Appendix B

Chapter Notes

Chapter 0 Introduction

The most complete work on light microscopy that I used in compiling these notes is that by Maksymilian Pluta - Advanced Light Microscopy: Volume 1 Principles and Basic Properties, and Volume 2 Specialized Methods. The third volume in the series, Measuring Techniques, I did not use. A more basic text on light microscopy is that by Robert F. Smith – Microscopy and Photomicrography, A Working Manual, 2nd edition. Included in this text is a color illustration containing images made of from an Abbe test plate. Abbe’s theory of image formation is beautifully illustrated in this series of images. Many microscopy texts contain some historical information. Here I will only mention Donald L. Padgitt, A short History of the Early American Microscopes, Harold Malies, A Short History of the English Microscope, and American Optical Company, Three American Microscope Builders.. This last is a quaint, out of print, text that retains the flavor of the early days of microscopy in the United States.

Chapter 1 Parts

The standard reference for terminology in light microscopy is the RMS Dictionary of Light Microscopy.

Chapter 2 Alignment

An excellent compilation of traditional illumination methods and of Köhler’s contribution is contained in the pamphlet titled “Köhler Illumination Centenary” prepared by the Royal Microscopical Society 1994. Included is an English translation of Köhler’s original paper. The pamphlet was generally available through Zeiss representatives and from the RMS (37/38 St Clemments, Oxford OX4 1AJ, Phone (0865) 248768, Fax: (0865) 791237.

Chapter 3 Cleaning

Shinya Inoué includes a section on inspecting and cleaning optics in his excellent book: Video Microscopy 2nd edition, pp 149-157. The pamphlet “How to use a microscope and take a photomicrograph” form NIKON Inc. contains a short section on cleaning lenses (pp 32-34). It should be available from your local NIKON representative. Another short section is contained in Optimizing Light Microscopy for Biological and Clinical Laboratories (pp 35-36) by Babara Foster.

Chapter 4 Optics,
Chapter 5 Illumination Path,
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Chapter 6 Image Path,
Chapter 7 Lenses

Most books on microscopy contain information on optics. Here I will mention only a few of those that I have used. Michael B. Wilson’s *The Science and Art of Basic Microscopy* has some of the best illustrations of geometric optics I have found. Saviel Bradbury’s *An Introduction to the Optical Microscope*, covers a lot of optics in a very small space. A much more detailed book is that by Wolfgang Zieler: *The Optical Performance of the Light Microscope* Vol 1. A classic work that has been reprinted is *The Practical Use of the Microscope* by George Herbert Needham. Unfortunately, out of print is *Optical Methods in Biology* by Elizabeth M. Slayter. Also, unfortunately out of print is *Fundamentals of Light Microscopy* by Michael Spencer. I highly recommend these two books. They are available in the UNC-CH Health Sciences Library. “The Star Test” as described in Ch. 7 is adapted from Needham. For those who prefer a mathematical treatment of optics in microscopy, I recommend *Light and Electron Microscopy* by E.M. Slayter and H. S. Slayter.

Chapter 8 Bright Field

By far the best descriptions of bright field microscopy methods are contained in the older, out of print texts. Beside those mentioned above an interesting one is *The Microscope* by Simon Henry Gage, 17th. While Gage apparently never accepted Abbe’s theory of image formation, his book contains a huge quantity of practical advice and many instructive exercises. *Contrast Techniques in Light Microscopy* by S. Bradbury and P.J. Evennett contains a short chapter on bright field. A good text on histological staining is *Theory and Practice of Histotechnology*, 2nd edition by Sheehan and Harpchak. Rheinberg Illumination is briefly described in volume 2 of a short series of books *Basics and Beyond Series* by Mortimer Abramowitz. A more detailed description is given by Bradbury and Evennett, and in Needham.

Chapter 9 Dark Field

*The Practical Use of the Microscope* by George Herbert Needham is the best source I have found for dark field microscopy. More recent publications include those by Robert F. Smith and Bradburry and Evennett.

Chapter 10 Phase Contrast

*Phase Microscopy, Principles and Applications* by A. Bennett, H. Osterberg, H. Jupnik, and O. Richards was my main source for this chapter. This out of print text contains a wealth of technical and practical information. *Advanced Light Microscopy vol. 2: Specialized Methods* by Maksymilian Pluta is the best current source. The physical properties of light involved in phase contrast are well described by Michael Spencer. I also found *Fundamentals of Optics* 4th edition by F.A. Jenkins and H.E. White to be of much help in understanding the underlying optical concepts.

