LAWRENCE R. GRIFFING

IMAGING LIFE

IMAGE ACQUISITION AND ANALYSIS IN BIOLOGY AND MEDICINE

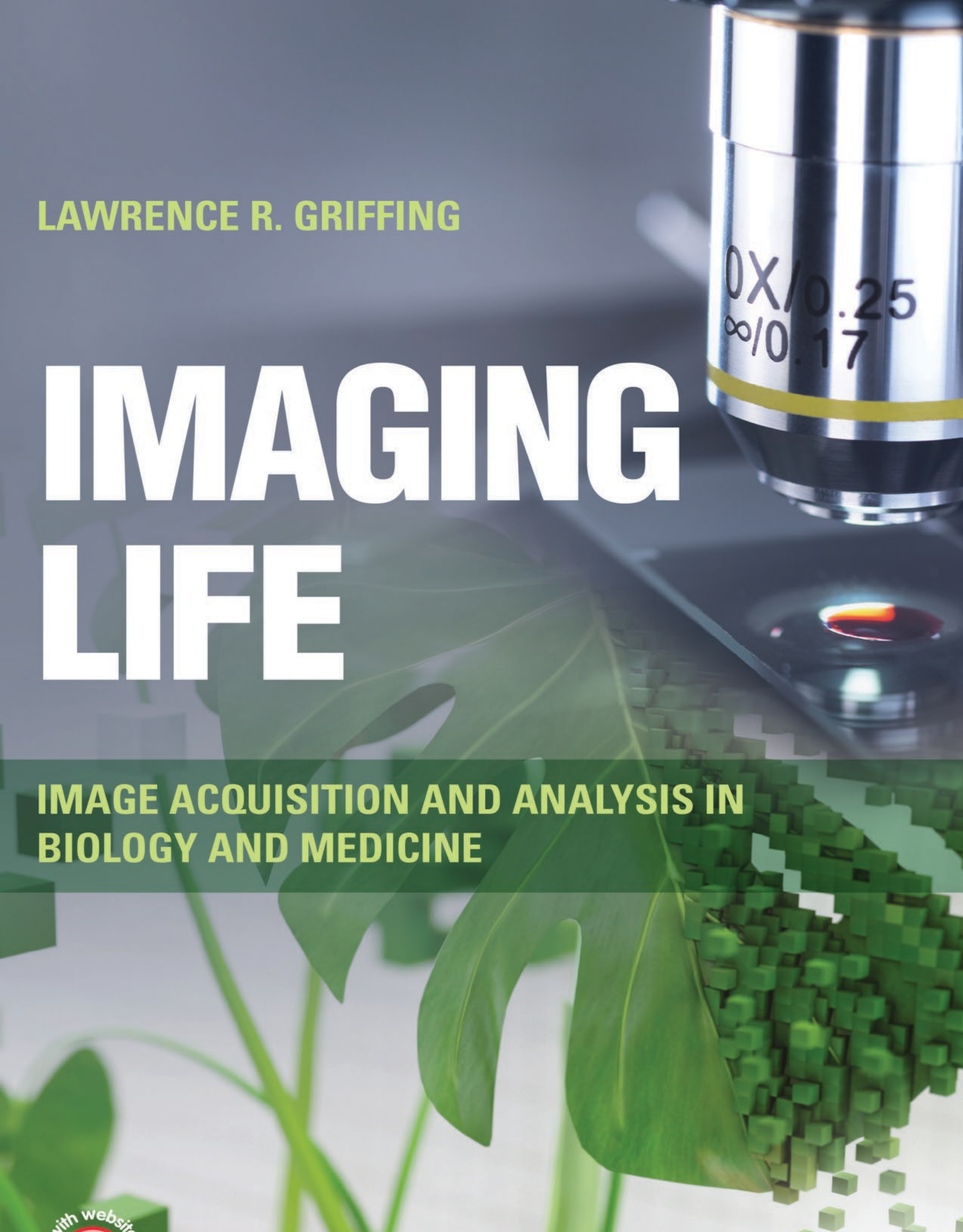

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Image Acquisition and Analysis in Biology and Medicine

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Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

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A catalogue record for this book is available from the Library of Congress

Hardback ISBN: 9781119949206; ePub ISBN: 9781119081579; ePDF ISBN: 9781119081593

Cover image(s): © Westend61/Getty Images; Yaroslav Kushta/Getty Images
Cover design: Wiley

Set in 9.5/12.5pt STIXTwoText by Integra Software Services Pvt. Ltd., Pondicherry, India

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Preface

Imaging Life Has Three Sections: Image Acquisition, Image Analysis, and Imaging Modalities

The first section, **Image Acquisition**, lays the foundation for imaging by extending prior knowledge about image structure (Chapter 1), image contrast (Chapter 2), and proper image representation (Chapter 3). The chapters on imaging by eye (Chapter 4), by camera (Chapter 5), and by scanners (Chapter 6) relate to prior knowledge of sight, digital (e.g., cell phone) cameras, and flatbed scanners.

