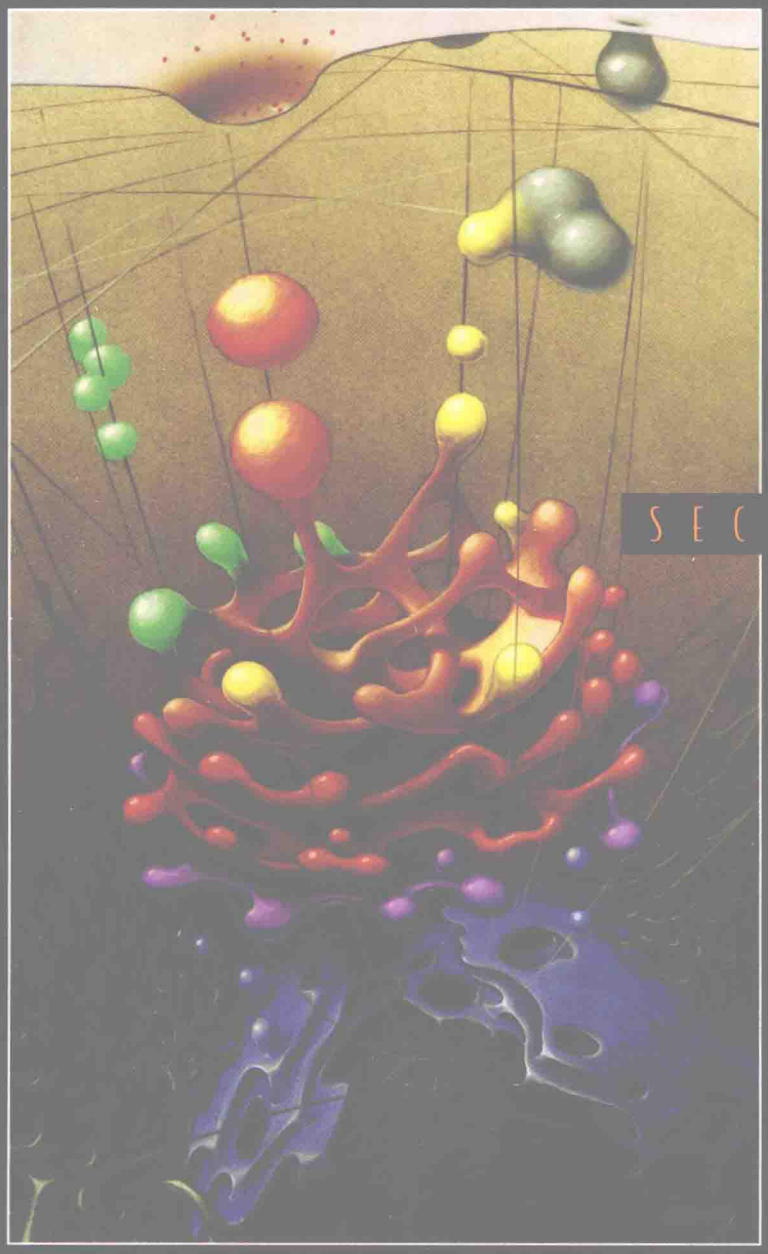

SECOND EDITION

Essential Histology

DAVID H. CORMACK

Essential Histology

Second Edition

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Preface to the Second Edition

Essential Histology is a core selection of histology relating microscopic and molecular details of tissues, cells, and key cellular products to medical cell biology. Its primary focus is tissue and organ function at the cellular level. Maintained from the first edition is the primary goal of presenting the essentials of histology concisely to medical students and others who are faced with the dual challenge of having a minimal background in medical cell biology and a restricted amount of time for study. Those who are entirely new to the subject will also find the logical progression from fundamentals to body systems adequately explained. The subject matter added to this edition remains appropriate for the time constraints experienced by most students who are studying histology.

The second edition of *Essential Histology* contains further molecular information to reflect its burgeoning importance. Keyed to the revised text are improved color plates for facilitating slide interpretation when supplementary atlases are not available. In relating histology further to medical disorders, I have endeavoured to keep this edition fully congruent and synergistic with my other text, *Clinically Integrated Histology* (Lippincott-Raven, 1998).

