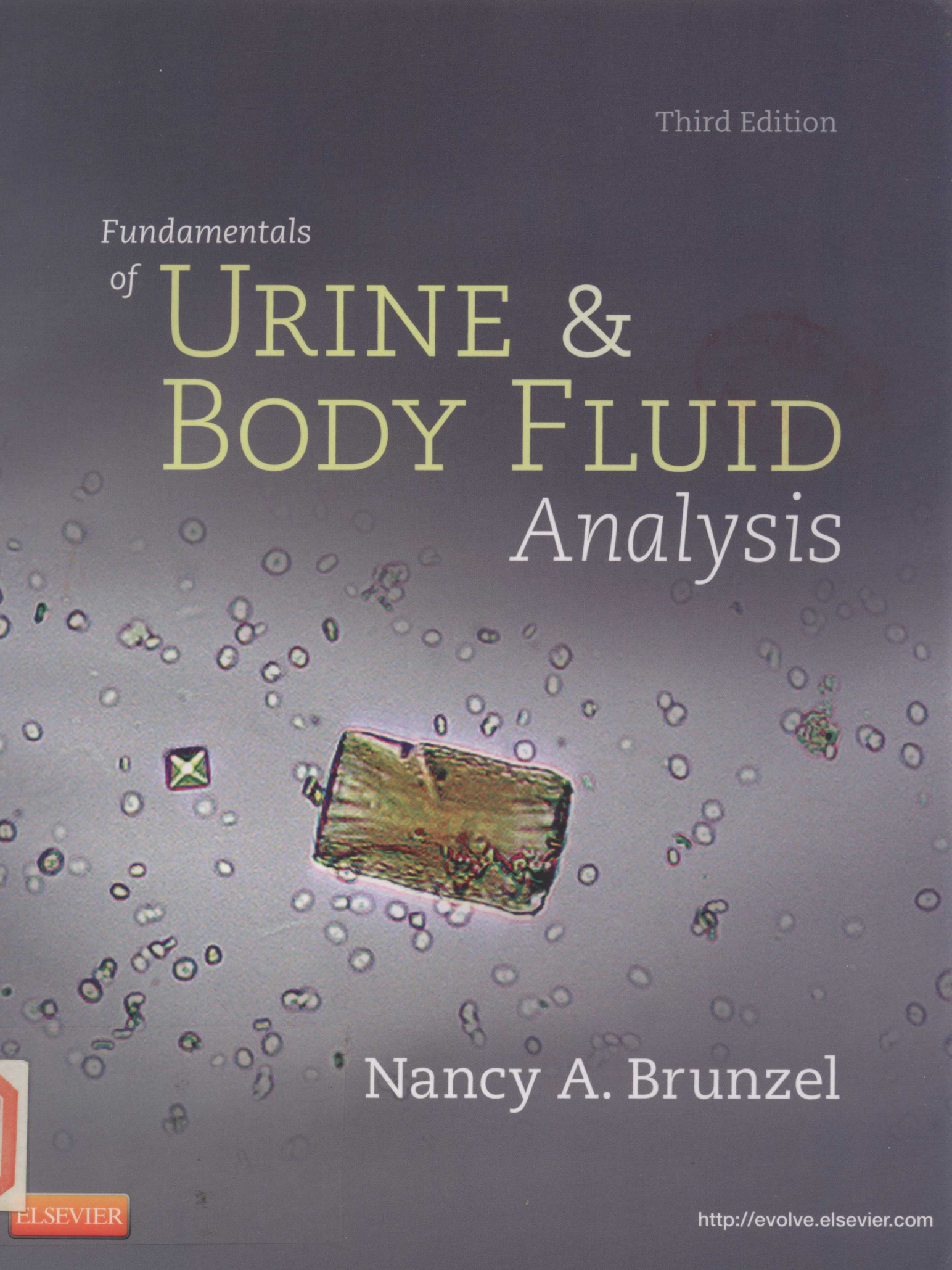
Third Edition

Fundamentals of URINE & BODY FLUID *Analysis*



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Fundamentals of URINE & BODY FLUID *Analysis*

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PREFACE

This book is designed as a teaching and reference text for the analysis of urine and body fluids. The intended audience is students in clinical/medical laboratory science programs and practicing laboratory professionals. However, other health care professionals—physicians, physician assistants, nurse practitioners, and nurses—can also benefit from the information provided.

As with previous editions, the task of achieving a balance in depth and breadth of content to meet all needs is challenging. I believe that to gain a true understanding of a subject requires more than the mere memorization of facts. Therefore, a guiding principle in the format and writing of this book was to present comprehensive information in a manner that arouses interest, enhances learning, and facilitates understanding and mastery of the content. Although the content is comprehensive and detailed, educators can easily adapt it to the level of content desired.

ORGANIZATION

The organization is similar to previous editions, with a couple of minor changes: the *Urine Sediment Image Gallery* has been centralized for easy access and two new chapters are present. As in previous editions, the first two chapters cover general topics that are important in laboratories that perform urine and body fluid analyses, namely microscopy, quality assurance, and safety. Chapter 1 describes various types of microscopy, proper microscope handling and care, including important do's and don'ts, as well as step-by-step instructions for properly adjusting a binocular microscope for optimal viewing (i.e., Kohler illumination). Chapter 2 reviews quality assurance and safety concerns that apply to laboratories that perform urine and body fluid analyses.

Chapters 3 through 9 focus on the study of urine—preanalytical considerations, analysis, and the role urinalysis plays in the diagnosis and monitoring of disorders. Chapter 3 provides a thorough discussion of urine specimen collection, handling, and preservation; chapters 4 and 5 review the anatomy and physiology of the urinary system. Together, these chapters set the stage for an in-depth discussion of the three components of a complete urinalysis, namely the physical examination (Chapter 6), chemical examination (Chapter 7), and microscopic examination (Chapter 8). Located between Chapters 8 and 9 is a *Urine Sediment Image Gallery* containing *more than 100* urine sediment photomicrographs to be used as a teaching tool and reference for the identification of urine sediment elements. Chapter 9 completes the study of urine with a discussion of the clinical features of renal and metabolic disorders and their associated urinalysis results.