The second section, **Image Analysis**, starts with how to select features in an image and measure them (Chapter 7). With this knowledge comes the realization that there are limits to image measurement set by the optics of the system (Chapter 8), a system that includes the sample and the light- and radiation-gathering properties of the instrumentation. For light-based imaging, the nature of the lighting and its ability to generate contrast (Chapter 9) optimize the image data acquired for analysis. A wide variety of image filters (Chapter 10) that operate in real and reciprocal space make it possible to display or measure large amounts of data or data with low signal. Spatial measurement in two dimensions (Chapter 11), measurement in time (Chapter 12), and processing and measurement in three dimensions (Chapter 13) cover many of the tenets of image analysis at the macro and micro levels.

The third section, **Imaging Modalities**, builds on some of the modalities necessarily introduced in previous chapters, such as computed tomography (CT) scanning, basic microscopy, and camera optics. Many students interested in biological imaging are particularly interested in biomedical modalities. Unfortunately, most of the classes in biomedical imaging are not part of standard biology curricula but in biomedical engineering. Likewise, students in biomedical engineering often get less exposure to microscopy-related modalities. This section brings the two together.

The book does not use examples from materials science, although some materials science students may find it useful.

Imaging Life Can Be Either a Lecture Course or a Lab Course

This book can stand alone as a text for a lecture course on biological imaging intended for junior or senior undergraduates or first- and second-year graduate students in life sciences. The annotated references section at the end of each chapter provides the URLs for supplementary videos available from iBiology.com and other recommended sites. In addition, the recommended text-based internet, print, and electronic resources, such as microscopyu.com, provide expert and in-depth materials on digital imaging and light microscopy. However, these resources focus on particular imaging modalities and exclude some (e.g., single-lens reflex cameras, ultrasound, CT scanning, magnetic resonance imaging [MRI], structure from motion). The objective of this book is to serve as a solid foundation in imaging, emphasizing the shared concepts of these imaging approaches. In this vein, the book does not attempt to be encyclopedic but instead provides a gateway to the ongoing advances in biological imaging.

The author's biology course non-linearly builds off this text with weekly computer sessions. Every third class session covers practical image processing, analysis, and presentations with still, video, and three-dimensional (3D) images. Although these computer labs may introduce Adobe Photoshop and Illustrator and MATLAB and Simulink (available on our university computers), the class primarily uses open-source software (i.e., GIMP2, Inkscape, FIJI [FIJI Is Just ImageJ], Icy, and Blender). The course emphasizes open-source imaging. Many open-source software packages use published and archived algorithms. This is better for science, making image processing more reproducible. They are also free or at least cheaper for students and university labs.

The images the students acquire on their own with their cell phones, in the lab (if taught as a lab course), or from online scientific databases (e.g., Morphosource.org) are the subjects of these tutorials. The initial tutorials simply introduce basic features of the software that are fun, such as 3D model reconstruction in FIJI of CT scans from Morphosource, and informative, such as how to control image size, resolving power, and compression for analysis and publication. Although simple, the tutorials address major pedagogical challenges caused by the casual, uninformed use of digital images. The tutorials combine the opportunity to judge and analyze images acquired by the students with the opportunity to learn about the software. They are the basis for weekly assignments. Later tutorials provide instruction on video and 3D editing, as well as more advanced image processing (filters and deconvolution) and measurement. An important learning outcome for the course is that the students can use this software to rigorously analyze and manage imaging data, as well as generate publication-quality images, videos, and presentations.

This book can also serve as a text for a laboratory course, along with an accompanying lab manual that contains protocols for experiments and instructions for the operation of particular instruments. The current lab manual is available on request, but it has instructions for equipment at Texas A&M University. Besides cell phones, digital single-lens reflex cameras, flatbed scanners, and stereo-microscopes, the first quarter of the lab includes brightfield transmitted light microscopy and fluorescence microscopy. Assigning Chapter 16 on transmitted light microscopy and Chapter 17 on epi-illuminated light microscopy early in the course supplements the lab manual information and introduces the students to microscopy before covering it during class time. Almost all the students have worked with microscopes before, but many have not captured images that require better set-up (e.g., Köhler illumination with a sub-stage condenser) and a more thorough understanding of image acquisition and lighting.

The lab course involves students using imaging instrumentation. All the students have access to cameras on their cell phones, and most labs have access to brightfield microscopy, perhaps with various contrast-generating optical configurations (darkfield, phase contrast, differential interference contrast). Access to fluorescence microscopy is also important. One of the anticipated learning outcomes for the lab course is that students can troubleshoot optical systems. For this reason, it is important that they take apart, clean, and correctly reassemble and align some optical instruments for calibrated image acquisition. With this knowledge, they can become responsible users of more expensive, multi-user equipment. Some might even learn how to build their own!

Access to CT scanning, confocal microscopy, multi-photon microscopy, ultrasonography, MRI, light sheet microscopy, superresolution light microscopy, and electron microscopy will vary by institution. Students can use remote learning to view demonstrations of how to set up and use them. Many of these instruments have linkage to the internet. Zoom (or other live video) presentations provide access to operator activity for the entire class and are therefore preferable for larger classes that need to see the operation of a machine with restricted access. Several instrument companies provide video demonstrations of the use of their instruments. Live video is more informative, particularly if the students read about the instruments first with a distilled set of instrument-operating instructions, so they can then ask questions of the operators. Example images from the tutorials for most of these modalities should be available for student analysis.