DAVID H. CORMACK

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Contents

CHAPTER 1

Introduction to Histology

OBJECTIVES

The information in this chapter should enable you to do the following:

- Define histology
- Explain the term **basic tissue**
- Recognize which components in a histologic section represent cells, extracellular matrix, or body fluids
- Draw a cross section of a cell and show which parts of it can be seen in histologic sections
- Use a microscope properly and study sections effectively
- Distinguish between basophilic and acidophilic staining and cite examples of each
- Interpret in three dimensions what you observe in sections
- Summarize the main similarities and differences between a light microscope and an electron microscope

Histology means the **science of the tissues** (Gk. *histos*, web or tissue; *logia*, branch of learning). By establishing the significance of distinctive microscopic features of cells and tissues, histologic studies elucidate the relationships between microscopic structure and function.

BASIC TISSUES

The word **tissue** (L. *texere*, to weave) was first used in an anatomical context by Bichat, a French surgeon who was impressed by the different textures found in the body parts he dissected. As a result, he described the body as being made up of a variety of different **tissues**. Instead of the many distinct tissues that Bichat originally proposed, only four basic tissues are currently recognized, each with variants. All body parts are made up of basic tissues and their variants in distinctive combinations. The first part of this book introduces the cell and the four basic tissues, i.e., epithelial tissue, connective tissue, nervous tissue, and muscle. The remainder describes the body's various organ systems, constructed of basic tissues, which perform essential functions for the body as a whole.

NONCELLULAR CONSTITUENTS OF TISSUES

Cells are essentially soft and gelatinous. If the body were composed entirely of cells, it would be too weak to support its weight. However, cells of connective tissue produce **intercellular (extracellular) matrix** constituents (L. *inter*, between; L. *extra*, outside of), some of which are remarkably strong. Bone tissue, for example, produces a hard extracellular matrix that is reinforced internally in the same way as concrete. Hence, in some respects, the body resembles a building made of extracellular matrix that is inhabited by various kinds of cells. These cells must also be supplied with nutrients and oxygen, and adequate arrangements are required for disposal of toxic byproducts. For these purposes, the body depends on yet another component, its **body fluids**. These include blood plasma, which is a complex fluid that circulates within the confines of the blood vascular system, and a few other extracellular fluids that will be considered in due course. The three primary components that make up the body tissues are 1) cells, 2) extracellular matrix, and 3) body fluids. The first step in recognizing various tissues is to appreciate the microscopic appearance of their individual cells and to learn how to identify the various extracellular matrix constituents. In addition, it is helpful to learn how to recognize the sites where body fluids were present during life. But before we consider the microscopic appearance of these primary tissue components, we should outline how tissues are prepared for routine light microscopy.

PREPARATION OF HISTOLOGIC SECTIONS

Much of our detailed knowledge of body structure comes from a study of small representative samples cut into very thin slices termed tissue **sections**. Light microscopic sections need to be thin enough to transmit plenty of light, which comes from underneath and must pass through the specimen, the objective lens, and then the eyepiece lens, before reaching the eye. In general, the thinner the section, the less is the likelihood that its components will appear superimposed. The optimal thickness of light microscopic sections (5 to 8 μm) is less than the diameter of a typical cell. Histologic sections are routinely prepared by the **paraffin technique.** Alternate procedures for preparing tissues are also described.

Paraffin Sections

The standard paraffin technique consists of the following stages.

Tissue Sampling

Tissue blocks (tissue samples cut <1 cm in each dimension) may be obtained through biopsy (diagnostic sampling), surgical excision, or postmortem dissection. To avoid misleading structural deterioration, postmortem samples should be taken as soon as possible, and to minimize tissue distortion, dissection instruments should be kept extremely sharp. Tissue blocks must be immersed in fixative immediately after removal.

Fixing

Chemical fixation, required to avoid unnecessary distortion, crosslinks certain proteins and denatures others through dehydration. The resulting coagulation of tissue proteins has a hardening effect on soft tissues. Fixation needs to be rapid enough to curtail release from dead cells of enzymes capable of digesting tissue constituents. If degradation by such enzymes is allowed to continue, it ruins microscopic detail, causing **postmortem degeneration.** Fixatives also lock into position a number of carbohydrate- and fat-containing macromolecules that otherwise would be lost during tissue processing. Moreover, fixation kills bacteria and other disease-causing agents, and its antiseptic action decreases risk of contamination when infected tissues are handled. Fixation can also enhance tissue staining. Special fixatives are used for some tissue components, but a 4% aqueous solution of formaldehyde, buffered to neutral pH, is suitable for routine work.