Chapter 11 DIC
Short descriptions of Normarski Differential Interference Contrast are given in Bradbury and Evennett and in Smith. By far the best treatment is in Pluta vol 2. Jenkins and White have a very good description of polarization, birefringence and the construction of Nicol and Wollaston prisms. The prisms used in DIC are modified Wollaston prisms. Hoffman Modulation Contrast was first described by Robert Hoffman in 1977. Barbara Foster gives a description of the technique, and Pluta vol 2 gives more technical details.

Chapter 12 Fluorescence
Anyone just starting in fluorescence microscopy should read *Introduction to Fluorescence Microscopy* by J.S.Ploem and H.J. Tanke. This small book covers the fundamentals of fluorescence microscopy and does so very well. A good comparison on episcopic and diascopic fluorescence is in “Fluorescence Microscopes” by Nikon Inc. *Fluorescence Microscopy of Living Cells in Culture part A and B* edited by Wang and Taylor covers most of the current techniques in biological fluorescence microscopy.

Chapter 13 Confocal Laser Scanning Microscopy
Handbook of Biological Confocal Microscopy 3rd edition ed. James B. Pawley is my primary source for this chapter. For information on video and digital imaging I use Inoué. Minsky has written a fascinating account of his invention of the confocal scanning microscope in the journal Scanning.

Chapter 14 Photomicrography
John Gustav Delly’s book *Photography Through the Microscope* is one of the best sources on this subject. I have also used *Modern PhotoMICROgraphy* by Bracegirdle and Bradbury and the Nikon brochure “How To Use A Microscope And Take A Photomicrograph”. A good reference on CCD chip design is in Inoue and Spring Video Microscopy 2nd edition. Handbook of Biological Confocal Microscopy 3rd edition ed. James B. Pawley also has more than you ever wanted to know about CCDs and digital camera technology.

Chapter 15 Polarized Light
A good, short introduction to polarized light microscopy is in Bradbury and Evennett *Contrast Techniques in Light Microscopy* chapter 5. A very good, thorough presentation of polarized light in light microscopy is *Qualitative Polarized Light Microscopy* by Robinson and Bradbury.

**References**

Mortimer Abramowitz, *Basics and Beyond Series* by published by Olympus Corporation. These books are generally available through Olympus dealers. Mortimer Abramowitz is also the principal editor of the extremely good web site for light microscopy learners called Molecular Expressions [http://micro.magnet.fsu.edu/](http://micro.magnet.fsu.edu/).


George Herbert Needham, *The Practical Use of the Microscope* originally published by Charles C Thomas Publisher, 1958, is now available as a reprint through McCrone.


Nikon Inc. “How To Use A Microscope And Take A Photomicrograph”, Nikon Inc. Instrument Group. This booklet is available from Nikon representatives.


**Companies**

Carolina Biological Supply Company, 2700 Youk Road, Burlington, NC 27215, Ph. 800-334-5551, Fax 800-222-7112 https://www2.carolina.com. The diatoms mentioned in the exercises in these notes are available on a single slide called “Deatom Test Plate”, catalog number K3-B25D.

Chroma Technology Corp. 10 Imtec Lane, PO Box 489, Rockingham, VT 05101 also provides filters for fluorescent microscopy. Their web address [www.chroma.com](http://www.chroma.com) has extensive information on filter sets.

Leica AG, Ch-9435 Heerbrugg, Seitzerland, Ph. 41 71 70 3 31, Fax 41 71 72 16 38, [http://www.bodan.net/leica/index_e.html](http://www.bodan.net/leica/index_e.html).

McCrone Accessories & Components, 850 Pasquinelli Drive, Westmont, IL 60559-5539, Ph 800-622-8122, Fax 630-887-7100, e-mail: mac@mccrone.com, web: [www.macrone.com](http://www.macrone.com).


Olympus Corp., Precision Instruments Division, 4 Nevada Drive, Lake Success, NY 11042 [http://www.olympus.com](http://www.olympus.com).

Omega Optical, Omega Optical, Inc Delta Campus Omega Drive Brattleboro, VT 05301 Toll free: (866) 488-1064 manufacturers interference filters for fluorescence microscopy. Their web site [www.omegafilters.com](http://www.omegafilters.com) has a very good tool (Curv-o-matic) for checking
the excitation and emission spectra of just about every known dye plus the Omega filter combination that is good for those dyes.


Software

ImageJ is free image acquisition and processing software available from the following web site http://rsb.info.nih.gov/ij/. It was written by Wayne Rasband at NIH and is one of the best image processing programs available. ImageJ runs on any computer equipped with Java (a standard feature of all web capable computers). ImageJ works with 24 bit (real color) images as well as gray scale images. This web site also contains links to many other sites that offer excellent image processing software some of which is either free or shareware.