Chapters 10 through 16 are dedicated to the study of body fluids (other than urine) frequently encountered in the clinical laboratory. Each chapter describes the physiology, normal composition, and clinical value associated with laboratory analysis of the fluid. Preanalytical factors in specimen collection and handling are discussed along with the significance of specific tests that provide clinically useful information. Note that laboratory tests routinely performed on one body fluid may not have clinical value when analyzing another body fluid.

Chapter 17 provides a snapshot of automation currently available for the analysis of urine and body fluids. Because of the robust and dynamic nature of laboratory instrumentation, the content of this chapter will quickly outdate. However, the intent is to provide an understanding of the analytical principles used in semi- and fully automated instruments. In this regard, the basic analytical principles for chemical (reflectance photometry) and microscopic (digital microscopy, flow cytometry) analyses have stood the test of time and will endure. Future improvements in the application of these analytical techniques for the analysis of urine and body fluids will undoubtedly bring to the marketplace new analyzers and manufacturers.

For a variety of reasons, manual cell counts of body fluids using a hemacytometer persist today. Chapter 18 and Appendix C are resources for the preparation of dilutions and the performance of manual body fluid cell counts. Pretreatment solutions and a variety of diluents for body fluids are discussed, as are step-by-step instructions and calculations for performing manual cell counts. This chapter ends with a discussion of cytocentrifugation and the preparation of slides for a leukocyte differential.

Three appendices are provided to complement the chapters. As previously stated, Appendix C *Body Fluid Diluent and Pretreatment Solutions* supplements Chapter 18 by providing detailed instructions for the preparation and use of diluents and pretreatment solutions. Appendix A, *Reagent Strip Color Charts*, supplements the chemical examination of urine (see Chapter 7) by providing figures of manufacturer color charts used to manually determine reagent strip results. These figures are a useful reference and assist in highlighting differences in reagent strip brands such as physical orientation of strip

to chart and variations in result reporting. Appendix B serves as a handy resource for the Reference Intervals that are provided in the various chapters.

The book ends with two additional sections, a Glossary and Answer Key. The glossary includes the key terms that are bolded in each chapter and additional important clinical and scientific terms that may be new to readers. The Answer Key provides the answers and explanations (when necessary) to the end-of-chapter study questions and cases in a convenient, readily accessible location.

NEW TO THIS EDITION

- Throughout the text, content has been updated and numerous tables have been revised or added to supplement and enhance mastery of the material.
- In Chapter 8, *Microscopic Examination of Urine Sediment,* some content was reorganized and figures added.
- The alphabetized *Urine Sediment Image Gallery* was relocated to follow Chapter 8, which will greatly assist users in quickly and easily locating additional photomicrographs for reference when performing microscopic examinations.
- *Quick Reference Tables* added to the inside front cover assist in finding figures (i.e., photomicrographs of interest).
- Returning to the third edition is a chapter discussing automation in the analysis of urine and other body fluids and the principles involved in automated systems (see Chapter 17, *Automation of Urine and Body Fluid Analysis*).
- A new Chapter 18 covers manual hemacytometer counts (previously an appendix) of body fluids and differential slide preparation using cytocentrifugation. This chapter is complemented by a new Appendix C, *Body Fluid Diluent and Pretreatment Solutions*, which provides detailed instructions for the preparation and use of diluents and pretreatment solutions.

LEARNING AIDS

Each chapter includes the following aids to enhance mastery of the content:

- *Learning Objectives* at three cognitive levels (Recall, Application, Analysis)
- *Key Terms* that are bold in the chapter and defined at the front of the chapter and in the Glossary
- Many *Tables* that capture and summarize content
- Numerous high-quality *Figures* in full color
- *Study Questions* at three cognitive levels (Recall, Application, Analysis)
- *Case Studies*, when applicable to content

EVOLVE INSTRUCTOR RESOURCES

New for this edition, downloadable instructor content specific to this text is available on the companion Evolve site (http://evolve.elsevier.com/Brunzel). This includes the following ancillary material for teaching and learning:

- *PowerPoint presentations* for all chapters to aid in lecture development
- *Test Banks* that tie exam questions directly to book content, making exam development easier and faster
- *Image Collection* that includes all illustrations in the book in various formats, offering a closer look at hundreds of microscopic slides.

ACKNOWLEDGMENTS

I have fond memories of performing my first urinalyses in a small hospital laboratory in Kansas City, Missouri. When I reflect on the beginnings of this love for the study of urine, one individual comes to mind—Mary Abts, MT(ASCP). She was my urinalysis instructor at the bench and she made it so exciting! So, I thank her for her guidance, professionalism, and infectious enthusiasm, which ignited the spark that eventually developed into this book.

Throughout the years, numerous colleagues have also blessed me personally and professionally. A special acknowledgement to Karen Karni—my mentor—who nurtured the writer and educator within me, a process that continues to impact what I do today. I am grateful to Charlotte Janita for sharing her expertise in body fluid analysis and contributing photomicrographs. To the many laboratory scientists and students that ask questions, provide feedback, and make suggestions, I take my hat off to you.

To Ellen Wurm-Cutter, a special thank you for her steadfast and patient guidance in directing this new edition, and to Beth LoGiudice, for her encouragement and capable assistance. Finally, I express my appreciation to Karen Rehwinkel and the entire production team at Elsevier for their expertise and creativity.

Nancy A. Brunzel

CONTENTS

APPENDICES

CHAPTER 1

Microscopy

LEARNING OBJECTIVES

After studying this chapter, the student should be able to:

1. Identify and explain the functions of the following components of a microscope:
   - Aperture diaphragm
   - Condenser
   - Eyepiece (ocular)
   - Field diaphragm
   - Mechanical stage
   - Objective
2. Describe Köhler illumination and the microscope adjustment procedure used to ensure optimal specimen imaging.
3. Describe the daily care and preventive maintenance routines for microscopes.
4. Compare and contrast the principles of the following types of microscopy:
   - Brightfield
   - Phase-contrast
   - Polarizing
   - Interference contrast
   - Darkfield
   - Fluorescence
5. List an advantage of and an application for each type of microscopy discussed.

