believe to be its inaccuracies, errors, and faulty arrangements. This is exactly how I felt, twenty years ago, when I struggled with the reference books on microtechnique which I was then using. You may decide, as I did, to try to write a better book.

You will find it a wearisome and disillusioning task. The research will, of course, be wholly delightful, but it will be followed by a period of brutal hard labor. Not only will you have to write, but then, if you are to produce a publishable book, it will have to be condensed and rewritten. Add to this the fact that the finished work has then to be reread four separate times as it goes through press, and you will join me in hoping that your activities do not too strongly resemble those of the dog mentioned in the Book of Proverbs.

This book would never have been completed without the help of the librarians of the University of Edinburgh, the Wood's Hole Marine Biological Laboratory, the University of Rochester, the Carnegie Library of Pittsburgh, and the University of Pittsburgh. I am especially indebted to Miss Lorena Garloch, and her assistants in the Reference Department of the University of Pittsburgh Library, for their extraordinary skill in tracking down obscure journals and securing them for me on inter-library loan.

The illustrations for this work, as for my *Handbook of Basic Microtechnique*, were prepared from my photographs and sketches by Mrs. Gloria Green Hirsch. I am glad that reviewers of the published book share my enthusiasm for her work.

The number of those, including the author and his wife, who have had a hand in typing this book is legion. It should be recorded, however, that Mrs. Mary Roman single-handed produced the first complete (1500 page) typescript

and that Mrs. Dolores Johnson, and Miss Kristine Pallesen, have stood by the author during the distressing hurly-burly known as "getting the book into press."

Dr. James Lackey, then scientific editor of the Blakiston Company, encouraged me over long periods to persevere in producing a publishable book. His successor, Mr. William Keller, approved what had been done and, with Mr. Willard Shoener converted my efforts to their present form. My debt to these gentlemen, and to their editorial assistants, is immense.

Acknowledgement is made with thanks to the American Optical Company for figures 56, 57, and 84, to the Fisher Scientific Company for figures 34 and 38, and to the Carbide and Chemical Corporation for some of the data in Chapter 25. Permission to reproduce copyright material of the R. R. Bowker Company and the Oxford University Press is specifically acknowledged at the places where these reproductions occur.

PETER GRAY

EDINBURGH 1933
PITTSBURGH 1953

List of Contents

Introduction

Scope of the Book

This work consists of two parts. Part I (Chapters 1–16) is a treatise on the art of making microscope slides from biological specimens. Part II (Chapters 17–28) is a classified list of the formulas and techniques used in this art.

Arrangement of Part I

Each chapter deals with a specific type of microscope slide and is divided into two parts. The first part discusses problems involved in the preparation of such a slide and the general methods by which these problems have been overcome. The second part is devoted to one or more specific examples which describe in detail the application of the general methods to the production of an actual slide. The few literature references in Part I are confined to places where the author is describing a method of which he lacks personal experience, or where he is giving opinions at variance with his own.

Arrangement of Part II

The chapters in Part II are devoted to specific types of formulas and give, where necessary, the techniques by which these formulas are used. Each chapter is subdivided decimally in accordance with a scheme given in full at the beginning of the chapter and explained in the first paragraph of the chapter. Every formula or technique is thus identified by a number which is used, together with two or three letters identifying the chapter, in all cross references. Thus:

DS 11.122 Mayer 1891

identifies a specific alum-hematoxylin of Mayer (he published five other alum-hematoxylin formulas) in any of the fifty places that reference is made to it. The formula is given only once and then in association with all the other alum-hematoxylin (DS 11.122) formulas in the book. These decimal reference numbers are added to the page numbers all through, thus making it easy to run down a given type of formula or technique.