Acknowledgments

Peter Hepler and Paul Green taught a light and electron microscopy course at Stanford University that introduced me to the topic while I was a graduate student of Peter Ray. After working in the lab of Ralph Quatrano, I acquired additional expertise in light and electron microscopy as a post-doc with Larry Fowke and Fred Constabel at the University of Saskatchewan and collaborating with Hilton Mollenhauer at Texas A&M University. They were all great mentors.

I created a light and electron microscopy course for upper-level undergraduates with Kate VandenBosch, who had taken a later version of Hepler's course at the University of Massachusetts. However, with the widespread adoption of digital imaging, I took the course in a different direction. The goals were to introduce students to digital image acquisition, processing, and analysis while they learned about the diverse modalities of digital imaging. The National Science Foundation and the Biology Department at Texas A&M University provided financial support for the course. No single textbook existing for such a course, I decided to write one. Texas A&M University graciously provided one semester of development leave for its completion.

Martin Steer at University College Dublin and Chris Hawes at Oxford Brookes University, Oxford, read and made constructive comments on sections of the first half of the book, as did Kate VandenBosch at the University of Wisconsin. I thank them for their help, friendship, and encouragement.

I give my loving thanks to my children. Alexander Griffing contributed a much-needed perspective on all of the chapters, extensively copy edited the text, and provided commentary and corrections on the math. Daniel Griffing also provided helpful suggestions. Beth Russell was a constant source of enthusiasm.

My collaborators, Holly Gibbs and Alvin Yeh at Texas A&M University, read several chapters and made comments and contributions that were useful and informative. Jennifer Lippincott-Schwartz, senior group leader and head of Janelia's four-dimensional cellular physiology program, generously provided comment and insight on the chapters on temporal operations and superresolution microscopy. I also wish to thank the students in my lab who served as teaching assistants and provided enthusiastic and welcome feedback, particularly Kalli Landua, Krishna Kumar, and Sara Maynard. The editors at Wiley, particularly Rosie Hayden and Julia Squarr, provided help and encouragement. Any errors, of course, are mine.

The person most responsible for the completion of this book is my wife, Margaret Ezell, who motivates and enlightens me. In addition to her expertise and authorship on early modern literary history, including science, she is an accomplished photographer. Imaging life is one of our mutual joys. I dedicate this book to her, with love and affection.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/griffing/imaginglife

Please note that the resources are password protected.

The resources include:

- Images and tables from the book
- Examples of the use of open source software to introduce and illustrate important features with video tutorials on YouTube
- Data, and a description of its acquisition, for use in the examples

Section 1

Image Acquisition

1

Image Structure and Pixels

1.1 The Pixel Is the Smallest Discrete Unit of a Picture

Images have structure. They have a certain arrangement of small and large objects. The large objects are often composites of small objects. The Roman mosaic from the House VIII.1.16 in Pompeii, the House of Five Floors, has incredible structure (Figure 1.1). It has lifelike images of a bird on a reef, fishes, an electric eel, a shrimp, a squid, an octopus, and a rock lobster. It illustrates Aristotle's natural history account of a struggle between a rock lobster and an octopus. In fact, the species are identifiable and are common to certain bays in the Italian coast, a remarkable example of early biological imaging.

It is a mosaic of uniformly sized square colored tiles. Each tile is the smallest picture element, or **pixel**, of the mosaic. At a certain **appropriate viewing distance** from the mosaic, the individual pixels cannot be distinguished, or resolved, and what is a combination of individual tiles looks solid or continuous, taking the form of a fish, or lobster, or octopus. When viewed closer than this distance, the individual tiles or pixels become apparent (see Figure 1.1); the image is **pixelated**. Beyond viewing it from the distance that is the height of the person standing on the mosaic, pixelation in this scene was probably further reduced by the shallow pool of water that covered it in the House of Five Floors.

The order in which the image elements come together, or render, also describes the image structure. This mosaic was probably constructed by tiling the different objects in the scene, then surrounding the objects with a single layer of tiles of the black background (Figure 1.2), and finally filling in the background with parallel rows of black tiles. This form of image construction is **object-order rendering**. The background rendering follows the rendering of the objects. **Vector graphic** images use object-ordered rendering. Vector graphics define the object mathematically with a set of vectors and render it in a scene, with the background and other objects rendered separately.

Vector graphics are very useful because any number of pixels can represent the mathematically defined objects. This is why programs, such as Adobe Illustrator, with vector graphics for fonts and illustrated objects are so useful: the number (and, therefore, size) of pixels that represent the image is chosen by the user and depends on the type of media that will display it. This number can be set so that the fonts and objects never have to appear pixelated. *Vector graphics are resolution independent*; scaling the object to any size will not lose its sharpness from pixelation.