CHAPTER OUTLINE

KEY TERMS

aperture diaphragm Microscope component that regulates the angle of light presented to the specimen. The diaphragm is located at the base of the condenser and changes the diameter of the opening through which source light rays must pass to enter the condenser.

birefringent (also called *doubly refractile*) The ability of a substance to refract light in two directions.

brightfield microscopy Type of microscopy that produces a magnified image that appears dark against a bright or white background.

chromatic aberration Unequal refraction of light rays by a lens that occurs because the different wavelengths of light refract or bend at different angles. As a result, the image produced has undesired color fringes.

condenser Microscope component that gathers and focuses the illumination light onto the specimen for viewing. The condenser is a lens system (a single lens or a combination of lenses) that is located beneath the microscope stage.

darkfield microscopy Type of microscopy that produces a magnified image that appears brightly illuminated against a dark background. A special condenser presents only oblique light rays to the specimen. The specimen interacts with these rays (e.g., refraction, reflection), causing visualization of the specimen. Darkfield microscopy is used on unstained specimen preparations and is the preferred technique for identification of spirochetes.

eyepiece (also called ocular) The microscope lens or system of lenses located closest to the viewer's eye. The eyepiece produces the secondary image magnification of the specimen.

field diaphragm Microscope component that controls the diameter of light beams that strike the specimen and hence reduces stray light. The diaphragm is located at the light exit of the illumination source. With Köhler illumination, the field diaphragm is used to adjust and center the condenser appropriately.

field number A number assigned to an eyepiece that indicates the diameter of the field of view, in millimeters, that is observed when using a 1× objective. This diameter is determined by a baffle or a raised ring inside the eyepiece and sometimes is engraved on the eyepiece.

field of view The circular field observed through a microscope. The diameter of the field of view varies with the eyepiece field number and the magnifications of the objective in use, plus any additional optics before the eyepiece. The field of view (FOV) is calculated using the following formula: FOV (in millimeters) = Field number/M, where *M* is the sum of all optics magnifications, except that of the eyepiece.

fluorescence microscopy Type of microscopy modified for visualization of fluorescent substances. Fluorescence microscopy uses two filters: one to select a specific wavelength of illumination light (excitation filter) that is absorbed by the specimen, and another (barrier filter) to transmit the different, longer-wavelength light emitted from the specimen to the eyepiece for viewing. The fluorophore (natural or added) present in the specimen determines the selection of these filters.

interference contrast microscopy Type of microscopy in which the difference in optical light paths through the specimen is converted into intensity differences in the specimen image. Three-dimensional images of high contrast and resolution are obtained, without haloing. Two types available are modulation contrast (Hoffman) and differential interference contrast (Nomarski).

Köhler illumination Type of microscopic illumination in which a lamp condenser (located above the light source) focuses the image of the light source (lamp filament) onto the front focal plane of the substage condenser (where the aperture diaphragm is located). The substage condenser sharply focuses the image of the field diaphragm (located at or slightly in front of the lamp condenser) at the same plane as the focused specimen. As a result, the filament image does not appear in the field of view, and bright, even illumination is obtained. Köhler illumination requires appropriate adjustments of the condenser and of the field and aperture diaphragms.

mechanical stage Microscope component that holds the microscope slide with the specimen for viewing. The stage is adjustable, front to back and side to side, to enable viewing of the entire specimen.

numerical aperture Number that indicates the resolving power of a lens system. The numerical aperture (NA) is derived mathematically from the refractive index (n) of the optical medium (for air, n = 1) and the angle of light (μ) made by the lens: $NA = n \times \sin \mu$.

objective The lens or system of lenses located closest to the specimen. The objective produces the primary image magnification of the specimen.

parcentered Term describing objective lenses that retain the same field of view when the user switches from one objective to another of a differing magnification.

parfocal Term describing objective lenses that remain in focus when the user switches from one objective to another of a differing magnification.

phase-contrast microscopy Type of microscopy in which variations in the specimen's refractive index are converted into variations in light intensity or contrast. Areas of the specimen appear light to dark with haloes of varying intensity related to the thickness of the component. Thin, flat components produce less haloing and the best-detailed images. Phase-contrast microscopy is ideal for viewing low-refractile elements and living cells.

polarizing microscopy Type of microscopy that illuminates the specimen with polarized light. Polarizing microscopy is used to identify and classify birefringent substances (i.e., substances that refract light in two directions) that shine brilliantly against a dark background.

resolution Ability of a lens to distinguish two points or objects as separate. The resolving power (R) of a microscope depends on the wavelength of light used (λ) and the numerical aperture of the objective lens. The greater the resolving power, the smaller the distance distinguished between two separate points.

spherical aberration Unequal refraction of light rays when they pass through different portions of a lens such that the light rays are not brought to the same focus. As a result, the image produced is blurred or fuzzy and cannot be brought into sharp focus.

A high-quality brightfield microscope is required for the microscopic examination of urine and other body fluids. One must give considerable care to its selection because its use is an integral part of laboratory work, and microscopes with quality objective lenses are costly. Because some brightfield microscopes can be modified to allow several types of microscopy from a single instrument—brightfield, phase-contrast, polarization—good planning ensures selection of the most appropriate instrument. Whereas acquiring a suitable microscope is of utmost importance, appropriate training on its use and proper maintenance and cleaning of the microscope are crucial to ensure maximization of its potential. The user must be familiar with each microscope component and its function, as well as with proper microscope adjustment and alignment procedures.

BRIGHTFIELD MICROSCOPE

A brightfield microscope (Figure 1-1) produces a magnified specimen image that appears dark against a brighter background. A simple brightfield microscope consisting of only one lens is known as a *magnifying glass*. In the clinical laboratory, however, compound brightfield microscopes predominate and consist of two lens systems. The first lens system, located closest to the specimen, is the objective mounted in the nosepiece. The objective produces the primary image magnification and directs this image to the second lens system, the eyepiece, or ocular.

The eyepiece further magnifies the image received from the objective lens. Total magnification of a specimen is the product of these lens systems, that is, multiplication of the objective lens magnification by the eyepiece lens magnification. For example, a 10× objective with a 10× eyepiece produces a 100× magnification. In other words, the viewed image is 100 times larger than its actual size.

The eyepiece also determines the diameter of the field of view (FOV) observed. This diameter is established by a round baffle or ridge inside the eyepiece and is indicated by the field number assigned to the eyepiece. Field numbers that predominate in clinical laboratory microscopes typically range from 18 to 26. This number indicates the diameter of the FOV, in millimeters (mm), when a 1× objective is used. To determine the diameter of any FOV the following equation is used

Equation 1-1

$$\text{FOV} = \frac{\text{Field number}}{\text{M}}$$

where *M* is the magnification of the objective and any additional optics. (*Note:* This sum does not include the eyepiece magnification.)