Dehydrating

Paraffin embedding replaces tissue water with paraffin wax, enabling the block to be cut readily. Because paraffin is not water soluble, water is removed from the fixed tissue by passing it through successively stronger solutions of ethyl alcohol, allowing enough time for thorough reagent penetration at each stage. Because paraffin is insoluble in alcohol, the next stage, clearing, involves replacing alcohol with a paraffin solvent that is miscible with alcohol.

Clearing

Xylene is routinely used for clearing tissues. The alcohol-permeated block is passed through several changes of this solvent to replace alcohol with xylene.

Embedding

The xylene-permeated block is passed through several changes of warm paraffin wax, which is soluble in xylene. Once the tissues become completely saturated, melted wax occupies spaces formerly occupied by water. On cooling, the wax hardens. Thin shavings can then be cut off the embedded tissues.

Sectioning

Surplus wax is trimmed away and the block is mounted on a cutting device called a **microtome**. The edges of the thin shavings (**sections**) coming off the microtome knife adhere to one another, producing a long ribbon from which single sections may readily be detached (Fig. 1-1).

Staining and Mounting

Aqueous solutions are usually used in staining. Prior to this, the wax must be dissolved and replaced with water. For this, the slide with the attached section is passed first through xylene to remove paraffin, then through

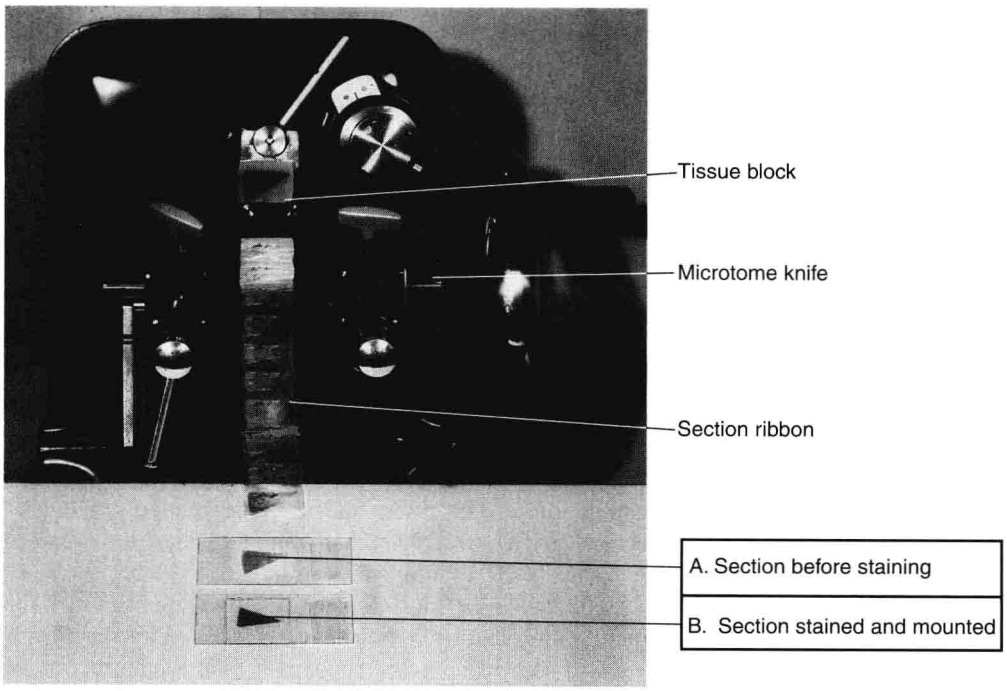

Figure 1-1
Paraffin sections are cut on a microtome for light microscopy.

absolute alcohol to remove xylene, followed by alcohols of decreasing strength and eventually, water. When prepared for staining, the section appears as in Figure 1-1, label *A*. After staining, the section is passed through alcohol solutions of increasing strength, absolute alcohol, and xylene. It is then covered with mounting medium dissolved in xylene. This medium minimizes refraction of the light passing through the section. Once a protective coverslip has been added and xylene has evaporated around its edges, dried mounting medium bonds the coverslip firmly to the slide (see Fig. 1-1, label *B*).