Pet Names

Some biologists have a pernicious habit of omitting literature references and using what Conn 1938 (20540b, 13:121) in a well-organized attack on them, calls "pet names." In cases where these pet names—such as *paracarmine* or *B 15*—have become embedded in the folklore of microtechnique, the present author puts them in italics, immediately after the decimal reference, thus:

DS 11.22 Mayer 1892 *paracarmine—compl. script.*

The appended *compl. script.* indicates that the word has occurred in a "great many writings." When only the originator of the technique appears to have used the pet name it is referred to with *auct.* Pet names should never be used in scientific writing but such sloppy scholarship as is inherent in a reference to "Bouin's Fluid" is almost worse. Bouin is the originator of many fixatives of which one happens to be popular at the moment; quite another was popular twenty years ago, when a casual reference to Bouin's Fluid meant a mercuric formaldehyde mixture.

Method of Giving Literature References

The author indicates, after every formula or technique, the source from which he is quoting. The form used varies according to the type of source and an example of each will be given.

DIRECT QUOTATIONS FROM BOOKS. The author's name, date, and page only are used, thus:

DS 11.123 Anderson Anderson 1929, 129

At the end of Part II there is a list of books cited where "Anderson 1929" is expanded to a full bibliographic reference.

INDIRECT QUOTATIONS FROM BOOKS. It too frequently happens that a book quotes a formula by name, either without giving a reference at all or with an incorrect reference. When the present author has been unable to find the original he uses the abbreviation *test.* Thus:

DS 11.123. Conklin *test.* **1930** Guyer Guyer 1930, 232.

This indicates that the volume in question contains, on page 232, a formula for Conklin's picro-hematoxylin but offers no information as to where the original can be checked.

Where the author of a book cited is quoting at second hand, the abbreviation *cit.* is used. Thus

DS 11.24 Vignal *test.* **1907 Böhm and Oppel** *cit.* Henneguy Böhm and Oppel 1907, 118

indicates that, on page 118 of the volume in question, there is a statement to the effect that Henneguy proposed, following the method of Vignal, to prepare a picro-carmine by this particular method.

Where the author of a book cites himself, or the authors cite themselves, without reference, the abbreviation used is *test. ips.* (standing for *teste ipso* or *testibus ipsis*). Thus:

MS 31.22 Cajal 1925 *test.* **1933** *ips.* Cajal and de Castro 1933, 262

The present author would plead in self defense that he has tracked more than a thousand such references to the originals and that these *test.* and *cit.* references are used only where he has failed to find the original or where the original is incorrectly quoted.

DIRECT QUOTATIONS FROM JOURNALS. The author has used, in place of the name of the journal, the number assigned to that journal in the *World List of Scientific Periodicals* (Oxford, The University Press, 1927). Thus:

DS 11.122 Carazzi 1911 23632, **28**:273

indicates that Carazzi's formula is given on page 273 of volume 28 of the *Zeitschrift für wissenschäftliche Mikroskopie und für mikroskopische Technik*. The full titles of the two-hundred-odd journals cited will be found immediately preceding the index. The use of this number not only saves space, an important consideration in a volume of this magnitude, but also permits exact identification of the journal. The author decided to use these numbers quite shortly after he started checking references in the *J. Anat.* (either of two journals) and the *J. Bot.* (any one of three journals).

INDIRECT QUOTATIONS FROM JOURNALS. The abbreviations *test.*, *test. cit.* and *test. ips.* are used with journal references exactly as described for book references.

UNPUBLISHED INFORMATION. The abbreviations *in verb.* and *in litt.* indicate that the author has received unpublished information either verbally or in a letter. Thus:

V 12.2 Fant 1932 *in verb.*

indicates that Mr. Fant told the author the unpublished composition of his sealing medium for glycerol mounts in 1932.

Slavonic Names

Where the author has cited Slavonic names from a Cyrillic alphabet original, he has transliterated according to the rules of the Library of Congress (Beetle, Clara, ed. *A. L. A. Cataloging Rules for Author and Title Entries*. Chicago, American Library Association, 1949, p. 246) without regard for the writer's preference as indicated in, say, a German summary of his paper. Thus *Yasvoyn*, not *Jasswoin*, is cited from the original. Slavonic names cited from a Latin alphabet original are transcribed directly even though this involves referring to the same individual by several names. Slavonic names cited at second hand are also transcribed directly, no matter how obviously they may have been mistransliterated.