For example, an eyepiece with a field number of 18 has a diameter of 18 mm when a 1× objective is used, a diameter of 1.8 mm when a 10× objective is used, and a diameter of 0.18 mm when a 100× objective is used. Some manufacturers engrave the field number on the eyepiece along with the eyepiece magnification. For those that do not, a small ruler can be placed on the stage to measure the diameter, or the manufacturer can be contacted. As the field number increases, so does the cost of the eyepiece. Before about 1990, microscopes used in the clinical laboratory had eyepieces with a field

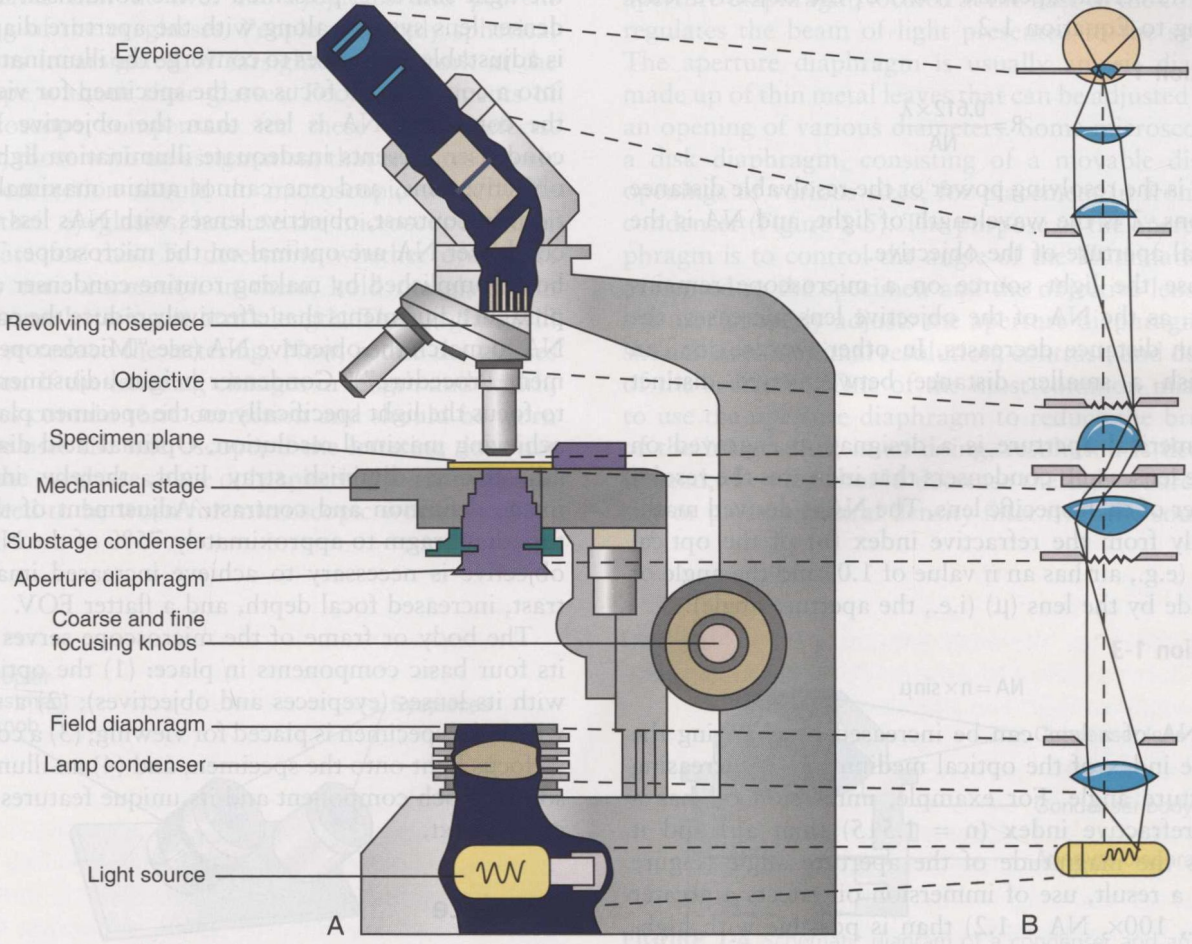


FIGURE 1-1 **A,** A schematic representation of a brightfield microscope and its components. **B,** Path of illumination using Köhler illumination.

number of 18. Most microscopes purchased after that time have field numbers of 20 or 22.

In areas of the laboratory where results are reported as the number of elements observed per FOV (e.g., urinalysis, number per high-power or low-power field), performing a microscopic examination on the same or equivalent microscopes is crucial. If this is not done, results depend on the microscope used and can be significantly different even with evaluation of the same specimen. In other words, two microscopes with the same objective magnification and the same eyepiece magnification (e.g., 10×) but with different field numbers will have different diameters for their FOVs. Therefore standardizing microscopic examinations reported as the number of elements observed per FOV requires that microscopes with the same eyepiece field number are used.

The purpose of the microscope lens system (i.e., eyepiece and objective) is to magnify an object sufficiently for viewing with maximum resolution. Resolution, or resolving power, describes the ability of the lens system to reveal fine detail. Stated another way, resolution is the smallest distance between two points or lines at which they are distinguished as two separate entities. Resolving power (R) depends on the wavelength (λ) of light used and the numerical aperture (NA) of the objective lens, according to Equation 1-2.

Equation 1-2

$$R = \frac{0.612 \times \lambda}{NA}$$

where *R* is the resolving power or the resolvable distance in microns, λ is the wavelength of light, and *NA* is the numerical aperture of the objective.

Because the light source on a microscope remains constant, as the NA of the objective lens increases, the resolution distance decreases. In other words, one can distinguish a smaller distance between two distinct points.

A numerical aperture is a designation engraved on objective lenses and condensers that indicates the resolving power of each specific lens. The NA is derived mathematically from the refractive index (n) of the optical medium (e.g., air has an n value of 1.0) and the angle of light made by the lens (μ) (i.e., the aperture angle).