Frozen Sections

If sections need to be examined without delay, **tissue freezing** becomes a preferable means of preparation. Frozen sections are particularly appropriate 1) if the course of a surgical procedure depends on rapid histologic assessment of the nature or spread of a diseased tissue, or 2) if a study of undenatured proteins or lipids in tissues requires avoidance of extraction or harsh fixation. Virtually everything present in living tissues remains represented in frozen sections, but these sections require prompt observation because their constituents are not preserved (subsequent fixation, though sometimes used, compromises certain advantages of the method). Moreover, frozen sections need to be cut slightly thicker (5 to 10 μm) than paraffin sections, and they are laborious to prepare in large batches.

The first step in preparing frozen sections is to freeze the block of fresh tissue as rapidly as possible, using liquid nitrogen. Sections are cut inside a refrigerated cabinet called a **cryostat**, which maintains the microtome knife at a subzero temperature. They are then suitably stained for tissue diagnosis or further microscopic observation.

Semithin Sections

Greater resolution is obtainable if light microscopic sections (**semithin sections**) are cut at 0.5 to 2 μm. This requires the use of an epoxy or acrylic resin as the embedding medium. Toluidine blue is usually used to stain these sections.

LIGHT MICROSCOPY

Microscopes 1) produce enlarged images of small objects and 2) reveal details. Whereas enlarging an optical image is called **magnification**, disclosing its fine details is called **resolution**.

The simplified light path in a monocular microscope is shown in Figure 1-2. Unlike the coarse and fine focusing knobs of older microscopes, which raise or lower the microscope tube and its 10× (magnification) **eyepiece (ocular)**, focusing adjustments on modern, binocular microscopes move the stage instead. The microscope stage is a flat plate with a central opening for the condenser that collects light from the lamp filament. At each magnification, the aperture of the iris diaphragm that regulates the diameter of the light beam entering the condenser should be restricted to two-thirds open. To obtain optimal illumination of the slide clipped to the stage, condenser height should also be adjusted. Temporary withdrawal of the top lens of the condenser from the optical path may be necessary when scanning power is used. Interchangeable **objectives** on a revolving disk at the lower end of the microscope tube provide magnifications of 10×, 40×, and 100×, respectively. The 10× objective, known as the **low-power objective**, provides tenfold magnification. Because the eyepiece enlarges the resulting image by a further factor of 10, total magnification with the low-power objective is ×100. Likewise, the 40× objective, called the **high-power objective**, together with the 10× eyepiece, gives a total magnification of ×400. The 100× objective, which is called the **oil-immersion objective**, used in combination with the 10× eyepiece, gives a total magnification of ×1000. When the oil-immersion objective is being used, however, it is necessary to replace the air