Citing Latin alphabet names from Cyrillic alphabet originals has involved even more uncertainty. Russian writers not only omit references but also follow varying rules of transliteration, some taking a phonetic and others a literal approach. The name *Huygens*, for example, can be transliterated into a Cyrillic form which can then be phonetically transliterated in German as *Geugantz*. Thus Roskin 1946 attributes to an individual whose name may be transliterated *Shteve, Steeve, Stieve,* or *Stive* a fixative which resembles, but is not identical with, the formula attributed, also without reference, to Stieve by Romeis 1948. The author has given the one as Stieve *test.* 1946 Roskin—and the other as Stieve *test.* 1948 Romeis. Faulty scholarship can certainly increase the confusion originated by the architects of the Tower of Babel.

Names of Dyes

The author has, with one exception, changed the names of all dyes to accord with the synonym preferred by Conn 1946 (Conn, H. J. *Biological Stains* 5th ed. Geneva, N. Y., Biotech, 1946). The author prefers, however, to use the name *magenta,* rather than *basic fuchsin,* to describe the mixture of magenta O, magenta I, magenta II now sold as basic fuchsin. There is no discussion of the chemistry and synonymy of dyes in the present work; reference should be made to Conn (*op. cit.*). The author has not given certification numbers or dye content in formulas, since they are available for so few.

Names of Reagents Other than Dyes

The author has in almost all cases followed the usage preferred by *The Merck Index* 6th ed. Rahway, N. J., Merck, 1952. The terms *chromic acid, osmic acid,* and *picric acid,* though technically incorrect, are so universal in biological literature that they have been retained. Similarly the terms *alcohol* and *absolute alcohol* (abbreviated in the formulas to alc. and abs. alc.) have been used in place of *ethanol.*

Chemical names used are those customarily found on the label of the reagent bottle and are not accompanied by chemical formulas, or otherwise qualified, unless the reagent is found equally commonly in several forms. Thus *copper sulfate* indicates the usual reagent $CuSO_4.5H_2O$. On the rare occasions when reference is made to the anhydrous salt, it is referred to as *copper sulfate, anhydr.* In any case of doubt, reference should be made to the Merck Index.

Proprietary Compounds

Proprietary compounds of known composition, such as *amidol* and *salvarsan* have been referred to by the name preferred in the *Merck Index.* Proprietary compounds of secret composition have no place in contemporary science and have been ignored. It is fantastic that purveyors of reagents should be permitted to sell nostrums of secret composition, thus indicating a contempt for technicians equal to that shown by medicine men for the yokels they gypped with snake oil. The author would make it very plain that he does not extend this attitude to "brands" of mixtures or reagents selected for technicians' use. Every maker of microscope slides is indebted to those firms which select and blend materials specially for his use.

Quantities and Measures

The abbreviations ml. and Gm. have been omitted. It is to be presumed that all liquids will be measured in milliliters and all solids in grams. Formulas have been adjusted to give a rational total (usually 100) in terms of standard ingredients, no matter how the original was presented. This has been wearisome labor applied to thousands of formulas. It is doubtless convenient to make up a solution by adding fifteen drops of a 2.5% solution of this to 30 drops of a 1.25% solution of that and then to dilute to 15 milliliters with 30% alcohol. As a published instruction, however, it does not commend itself to writers of textbooks struggling to avoid duplication.

Index

The last section of the book is a single-alphabet, fully expanded, index, alphabetized according to the rules of the American Library Association (Beetle, 1949, *op. cit.*). These terms may require explanation.

A *single-alphabet index* is one in which all entries are placed in the same index. There are not separate indexes for authors, stains, etc. *Fully expanded* means that more than one entry leads to the information sought. For example, "Grenacher's alcoholic-borax-carmine" may be found whether the reader consults the word "Grenacher," "borax-carmine," alcoholic-borax-carmine," or "carmine." A condensed index, which saves the author work and the publisher money without regard to the reader's feelings, has "borax carmine *see* Grenacher," "carmine staining solutions, *see* author's name," etc. etc.

Those who do not think it necessary to have rules of alphabetization might try indexing *del Rio-Hortega, 2BD fixative, CS 13 mountant,* and *van't Hooft.* The rules of the A.L.A. (*op. cit.*) may not be perfect but they are at least clearly expressed and easily available.