Another way to make the mosaic would be to start from the top upper left of the mosaic and start tiling in rows. One row near the top of the mosaic contains parts of three fishes, a shrimp, and the background. This form of image structure is **image-order rendering**. Many scanning systems construct images using this form of rendering. A horizontal scan line is a **raster.** Almost all computer displays and televisions are raster based. They display a rasterized grid of data, and because the data are in the form of bits (see Section 2.2), it is a bitmap image. As described later, *bitmap graphics are resolution dependent*; that is, as they scale larger, the pixels become larger, and the images become pixelated.

Even though pixels are the smallest discrete unit of the picture, it does have structure. The fundamental unit of visualization is the **cell** (Figure 1.3). A **pixel** is a two-dimensional (2D) cell described by an ordered list of four points (its corners or vertices), and geometric constraints make it square. In three-dimensional (3D) images, the smallest discrete unit of the volume is the voxel. A **voxel** is the 3D cell described by an ordered list of eight points (its vertices), and geometrics constraints make it a cube.

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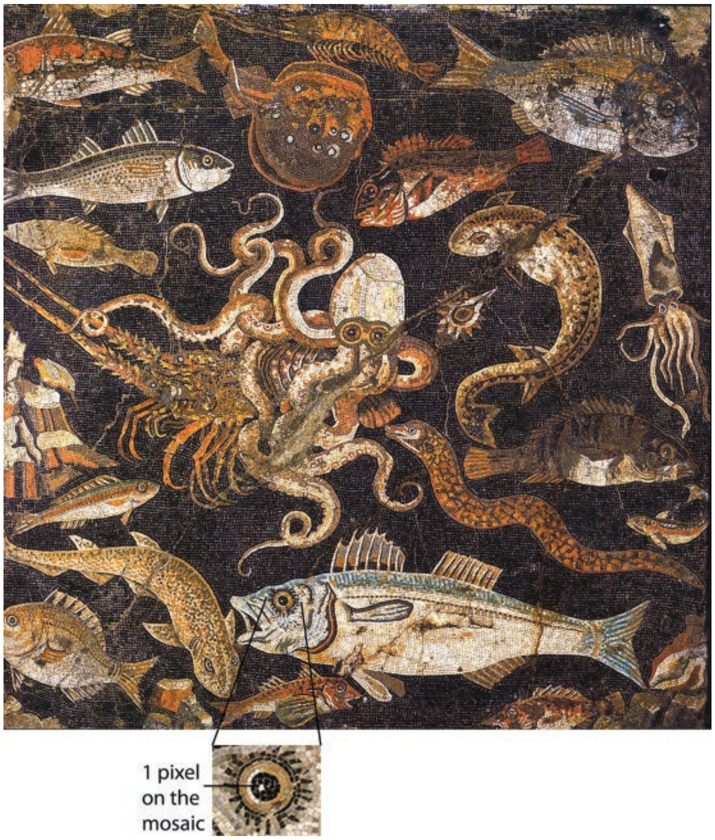

Figure 1.1 The fishes mosaic (second century BCE) from House VII.2.16, the House of Five Floors, in Pompeii. The lower image is an enlargement of the fish eye, showing that light reflection off the eye is a single tile, or pixel, in the image. Photo by Wolfgang Rieger, http://commons.wikimedia.org/wiki/File:Pompeii_-_Casa_del_Fauno_-_MAN.jpg and is in the public domain (PD-1996).

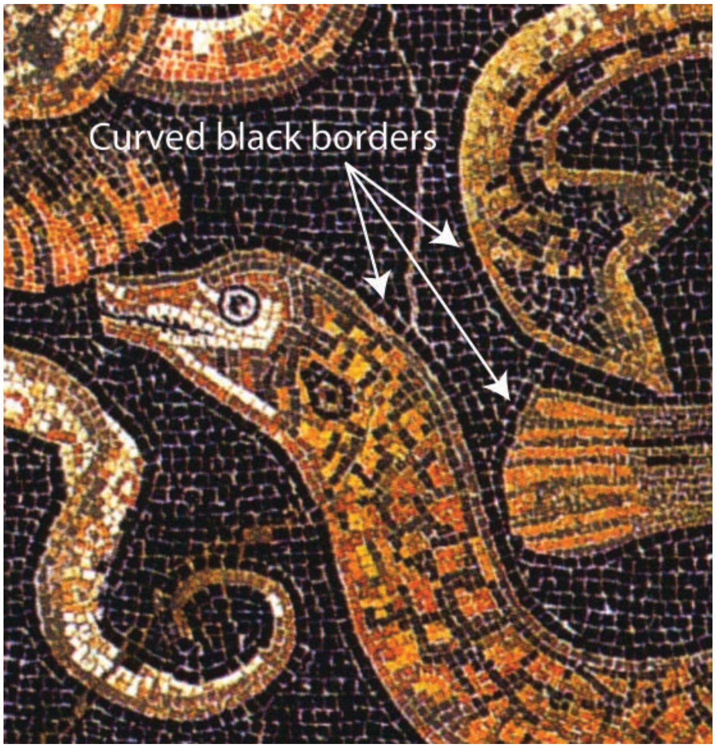

Figure 1.2 Detail from Figure 2.1. The line of black tiles around the curved borders of the eel and the fish are evidence that the mosaic employs object-order rendering.