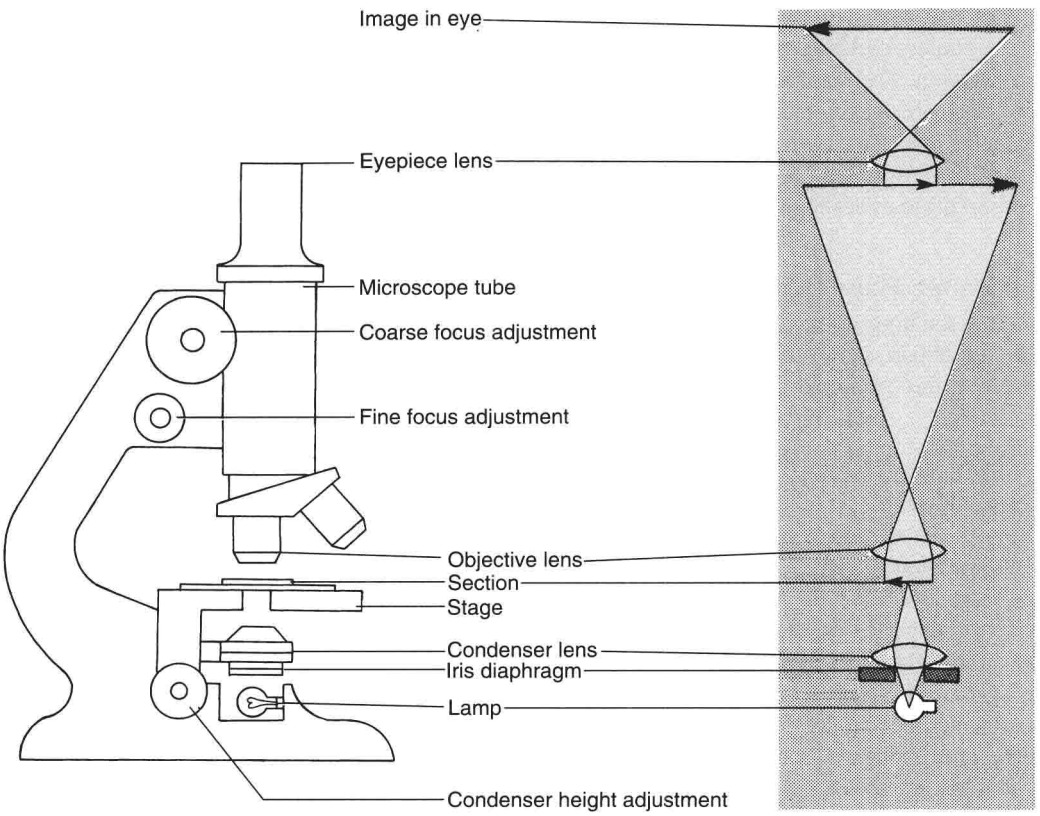

Figure 1-2
Optical components and imaging path of a light microscope (simplified).

between the objective lens and the coverslip with immersion oil of appropriate refractive index. Focus is unobtainable with this objective if the requirement for oil is overlooked. The need for extreme caution when using the oil-immersion objective is noted below.

Modern microscopes have an additional objective that is known as the **scanning lens** because of its low magnification. Such a very low power objective ensures that a relatively large area of the slide comes into view. The *extent* (i.e., diameter) of a given field of view *decreases* in direct proportion to the magnification used. The amount of *detail* that is obtained, however, *increases* with effective magnification up to a maximum of about ×1400.

STUDYING HISTOLOGIC SECTIONS

Before each slide is positioned on the microscope stage, it should be held up to the light and inspected directly, without magnification. Rapid confirmation of the surface of the slide bearing the section serves as a precaution against getting the slide upside down (labels are sometimes inadvertently mounted on the wrong side) and may reveal dirt or oil on the coverslip. Preliminary direct observation often helps in recognition of tissues by those experienced in interpreting histologic sections.

Low-Power Magnification

Most beginners enthusiastically grasp an opportunity to examine tissues under the highest possible magnification. The main advantage of starting at scanning or low-power magnification is that relatively large areas can be seen each time. Also, the slide may be surveyed thoroughly by racking it back and forth in a systematic manner. Meaningful details confined to certain areas may be found only in low-power searches of entire sections. Initial surveys under low power also pinpoint areas for observation under higher magnification.

High-Power Magnification

Swinging the high-power objective into position to examine areas selected under low power should not be a problem. However, if adequate focus cannot be obtained, the slide may be upside down and the section lies too far away to be brought into focus.

Oil-Immersion Magnification

To obtain focus at this magnification, the objective must be brought uncomfortably close to the coverslip over the section. Most oil-immersion objectives are therefore spring-loaded in design. Nevertheless, to avoid damaging the objective or breaking the coverslip, focusing should be done in a cautious manner. Before the oil-immersion objective is swung into place to observe an area preselected under high power, a small drop of immersion oil is placed on the coverslip. Unless it is known that the microscope is parfocal, the free end of the oil-immersion objective, viewed *from one side of the microscope*, is then brought into contact with the oil through use of the coarse focus adjustment. Once contact is established, as indicated by a brief flash of light, focusing is completed through use of the fine focus adjustment. However, if focusing requires a number of turns, make sure that part of the section is aligned with the tiny objective aperture, in which case some color should be visible. Considerable caution is necessary until some experience has been gained.

Cleaning Lenses

If the field of view appears irregularly clouded, distorted, or covered with specks, 1) the coverslip may be dirty, 2) the objective lens may be smeared with immersion oil (this is fairly common in the case of the 40× objective), 3) an eyepiece lens may be dirty, or 4) the top lens of the condenser may be dirty. If the distortion or specks turn when an eyepiece is rotated, the problem is a dirty eyepiece lens. An effective way to clean this lens is to breathe on it lightly and polish it *very gently* with lens paper. Oil on a coverslip or objective may be removed with lens paper (moistened, if required, with a drop of xylene).

HISTOLOGIC STAINS

Tissue components are difficult to distinguish with an ordinary light microscope because their optical densities are so similar. However, many of them can be rendered visible through the selective absorption of dyes. **Histologic stains** reveal tissue components either by coloring them selectively or by increasing their optical densities to different extents. **Electron microscopic stains** increase the electron density of particular tissue components without imparting any colors.

