Chapter 1
Fundamentals of the Microscope

INTRODUCTION

Prior to describing specific methods and applications using optical microscopes of any kind, it is important to understand some fundamentals that unify the technology. This fundamentals chapter is designed with this in mind. We start with Christian Combs' article on current methods, which summarizes most of the currently available tools included in this book. This first article details the primary methods, defining the main advantages and disadvantages of each, and addressing key questions regarding the resolution limits, expense, and difficulty levels of the respective approaches. The goal of this survey article is to provide readers with the basic information they require in order to select the most suitable approach to answer their scientific question, and also to enable the reader to target the appropriate section of the book in a facile way. This first article is followed by five articles covering the "essentials." These articles describe the important components of the fluorescence microscope and include information about how to align and clean each component. Embedded within these articles is a fundamental discussion of objective, filter, and camera choices. This information will hopefully ensure that any purchase decisions are made such that the microscope is well suited to its final scientific task.

The goal of the Microscope Objective article written by Joe Lobiondo is to give an in-depth understanding of the limits and advantages of each objective family, and is arguably one of the most valuable parts of the book. Choosing the right objective lens, regardless of the optical platform, is perhaps the most important decision to make when buying or using a microscope. The importance of numeric aperture cannot be understated, whereas the importance of magnification is commonly overstated. Topics of discussion in this article include the aberrations that occur in an objective and the relative importance of chromatic aberration, spherical aberration, or flatness of field in your particular experiment; how aberrations impact the final image; what ultimately limits the resolution and brightness of a microscope objective; the difference between finite and "infinity" correction in an objective and why these two types of objective cannot be used together. Finally, and perhaps most usefully, the numbers, labels, and scripts that occur on the barrel of every objective are decoded such individuals can make the right choice of lens for the experiment being conducted.

Beyond choosing the correct objective for the question under study, basic alignment of the light microscope is perhaps the most critical and neglected issue when attempting to generate high-quality image data. This is true for both transmitted and fluorescent mode imaging, but is absolutely essential for the former. Ted Salmon's article is a complete and thorough treatment of alignment needed for each technical approach. The article begins with a detailed description of the essential components of the microscope. The authors then define Köhler illumination and explain exactly how to get perfect illumination in transmitted and fluorescence microscopy. These sections are followed by alignment procedures for approaches that are essential for fluorescence microscopy, phase microscopy, differential interference contrast (DIC) imaging, and dark-field imaging. Other valuable topics, beyond alignment, covered in this article are a pragmatic description of test specimens for both the standard transmitted light and fluorescence methods, and appropriate methods for cleaning individual microscope components.

In addition to the objectives, key components of the fluorescent microscope also include the filter cubes and detectors. Turan Erdogan's article is a complete treatment of

how thin-film filters are designed, made, and developed for fluorescence microscopy. Discussion points include choosing filters for multicolor imaging, including fluorescence resonance energy transfer (FRET) and ratiometric imaging, and dealing with cross-talk when collecting multicolor images. This article also addresses the advantages and disadvantages of multicolor filter sets and issues with image registration, and presents an important description of the impact of the fluorescence filters on optical system performance (e.g., the effects of an uneven substrate surface, and/or an incorrect mounting angle). Finally, the opportunities and problems presented by optical tunable filters are discussed.

The final thing to consider when building or using a light microscope is the detector. There have been a number of recent advances in detector technology resulting in rich selection of device types. Each detector has specific advantages and disadvantages with respect to signal-to-noise, gain, spectral sensitivity, and pixel count, and thus the final choice of device will have a critical impact not only on the quality, but also on the quantitative aspects of the recorded image. Deepak Sharma's article deals with the principal aspects of detector design, particularly noise, and highlights the important factors determining final resolution (i.e., the coupler between the microscope and camera). Lastly, the pragmatic aspects of how to maintain and clean a camera are discussed.