Part I

The Art of Making Microscope Slides

Foreword to Part I

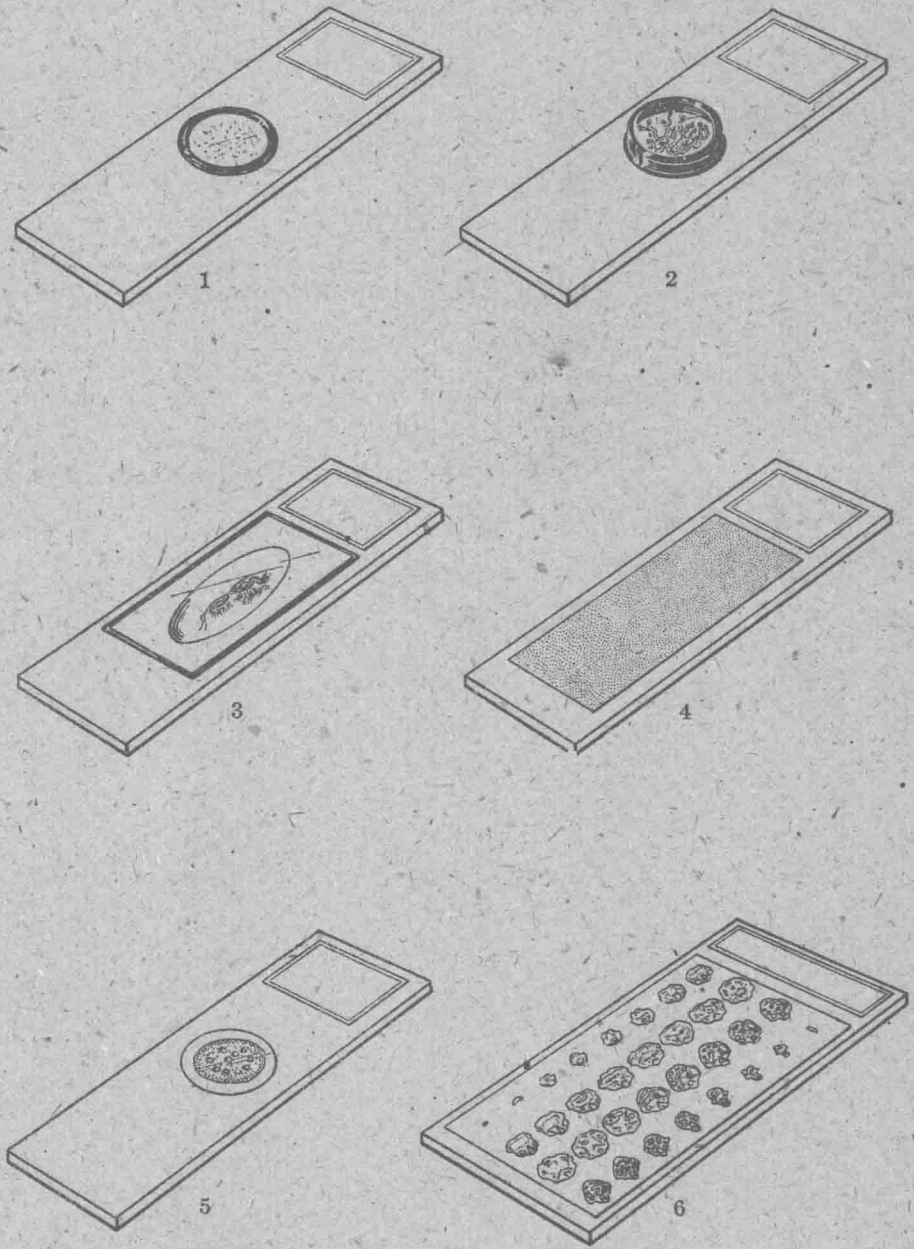
The preparation of objects for microscopic examination—more colloquially known as "making microscope slides"—has a twofold purpose. On the one hand it may be desired to preserve in permanent form objects too small or too delicate to be handled by the ordinary methods of museum preparation. Second, and far more important, it may be necessary to make permanent preparations of objects and tissues in such a manner that their structure may be more clearly seen under the microscope. In both cases, the object is mounted on a slide, which is nowadays a standardized 3″ × 1″ strip of thin glass.