Color is a subpixel component of electronic displays; printed material; and, remarkably, some paintings. Georges Seurat (1859–1891) was a famous French post-impressionist painter. Seurat communicated his impression of a scene by constructing his picture from many small dabs or points of paint (Figure 1.4); he was a **pointillist**. However, each dab of paint is not a pixel. Instead, when standing at the appropriate viewing distance, dabs of differently colored paint combine to form a new color. Seurat pioneered this practice of **subpixel color**. Computer displays use it, each pixel being made up of stripes (or dots) of red, green, and blue color (see Figure 1.4). The intensity of the different stripes determines the displayed color of the pixel.

For many printed images, the half-tone cell is the pixel. A half-tone cell contains an array of many black and white dots or dots of different colors (see Figure 1.10); the more dots within the half-tone cell, the more shades of gray or color that are possible. Chapter 2 is all about how different pixel values produce different shades of gray or color.

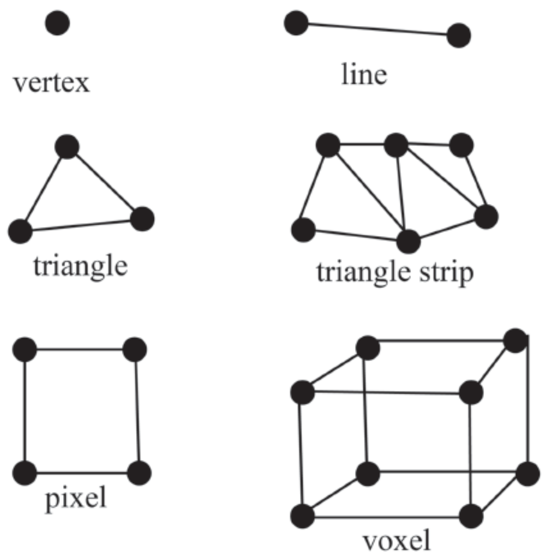

Figure 1.3 Cell types found in visualization systems that can handle two- and three-dimensional representation. Diagram by L. Griffing.

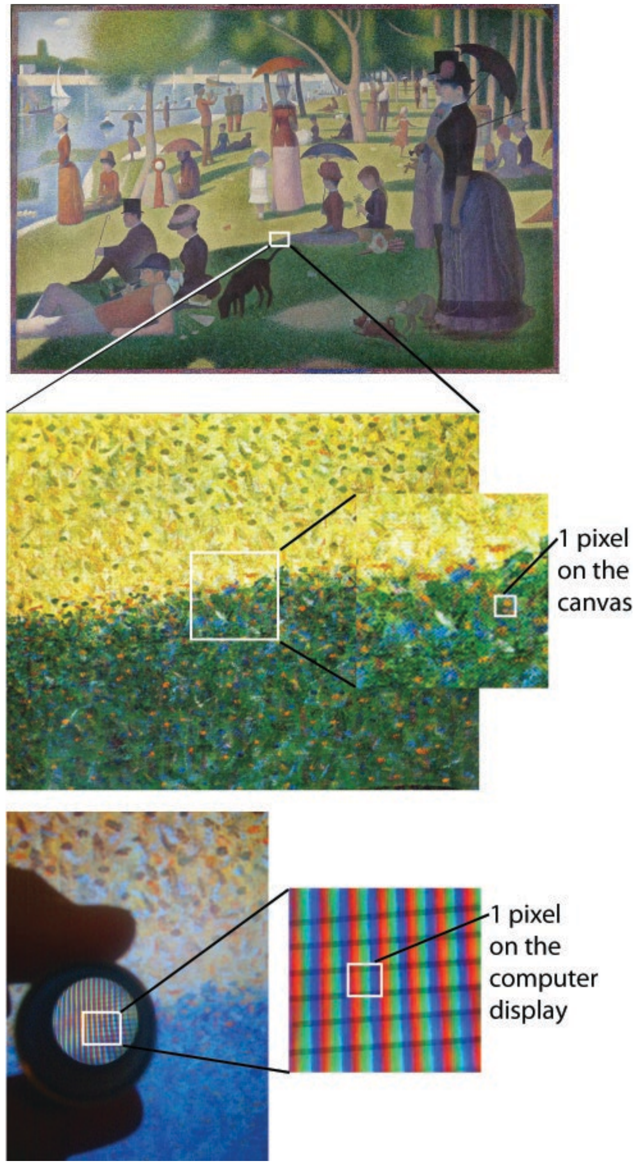

Figure 1.4 This famous picture *A Sunday Afternoon on the Island of La Grande Jatte* (1884–1886) by Georges Seurat is made up of small dots or dabs of paint, each discrete and with a separate color. Viewed from a distance, the different points of color, usually primary colors, blend in the mind of the observer and create a canvas with a full spectrum of color. The lower panel shows a picture of a liquid crystal display on a laptop that is displaying a region of the Seurat painting magnified through a lens. The view through the lens reveals that the image is composed of differently illuminated pixels made up of parallel stripes of red, green, and blue colors. The upper image is from https://commons.wikimedia.org/wiki/File:A_Sunday_on_La_Grande_Jatte,_Georges_Seurat,_1884.jpg. Lower photos by L. Griffing.