Equation 1-3

$$NA = n \times \sin\mu$$

The NA of a lens can be increased by changing the refractive index of the optical medium or by increasing the aperture angle. For example, immersion oil has a greater refractive index (n = 1.515) than air, and it increases the magnitude of the aperture angle (Figure 1-2). As a result, use of immersion oil effects a greater NA (e.g., 100×, NA = 1.2) than is possible with high-power dry lenses. An increase in NA equates with greater magnification and resolution.

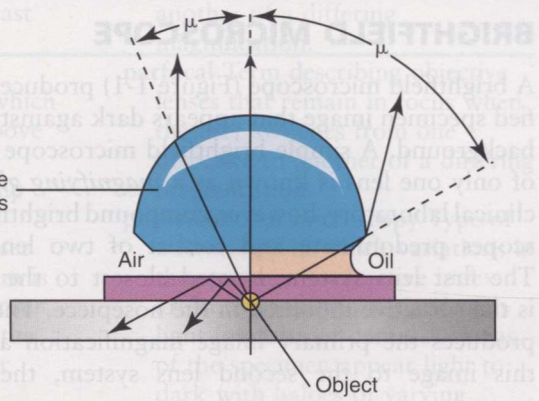


FIGURE 1-2 Drawing depicting changes in numerical aperture. Note the increase in light angle (μ) attained and therefore in the numerical aperture when immersion oil is used.

As previously discussed, the ability of a lens to resolve two points increases with the NA increases. However, to achieve the maximal resolution of a microscope the NA of the microscope condenser must be equal to or slightly greater than the NA of the objective lens used. This requirement is necessary to ensure adequate illumination to the objective lens and can be understood better by reviewing the dynamics involved. Illumination light from the light source is presented to the condenser. The condenser lens system, along with the aperture diaphragm, is adjustable and serves to converge the illumination light into a cone-shaped focus on the specimen for viewing. If the condenser NA is less than the objective NA, the condenser presents inadequate illumination light to the objective lens, and one cannot attain maximal resolution. In contrast, objective lenses with NAs less than the condenser NA are optimal on the microscope. This can be accomplished by making routine condenser and diaphragm adjustments that effectively reduce the condenser NA to match the objective NA (see "Microscope Adjustment Procedure"). Condenser height adjustments serve to focus the light specifically on the specimen plane, thus achieving maximal resolution. Optimal field diaphragm adjustments diminish stray light, thereby increasing image definition and contrast. Adjustment of the aperture diaphragm to approximately 75% of the NA of the objective is necessary to achieve increased image contrast, increased focal depth, and a flatter FOV.

The body or frame of the microscope serves to hold its four basic components in place: (1) the optical tube with its lenses (eyepieces and objectives); (2) a stage on which the specimen is placed for viewing; (3) a condenser to focus light onto the specimen; and (4) an illumination source. Each component and its unique features are discussed next.

Eyepiece

Whereas some microscopes have only one eyepiece (monocular), those used in most clinical laboratories have

two eyepieces (binocular). However when using a monocular microscope, always view with both eyes open to reduce eyestrain. Initially this may be difficult, but with practice the image seen by the unused eye will be suppressed. With a binocular microscope, adjustments to the oculars are necessary to ensure optimal viewing. The interpupillary distance of the eyepiece tubes is adjusted by simply sliding them together or apart. Because vision in both eyes usually not the same, each individual eyepiece is adjustable to compensate using the diopter adjustment. To adjust the eyepieces, first view the image using only the right eye and eyepiece. Look at a specific spot on the specimen, and bring it into sharp focus using the fine adjustment knob. Next, close the right eye, and while looking with the left eye through the left eyepiece, rotate the diopter adjustment ring on this eyepiece until the same spot on the specimen is also in sharp focus (Figure 1-3). Each technologist must make the interpupillary and diopter adjustments to suit his or her eyes. To eliminate eyestrain or tired eyes when performing microscopic work, always look through the microscope with eyes relaxed, and continually focus and re-focus the microscope as needed using the fine adjustment control.

Eyeglass wearers should consider keeping their glasses on when performing microscopic work. Rubber guards are available that fit over the eyepieces and prevent scratching of the eyeglasses. People with only spherical corrections (nearsighted or farsighted) can work at the microscope without their glasses. Focus adjustments of the microscope compensate for these visual defects. However, those with an astigmatism that requires a toric lens for correction should do microscopic work while wearing their eyeglasses, because the microscope cannot compensate for this. To determine whether eyeglasses have any toric correction in them, hold the glasses in front of some lettering at arm's length. The eyeglass will magnify or reduce the lettering. Now, rotate the glasses 45 degrees. If the lettering changes in length and width, the glasses contain toric correction and should be worn when one uses the microscope; if the lettering does not change, the eyeglasses have only spherical correction and do not need to be worn for microscopic work.

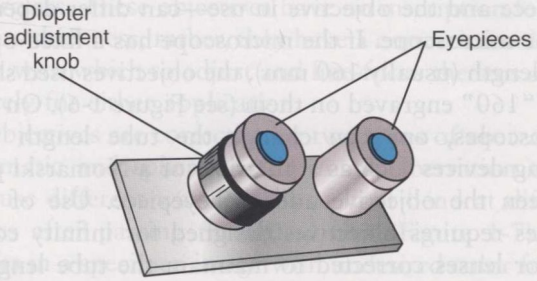


FIGURE 1-3 Schematic representation of a binocular eyepiece shows the location of the diopter adjustment ring.

Mechanical Stage

The microscope mechanical stage is designed to hold firmly in place the slide to be examined. The stage has conveniently located adjustment knobs to move the slide front to back and side to side. When viewing the slide, the user views the image upside down. Moving the slide in one direction causes the image to move in the opposite direction. Some stages have a vernier scale on a horizontal and a vertical edge to facilitate relocation of a particular FOV. By recording the horizontal and vertical vernier scale values, the slide can be removed and at a later time be placed back onto the stage and the identical FOV can be found and reexamined.