Originally microscope slides were very different and were usually made by taking a slip of ivory, about 2″ × ½″, and drilling through it a hole of about ⅜″ in diameter. This hole was then enlarged from each side, about a third of the way through, to a ½″ diameter, thus leaving a ridge of ivory in the center. The depression on each side of the slide was fitted with a ring of spring steel and several disks of mica of a half-inch diameter were furnished with each slide. To make a mount, a piece of mica was inserted from one side and held in place by the slip ring, the object was placed on it and another disk of mica was then inserted from the other side and, in its turn, held in by a slip ring. This was the only type of slide available until about the middle of the 18th century when glass slides first made their appearance. These glass slides were, however, of very little use, with their talc covers which remained the greatest bar to the progress of microtomy.

Toward the close of the first half of the 19th century Messrs. Chance, Birmingham, England discovered how to make thin glass coverslips. They were for many years (Queckett 1855, 287) the only manufacturers, and until the discovery of oil immersion objectives microscopists were entirely dependent upon the increasing thinness with which these glasses could be supplied. Microscopists were still seeking for magnification rather than resolution, and by 1880 (Beale 1880, 351) a coverslip had been made sufficiently thin to permit the use of a ⅙₀-inch "high dry" objective. Coverslips are now taken so much for granted that the contribution made to the development of biology through the introduction of thin glass is often overlooked. The earliest method of using these thin coverslips with glass slides was by holding the cover in place with the aid of a paper label which covered all the slide except the area immediately over the object. These labels were often fancifully engraved to the design of the individual technician and an excellent and well-illustrated description of their use is to be found in Martin 1872, pp. 46–52.

Microscope mounts, as made today, consist of three types. These are, first, *wholemounts*, in which organisms or pieces of organisms are mounted under a coverslip on a slide; second, *smear preparations*, in which either a cut surface or a viscous fluid is smeared on a slide to form a thin layer which is subsequently preserved under a cover glass; third, *sections*, in which thin slices of objects are mounted under a coverslip. Where objects are cut into a series of sections, each of which is mounted in consecutive order on a slide, the preparation is known as a *serial section*.

The simplest slide to prepare is that in which the object is mounted dry. An example of this is shown in Fig. 1 where a series of diatoms have been spread on a slide, and a coverslip placed over them. This coverslip is held in place by a ring of cement which is prevented from running under the edges of the coverslip by some



Figs. 1 to 6. Types of microscopical preparation. *1. Dry wholemount of diatoms. 2. Freshwater bryzoan in deep cell of formaldehyde. 3. Crustacean in oval cavity in glycerol jelly. 4. Smear preparation. 5. Single section of plant stem. 6. Serial section of embryo.*

form of thin cell, which may be either of cement or paper, and which serves the additional purpose of preventing the crushing of the object by the coverslip. Most wholemounts are, however, prepared in a preservative medium, which may be either aqueous, colloidal, or resinous. Many of these whole objects are relatively thick so that some method must be adopted of providing space for them under the coverslip. Fig. 2 shows an object mounted in a deep cell of glass, while Fig. 3 shows an alternative method in which a relatively thick slide has had an oval cavity ground into it. As will be seen from the figure these mounts are heavily varnished at the edges to prevent the evaporation of fluid or the withdrawal of water from the colloidal medium. Wholemounts prepared with resinous media, which harden and thus hold the coverslip in place, are frequently not varnished at the edges though some case can be made out (Gray 1936, *Microsc. Rec.*, 38) for the application of a ring of varnish around the edge of balsam mounts.

Smear preparations (Fig. 4) are almost invariably prepared in resinous media and equally invariably the edges of the coverslips are not varnished. Sections (Figs. 5 and 6), either single or serial, are universally mounted in resinous media and the edges of the coverslip are practically never varnished.