1.2 The Resolving Power of a Camera or Display Is the Spatial Frequency of Its Pixels

In biological imaging, we use powerful lenses to resolve details of far away or very small objects. The round plant protoplasts in Figure 1.5 are invisible to the naked eye. To get an image of them, we need to use lenses that collect a lot of light from a very small area and magnify the image onto the chip of a camera. Not only is the power of the lens important but also the power of the camera. Naively, we might think that a powerful camera will have more pixels (e.g., 16 megapixels [MP]) on its chip than a less powerful one (e.g., 4 MP). Not necessarily! The 4-MP camera could actually be more powerful (require less magnification) if the pixels are smaller. The size of the chip and the pixels in the chip matter.

The power of a lens or camera chip is its **resolving power**, the number of pixels per unit length (assuming a square pixel). It is not the number of total pixels but the number of pixels per unit space, the **spatial frequency** of pixels. For example, the eye on the bird in the mosaic in Figure 1.1 is only 1 pixel (one tile) big. There is no detail to it. Adding more tiles to give the eye some detail requires smaller tiles, that is, the number of tiles within that space of the eye increases – the spatial frequency of pixels has to increase. Just adding more tiles of the original size will do no good at all. Common measures of spatial frequency and resolving power are **pixels per inch** (ppi) or **lines per millimeter** (lpm – used in printing).

Another way to think about resolving power is to take its inverse, the inches or millimeters per pixel. Pixel size, the inverse of the resolving power, is the image **resolution**. One bright pixel between two dark pixels resolves the two dark pixels. Resolution is the minimum separation distance for distinguishing two objects, d_{min}. Resolving power is $1/d_{min}$. Note: Usage of the terms *resolving power* and *resolution* is not universal. For example, Adobe Photoshop and Gimp use *resolution* to refer to the spatial frequency of the image. Using *resolving power* to describe spatial frequencies facilitates the discussion of spatial frequencies later.

As indicated by the example of the bird eye in the mosaic and as shown in Figure 1.5, the resolving power is as important in image display as it is in detecting the small features of the object. To eliminate pixelation detected by eye, the resolving power of the eye should be less than the pixel spatial frequency on the display medium when viewed from an **appropriate viewing distance**. The eye can resolve objects separated by about 1 minute (one 60th) of 1 degree of the almost 140-degree field of view for binocular vision. Because things appear smaller with distance, that is, occupy a

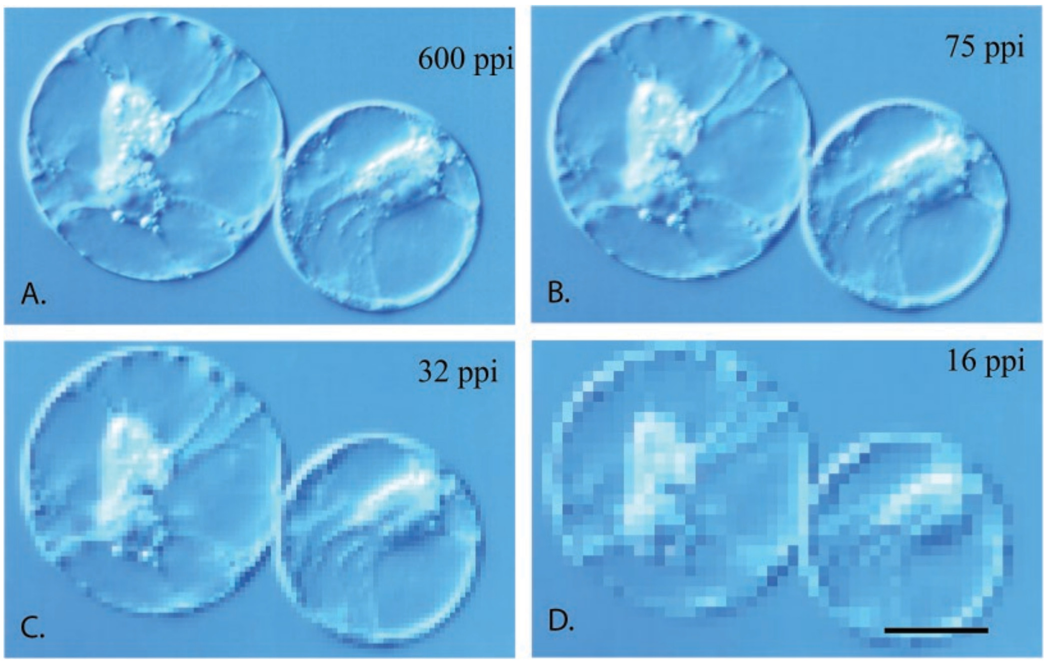

Figure 1.5 Soybean protoplasts (cells with their cell walls digested away with enzymes) imaged with differential interference contrast microscopy and displayed at different resolving powers. The scale bar is 10 μm long. The mosaic pixelation filter in Photoshop generated these images. This filter divides the spatial frequency of pixels in the original by the "cell size" in the dialog box (filter > pixelate > mosaic). The original is 600 ppi. The 75-ppi images used a cell size of 8, the 32-ppi image used a cell size of 16, and the 16-ppi image used a cell size of 32. Photo by L. Griffing.