Condenser

The condenser, located beneath the mechanical stage, consists of two lenses (Figure 1-4). The purpose of the condenser is to evenly distribute and optimally focus light from the illumination source onto the specimen. This is achieved by adjusting the condenser up or down using the condenser adjustment knob. The correct position of the condenser is always at its uppermost stop; it is slightly lowered only with Köhler illumination. The aperture diaphragm, located at the base of the condenser, regulates the beam of light presented to the specimen. The aperture diaphragm is usually an iris diaphragm made up of thin metal leaves that can be adjusted to form an opening of various diameters. Some microscopes use a disk diaphragm, consisting of a movable disk with openings of various sizes, for placement in front of the condenser (Figure 1-5). The purpose of the aperture diaphragm is to control the angle of the illumination light presented to the specimen and the objective lens. When the user properly adjusts the aperture diaphragm, he or she achieves maximal resolution, contrast, and definition of the specimen. One of the most common mistakes is to use the aperture diaphragm to reduce the brightness of the image field; in so doing, resolution is decreased. Instead, the user should decrease the light source intensity or place a neutral density filter over the source.

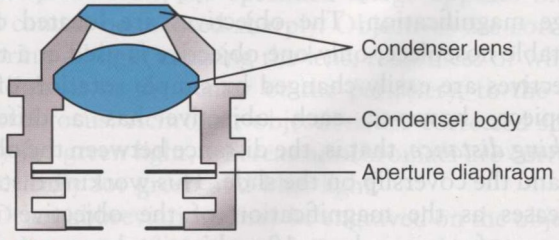


FIGURE 1-4 Schematic diagram of a condenser and an aperture diaphragm located beneath the mechanical stage of the microscope.

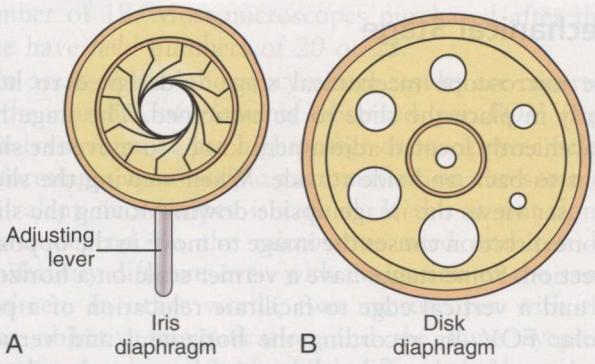


FIGURE 1-5 Two types of aperture diaphragms. **A,** An iris diaphragm. **B,** A disk diaphragm.

Illumination System

Microscopes today usually have a built-in illumination system. The light source is a tungsten or tungsten-halogen lamp located in the microscope base. These lamps often are manufactured specifically to ensure alignment of the lamp filament when a bulb requires changing. Dual controls are usually available: one to turn the microscope on, and another to adjust the intensity of the light. One should adjust the illumination intensity at the light source by turning down the lamp intensity, or by placing neutral density filters over the source. Neutral density filters do not change the color of the light but reduce its intensity. The filters are marked to indicate the reduction made, for example, a neutral density of 25 allows 75% of the light to pass. Some microscopes come with a daylight blue filter that makes the light slightly bluish. This color has been found to be restful to the eyes and is desirable for prolonged microscopic viewing.

Most clinical microscopes have a field diaphragm located at the light exit of the illumination source. The purpose of this diaphragm is to control the diameter of the light beam that strikes the specimen. The diaphragm is an iris type that when properly adjusted is just slightly larger than the FOV and serves to reduce stray light. See Box 1-1 for a procedure used to properly adjust a microscope for optimal viewing.

Objectives

The objectives are the most important optical components of the microscope because they produce the primary image magnification. The objectives are located on a rotatable nosepiece; only one objective is used at a time. Objectives are easily changed by simple rotation of the nosepiece; however, each objective has a different *working distance,* that is, the distance between the objective and the coverslip on the slide. This working distance decreases as the magnification of the objective used increases, for example, a 10× objective has a working distance of 7.2 mm, whereas a 40× objective has only 0.6 mm clearance. Therefore to prevent damage to the objective or to the slide that is being observed, care must be taken when changing or focusing objectives.

FIGURE 1-6 Engravings on this objective indicate that it is a planachromat lens (SPlan); the initial magnification is 40×; the numerical aperture is 0.70; the objective is designed for a microscope with an optical tube length of 160 mm; and the coverslip thickness should be 0.17 ± 0.01 mm.

A microscope has coarse and fine focus adjustment knobs. The user can focus the microscope by moving the mechanical stage holding the specimen up and down. Coarse focusing adjustments are made first, followed by any necessary fine adjustments.

Various engravings found on the objective indicate its magnification power, the NA, the optical tube length required, the coverglass thickness to be used, and the lens type (if not an achromat). Most often, the uppermost or largest number inscribed on the objective is the magnification power. Following this number is the NA, inscribed on the same line or just beneath it (Figure 1-6). As already discussed, the objective produces the primary magnification of the specimen, and the NA mathematically expresses the resolving power of the objective. Most objectives, designed for use with air between the lens and the specimen, are called *dry objectives.* In contrast, some objectives require immersion oil to achieve their designated NA. These objectives are inscribed with the term *oil* or *oel.*

The optical tube length—the distance between the eyepiece and the objective in use—can differ depending on the microscope. If the microscope has a fixed optical tube length (usually 160 mm), the objectives used should have "160" engraved on them (see Figure 1-6). On some microscopes, one can change the tube length when placing devices such as a polarizer or a Nomarski prism between the objective and the eyepiece. Use of these devices requires objectives designed for infinity correction or lenses corrected to maintain the tube length of 160 mm optically. Objectives designed for infinity correction have an infinity symbol (∞) engraved on them.

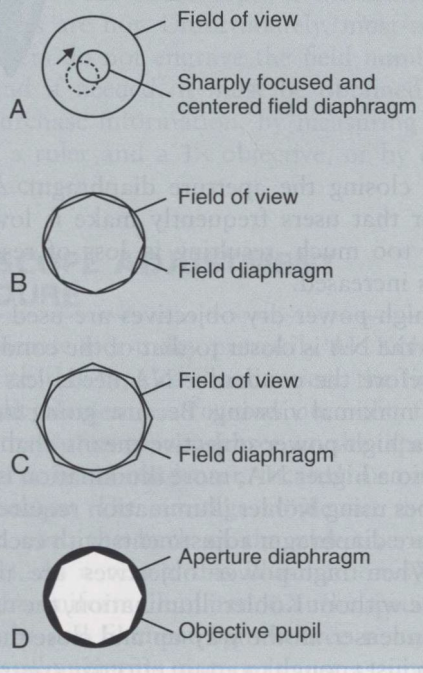
BOX 1-1 Binocular Microscope Adjustment Procedure With Köhler Illumination

Preparing the Microscope

1. Turn on the light source. Adjust the intensity to a comfortable level.
2. Position the low-power (10×) objective in place by rotating the nosepiece.
3. Place a specimen slide on the mechanical stage. Be sure the slide is seated firmly in the slide holder. Position the specimen on the slide directly beneath the objective using the mechanical stage adjustment knobs.