Sections are commonly stained with 1) a dye that imparts a bright color to certain components and 2) a counterstain that imparts a contrasting color to the remainder. **H&E-stained sections** are stained with hematoxylin and eosin. **Hematoxylin** is a dye called **hematein** (obtained from the logwood tree) used in combination with Al^{3+} ions. **Eosin** imparts a pink to red color to most components not stained a bluish purple by hematoxylin. However, many factors influence H&E staining, and the colors obtained depend on staining expertise and the stain batches used.

Basophilic and Acidophilic Staining

Basophilic components take up **basic stains**, whereas **acidophilic** components take up **acid stains.**

Both kinds of stains represent neutral salts. The **acid radical** of a salt is capable of combining with hydrogen to form an *acid*, whereas its **basic radical** is capable of combining with a hydroxyl group to form a *base.* If the color-imparting part of a dye molecule resides in its acid radical, the dye is an **acid stain**; if it lies in the basic radical, the dye is a **basic stain**. Hematoxylin is a basic stain because its color-imparting constituent (hematein + Al^{3+} ions) is its basic radical. Components stained by hematoxylin are therefore described as **basophilic**. Because eosin is an acid stain, components stained by eosin are correspondingly described as **acidophilic** or **eosinophilic**.

Another basis for stain classification is whether the color-imparting constituent is 1) the positively charged cation or 2) the negatively charged anion of the salt. If the color is imparted by the acid radical, which in ionic form bears a negative charge, the stain is an **anionic stain**. Conversely, if the color is imparted by the positively charged (cationic) basic radical, the stain is a **cationic stain.** Thus, acid stains such as eosin are anionic stains, and basic stains such as hematoxylin are cationic stains.

Stains can provide two different colors if their anion imparts one color to acidophilic components and their cation imparts another color to basophilic components. Such **neutral stains** are used primarily to stain blood cells. Alternative staining methods have been devised for tissue components that have weak affinity for ordinary stains. These special methods will be described in the context of the tissues for which they are used.

Interpreting the Colors Seen in Histologic Sections

The composition of tissue components is, of course, more relevant than their colors when stained. However, in a few instances these colors do indicate chemical composition. In **histochemical staining,** established color reactions are used to detect specific chemical groups in tissue components. An example (the PAS reaction) is given later in this chapter. However, histochemical staining is a special case; ordinary stains such as hematoxylin and eosin yield only nonspecific information about the chemical composition of components that they color, as we shall now explain.

Tissue components stain with a basic stain such as hematoxylin, or with an acid stain such as eosin, only if they carry a sufficient number of charged sites to enable them to bind colored dye radicals bearing the opposite charge. Basophilic components possess anionic (negative) sites, and bind the colored cations of hematoxylin (hematein complexed with Al^{3+} ions), whereas acidophilic components possess cationic (positive) sites, and bind the colored anions of eosin. However, the anion- or cation-binding sites are usually present on more than one sort of molecule, and their relative numbers vary according to fixation and staining conditions, so the resulting colors are seldom consistent. Basic and acid stains therefore provide a general indication of chemical composition, but this is not very specific.

Students should guard against becoming over-dependent on colors for routine tissue identification because these can vary. Furthermore, the importance of colors in this connection is often exaggerated. Color-blind students can become proficient at recognizing stained tissues, and stained sections may be usefully compared with black and white photomicrographs. Indeed, such comparisons are helpful preparation for the study of electron micrographs, which are always taken in black and white because an electron beam possesses no color spectrum. In tissue recognition, ample use should always be made of any additional confirming evidence such as size, location, shape, number, and association with other components.

In black and white photomicrographs, blue to purple staining appears as black tones whereas pink to red staining appears as shades of gray. Hence, darker tones indicate hematoxylin staining and lighter tones indicate eosin staining. Contrast between comparable depths of blue and red may be enhanced optically through the use of suitable color filters.

Unstained tissue components are hard to distinguish with an ordinary light microscope because they have comparable optical densities, i.e., they obstruct light to a similar extent. The degree to which they change the *phase* of light, however, varies. By disclosing phase differences as optical density differences, the **phase contrast microscope** reveals various components as gradations of black and white even without fixation or staining, allowing tissues to be observed in the living state.

Our next consideration is interpretation of what is seen in H&E-stained sections.