Table 1.1 Laptop, Netbook, and Tablet Monitor Sizes, Resolving Power, and Resolution.

Size (Diagonal)	Horizontal × Vertical Pixel Number	Resolving Power: Dot Pitch (ppi)	Resolution or Pixel Size (mm)	Aspect Ratio (W:H)	Pixel Number ($\times 10^6$)
6.8 inches (Kindle Paperwhite 5)	1236 × 1648	300	0.0846	4:3	2.03
11 inches (iPad Pro)	2388 × 1668	264 (retina display)	0.1087	4:3	3.98
10.1 inches (Amazon Fire HD 10 e)	1920 × 1200	224	0.1134	16:10	2.3
12.1 inches (netbook)	1400 × 1050	144.6	0.1756	4:3	1.4
13.3 inches (laptop)	1920 ×1080	165.6	0.153	16:9	2.07
14 inches (laptop)	1920 × 1080	157	0.161	16:9	2.07
	2560 × 1440	209.8	0.121	16:9	3.6
15.2 inches (laptop)	1152 × 768	91	0.278	3:2	0.8
15.6 inches (laptop)	1920 × 1200	147	0.1728	8:5	2.2
	3840 × 2160	282.4	0.089	16:9	8.2
17 inches (laptop)	1920 × 1080	129	0.196	16:9	2.07

smaller angle in the field of view, even things with large pixels look non-pixelated at large distances. Hence, the pixels on roadside signs and billboards can have very low spatial frequencies, and the signs will still look non-pixelated when viewed from the road.

Appropriate viewing distances vary with the display device. Presumably, the floor mosaic (it was an interior shallow pool, so it would have been covered in water) has an ideal viewing distance, the distance to the eye, of about 6 feet. At this distance, the individual tiles would blur enough to be indistinguishable. For printed material, the closest point at which objects come into focus is the **near point**, or 25 cm (10 inches) from your eyes. Ideal viewing for typed text varies with the size of font but is between 25 and 50 cm (10 and 20 inches). The ideal viewing distance for a television display, with 1080 horizontal raster lines, is four times the height of the screen or two times the **diagonal screen dimension**. When describing a display or monitor, we use its diagonal dimension (Table 1.1). We also use numbers of pixels. A 14-inch monitor with the same number of pixels as a 13.3-inch monitor (2.07×10^6 in Table 1.1) has larger pixels, requiring a slightly farther appropriate viewing distance. Likewise, viewing a 24-inch HD 1080 television from 4 feet is equivalent to viewing a 48-inch HD 1080 television from 8 feet.

There are different display standards, based on **aspect ratio**, the ratio of width to height of the displayed image (Table 1.2). For example, the 15.6-inch monitors in Table 1.1 have different aspect ratios (Apple has 8:5 or 16:10, while Windows has 16:9). They also use different standards: a 1920 × 1200 monitor uses the WUXGA standard (see Table 1.2), and the 3840 × 2160 monitor uses the UHD-1 standard (also called 4K, but true 4K is different; see Table 1.2). The UHD-1 monitor has half the pixel size of the WUXGA monitor. Even though these monitors have the same diagonal dimension, they have different appropriate viewing distances. The standards in Table 1.2 are important when generating video (see Sections 5.8 and 5.9) because different devices have different sizes of display (see Table 1.1). Furthermore, different video publication sites such as YouTube and Facebook and professional journals use standards that fit multiple devices, not just devices with high resolving power. We now turn to this general problem of different resolving powers for different media.

1.3 Image Legibility Is the Ability to Recognize Text in an Image by Eye

Image legibility, or the ability to recognize text in an image, is another way to think about resolution (Table 1.3). This concept incorporates not only the resolution of the display medium but also the resolution of the recording medium, in this case, the eye. Image legibility depends on the eye's *inability* to detect pixels in an image. In a highly legible image, the eye does not see the individual pixels making up the text (i.e., the text "looks" smooth). In other words, for text to be highly legible, the pixels should have a spatial frequency near to or exceeding the resolving power of the eye.

At near point (25 cm), it is difficult for the eye to resolve two points separated by 0.1 mm or less. An image that resolves 0.1 mm pixels has a resolving power of 10 pixels per mm (254 ppi). Consequently, a picture reproduced at 300 ppi would

Table 1.2 Display Standards.