Interpupillary Adjustment

4. Looking through the eyepieces with both eyes, adjust the interpupillary distance until perfect binocular vision is obtained (i.e., left and right images are fused together). Using coarse and fine adjustment knobs, bring the specimen into focus.



Diopter Adjustment

5. While closing the left eye, look through the right eyepiece with the right eye and bring the specimen into sharp focus using the fine adjustment knob.
6. Now, using only the left eye, bring the image into sharp focus by rotating the diopter adjustment ring located on the left eyepiece. (Do not use the adjustment knobs.)

Condenser Adjustment

(Note: If at any point during adjustment, the light intensity is very bright and uncomfortable, decrease the lamp voltage or insert a neutral density filter.)

Condenser Height and Centration

1. Close the field diaphragm.
2. Using the condenser height adjustment knob, bring the edges of the diaphragm into sharp focus.
3. Center the condenser, if necessary, using the condenser centration knobs (see Figure A).

Field Diaphragm Adjustment

4. Open the field diaphragm to just inside the edges of the field of view to confirm adequate centration (see Figure B). Re-center if necessary.
5. Now, open the field diaphragm until it is slightly larger than the field of view (see Figure C).

Condenser Aperture Diaphragm Adjustment

6. Depending on the microscope, perform one of the following:
   a. On microscopes with a numerical scale on the condenser, open the diaphragm to 70% or 80% of the objective NA (e.g., for 80% of 40× NA 0.65, adjust condenser aperture to 0.52).
   b. Alternatively, remove one eyepiece from the observation tube. While looking down the tube at the back of the objective, adjust the diaphragm until 70% to 80% of the field is visible (approximately 25% less than fully opened). See Figure D.
7. Note that each time an objective is changed, both field and aperture diaphragms should be readjusted.

Some objectives are designed to be used with a coverglass. If a coverglass is required, its thickness is engraved on the lens after the optical tube length (e.g., 160/0.17). Objectives that do not use a coverglass are designated with a dash (e.g., ∞/– or 160/–). A third type of objective is designed to be used with or without a coverglass. These objectives have no inscription for coverglass thickness; rather, they have a correction collar on them with which to adjust and fine-focus the lens appropriately for either application.

Objectives are corrected for two types of aberrations: chromatic and spherical. Chromatic aberration occurs because different wavelengths of light bend at different angles after passing through a lens (Figure 1-7). This results in a specimen image with undesired color fringes. Objectives corrected to bring the red and blue components of white light to the same focus are called *achromats* and may not have a designation engraved on them. Objectives that bring red, blue, and green light to a common focus are termed *apochromats* and are identified by the inscription "apo." Spherical aberration occurs when light rays pass through different parts of the lens and therefore are not brought to the same focus (Figure 1-8). As a result, the specimen image appears blurred and cannot be focused sharply. Objectives are corrected to bring all light entering the lens, regardless of whether the light is at the center or the periphery, to the same central focus. Achromat objectives are corrected spherically for green light, whereas apochromats are corrected spherically for green and blue light.

Other abbreviations may be engraved on the objective to indicate specific lens types. For example, "Plan" indicates that the lens is a planachromat, achromatically corrected and designed for a flat FOV over the entire

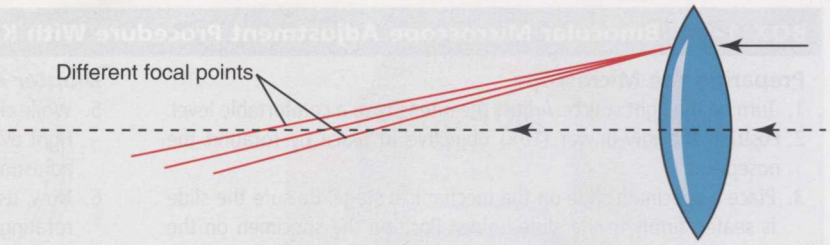


FIGURE 1-7 An illustration of chromatic aberration. Each wavelength of light is bent to a different focal point after passing through an uncorrected lens.

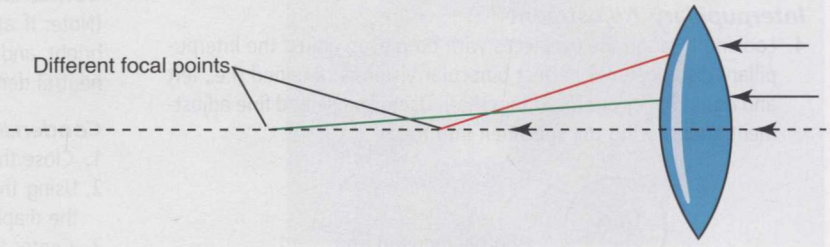


FIGURE 1-8 An illustration of spherical aberration. Each light ray is bent toward a different focal point, depending on where the ray enters an uncorrected lens.

area viewed. "Ph" indicates that the objective lens is for phase-contrast microscopy. Regardless of the manufacturer, the same basic information is engraved on all objective lenses, with only the format varying slightly. To ensure a compatible system, use of objectives and eyepieces designed by the same manufacturer that designed the microscope is advisable.

Two final features of objective lenses need to be discussed. The first characteristic is termed *parcentered* and relates to the ability of objective lenses to retain the same central FOV when the user switches from one objective to another. In other words, when an objective is changed to one of higher magnification for a closer look, the object does not move from the center of the FOV. The second feature, termed *parfocal,* refers to the ability of objectives to remain in focus regardless of the objective used. This allows initial focusing at low power; changing to other magnifications requires only minimal fine focus adjustment. Whereas both of these features are taken for granted today, in the recent past, each objective required individual centering and focusing.