Aspect Ratio (Width:Height in Pixels)			
4:3	8:5 (16:10)	16:9	Various
QVGA	CGA		
320 × 240	320 × 200		
SIF/CIF			
384 × 288			
352 × 288			
VGA	WVGA (5:3)	WVGA	
640 × 480	800 × 480	854 × 480	
PAL		PAL	
768 × 576		1024 × 576	
SVGA		WSVGA	
800 × 600		1024 × 600	
XGA	WXGA	HD 720	
1024 × 786	1280 × 800	1280 × 720	
SXGA+	WXGA+	HD 1080	SXGA (5:4)
1400 × 1050	1680 × 1050	1920 × 1080	1280 × 1024
UXGA	WUXGA	2K (17:9)	UWHD (21:9)
1600 × 1200	1920 × 1200	2048 × 1080	2560 × 1080
QXGA	WQXGA	WQHD	QSXGA (5:4)
2048 × 1536	1560 × 1600	2560 × 1440	2560:2048
		UHD-1	UWQHD (21:9)
		3840 × 2160	3440 × 1440
		4K (17:9)	
		4096 × 2160	
		8K	
		7680 × 4320	

Table 1.3 Image Legibility.

Resolving Power			
ppi	**lpm**	**Legibility**	**Quality**
200	8	Excellent	High clarity
100	4	Good	Clear enough for prolonged study
50	2	Fair	Identity of letters questionable
25	1	Poor	Writing illegible

lpm, lines per inch; ppi, pixels per inch.

have excellent text legibility (see Table 1.3). However, there are degrees of legibility; some early computer displays had a resolving power, also called **dot pitch**, of only 72 ppi. As seen in Figure 1.5, some of the small particles in the cytoplasm of the cell vanish at that resolving power. Nevertheless, 72 ppi is the borderline between good and fair legibility (see Table 1.3) and provides enough legibility for people to read text on the early computers.

The average computer is now a platform for image display. Circulation of electronic images via the web presents something of a dilemma. What should the resolving power of web-published images be? To include computer users who use old displays,

Table 1.4 Resolving Power Required for Excellent Images from Different Media.

Imaging Media	Resolving Power (ppi)
Portable computer	90–180
Standard print text	200
Printed image	300 (grayscale)
	350–600 (color)
Film negative scan	1500 (grayscale)
	3000 (color)
Black and white line drawing	1500 (best done with vector graphics)

the solution is to make it equal to the lowest resolving power of any monitor (i.e., 72 ppi). Images at this resolving power also have a small file size, which is ideal for web communication. However, most modern portable computers have larger resolving powers (see Table 1.1) because as the numbers of horizontal and vertical pixels increase, the displays remain a physical size that is portable. A 72-ppi image displayed on a 144-ppi screen becomes half the size in each dimension. Likewise, high-ppi images become much bigger on low-ppi screens. This same problem necessitates reduction of the resolving power of a photograph taken with a digital camera when published on the web. A digital camera may have 600 ppi as its default output resolution. If a web browser displays images at 72 ppi, the 600-ppi image looks eight times its size in each dimension.

This brings us to an important point. *Different imaging media have different resolving powers*. For each type of media, the final product must look non-pixelated when viewed by eye (Table 1.4). These values are representative of those required for publication in scientific journals. Journals generally require grayscale images to be 300 ppi, and color images should be 350–600 ppi. The resolving power of the final image is not the same as the resolving power of the newly acquired image (e.g., that on the camera chip). The display of images acquired on a small camera chip requires enlargement. How much is the topic of the next section.

1.4 Magnification Reduces Spatial Frequencies While Making Bigger Images

As discussed earlier, images acquired at high resolving power are quite large on displays that have small resolving power, such as a 72-ppi web page. We have magnified the image! As long as decreasing the spatial frequency of the display does not result in pixelation, the process of magnification can reveal more detail to the eye. As soon as the image becomes pixelated, any further magnification is **empty magnification**. Instead of seeing more detail in the image, we just see bigger image pixels.

In film photography, the **enlargement latitude** is a measure of the amount of negative enlargement before empty magnification occurs and the image pixel, in this case the photographic grain, becomes obvious. Likewise, for chip cameras, it is the amount of enlargement before pixelation occurs. Enlargement latitude is

$$E = R / L, \tag{1.1}$$

in which E is enlargement magnification, R is the resolving power (spatial frequency of pixels) of the original, and L is the acceptable legibility.

For digital cameras, it is how much digital zoom is acceptable (Figure 1.6). A sixfold magnification reducing the resolving power from 600 to 100 ppi produces interesting detail: the moose calves become visible, and markings on the female become clear. However, further magnification produces pixelation and empty magnification. Digital zoom magnification is common in cameras. It is very important to realize that digital zoom reduces the resolving power of the image. For scientific applications, it is best to use only optical zoom in the field and then perform digital zoom when analyzing or presenting the image.

The amount of final magnification makes a large difference in the displayed image content. The image should be magnified to the extent that the subject or **region of interest (ROI)** fills the frame but without pixelation. The ROI is the image area of the most importance, whether for display, analysis, or processing. Sometimes showing the environmental context of a feature is important. Figure 1.7 is a picture of a female brown bear being "herded" by or followed by a male in the spring (depending