When using a microscope, adjustments must be made with each objective to produce optimal viewing. These adjustments strive to equate the NA of the objective lens in use (e.g., 10×, NA 0.25) with the condenser NA (NA 0.9), thereby achieving maximal magnification and resolution. On current microscopes that use Köhler illumination, once the condenser height adjustment is made, it remains unchanged regardless of the objective used. The user lowers the effective NA of the condenser by decreasing the light the condenser receives (i.e., closing the field diaphragm) and by adjusting the aperture diaphragm for the objective. On microscopes with which Köhler illumination is not possible, use of low-power objectives may require (1) reducing the illumination source light, if possible; (2) slightly lowering (by approximately 1.0 mm) the condenser from its uppermost position; or (3) minimally closing the aperture diaphragm. An adjustment error that users frequently make is lowering the condenser too much, resulting in loss of resolution as contrast is increased.

When high-power dry objectives are used (e.g., 40×, NA 0.65), the NA is closer to that of the condenser (NA 0.9). Therefore the condenser NA needs less reduction to achieve maximal viewing. Because going from a low-power to a high-power objective means changing from a low NA to a higher NA, more illumination is required. Microscopes using Köhler illumination require only field and aperture diaphragm adjustments with each objective change. When high-power objectives are used on a microscope without Köhler illumination, the user should put the condenser all the way up and close the aperture diaphragm just enough to attain effective contrast. Never use the condenser or the aperture diaphragm to reduce image brightness; rather, decrease the illumination intensity or use neutral density filters.

OCULAR FIELD NUMBER

The eyepieces or oculars together with the objective lenses perform two important functions. They determine (1) the diameter of the **field of view** (FOV) and (2) the total magnification of a specimen. The diameter of the FOV is determined by the round baffle or ridge inside each ocular, and its numerical value is known as the ocular **field number.** Before 1990, most laboratory microscopes had an ocular field number of 18, which means that the diameter of the FOV when a 1× objective is used is 18 mm (or 1.8 mm with a 10× objective). In other words, if a metric ruler were placed on the stage and a 1× objective used, the diameter of the circle of view observed when looking through the eyepieces would measure 18 mm. Typically, the higher the field number, the more expensive the microscope. Areas of

high microscope use, such as hematology and pathology laboratories, may be able to justify the expense of microscopes with even higher ocular field numbers of 22 to 26 or larger.

When multiple microscopes are used in the laboratory for urine sediment examination, it is of paramount importance that their FOVs are the same, because clinically significant sediment components are reported as the number present per low-power field or per high-power field. The larger the ocular field number, the larger the FOV, and the greater the number of components that may be observed. Note that two microscopes with equivalent magnifying power (e.g., 100× and 400×) can have FOVs that differ! In other words, the magnification of the oculars on both microscopes is the same, but their field numbers are not. Unfortunately, most microscope manufacturers do not engrave the field number on the oculars, and if needed, it must be obtained from the original purchase information, by measuring the diameter using a ruler and a 1× objective, or by contacting the manufacturer.

MICROSCOPE ADJUSTMENT PROCEDURE

Clinical microscopes today primarily use Köhler illumination. With this type of illumination, the light source image (light filament) is focused onto the front focal plane of the substage condenser at the aperture diaphragm by a lamp condenser, located just in front of the light source. The substage condenser then focuses this image onto the back of the objective in use (see Figure 1-1). As a result, this illumination system produces bright, uniform illumination at the specimen plane even when a coil filament light source is used. Proper use of this illuminating system is just as important as selection of a microscope and its objectives. To use a microscope with Köhler illumination, the microscopist must know how to set up and optimally adjust the condenser and the field and aperture diaphragms. Manufacturers supply instructions with the microscope that are clear and easy to follow. In addition, online interactive tutorials are available that demonstrate the improved optical performance of a microscope when adjusted to achieve Köhler illumination.[1] Box 1-1 gives a basic procedure for adjustment of a typical binocular microscope with a Köhler illumination system. Whereas initially these steps may feel cumbersome, with use they become routine. When using other types of microscopy, additional adjustment procedures may be necessary to ensure optimal viewing. For example, phase-contrast microscopy requires that the phase rings are checked and aligned, if necessary.

Each day, before setting up and adjusting the microscope, the user should check it to ensure that it is clean. The microscopist should look for dust or dirt on the illumination source port, the filters, and the upper condenser lens. The user should check the eyepiece and the objectives, especially any oil immersion objective, to be sure that they are clean and free of oil and fingerprints. By routinely inspecting the microscope and optical surfaces before adjustment, valuable time can be saved in microscope setup and in troubleshooting problems. In laboratories in which the entire staff uses the same microscope, inspection before use helps identify people who need to be reminded of proper microscope care and maintenance.

CARE AND PREVENTIVE MAINTENANCE

The microscope is a precision instrument. Therefore, ensuring long-term mechanical and optical performance requires care, including routine cleaning and maintenance. Dust is probably the greatest cause of harm to the mechanical and optical components of a microscope. Dust settles in mechanical tracks and on lenses. Although dust can be removed from lenses by cleaning, the less cleaning of lenses performed, the better. To remove dust, dirt, or other particulate matter, the microscopist should use a grease-free brush (camel hair) or an air syringe (e.g., an infant's ear syringe). If compressed air is used, the air should be filtered (e.g., with cotton wool) to remove any contaminating residues or moisture. Using a microscope dust cover when the instrument is not in use or placing it in a storage cabinet eliminates dust buildup.

On microscopes, all mechanical parts are lubricated with special long-lasting lubricants. Therefore the user should never use grease or oils to lubricate the microscope. When mechanical parts are dirty, cleaning and regreasing should be performed by the manufacturer or by a professional service representative.

In climates in which the relative humidity is consistently greater than 60%, precautions must be taken to prevent fungal growth on optical surfaces. In these areas, a dust cover or a storage cabinet may reduce ventilation and enhance fungal growth. Therefore microscopes may require storage with a desiccant, or in an area with controlled temperature and air circulation. In addition to high humidity, microscopes should be protected from direct sunlight and high temperatures.

When handling the microscope, for example, when removing it from a storage cupboard or when changing work areas, the user must always carry it firmly using both hands and must avoid abrupt movements. The counter on which the microscope is placed should be vibration free. This eliminates undesired movement in the FOV when viewing wet preparations as well as the detrimental effects that long-term vibration can have on precision equipment.

All optical surfaces must be clean to provide crisp brilliant images. Because the nosepiece is rotated by hand, the objectives are constantly in danger of becoming smeared with skin oils. The user should avoid all