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.

Imaging Life: Image Acquisition and Analysis in Biology and Medicine, First Edition. Lawrence R. Griffing.

Companion Website: www.wiley.com/go/griffing/imaginglife

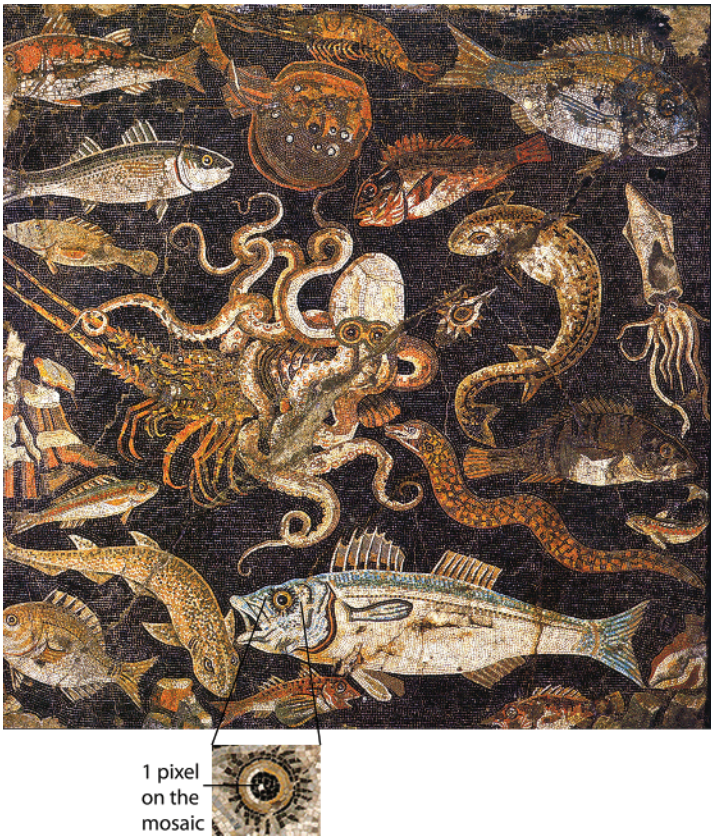


Figure 1.1 The fishes mosaic (second century BCE) from House VII.2.16, the House of Five Floors, in Pompeii. The lower image is an enlargement of the fish eye, showing that light reflection off the eye is a single tile, or pixel, in the image. Photo by Wolfgang Rieger, http://commons.wikimedia.org/wiki/File:Pompeii_-_Casa_del_Fauno_-_MAN.jpg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

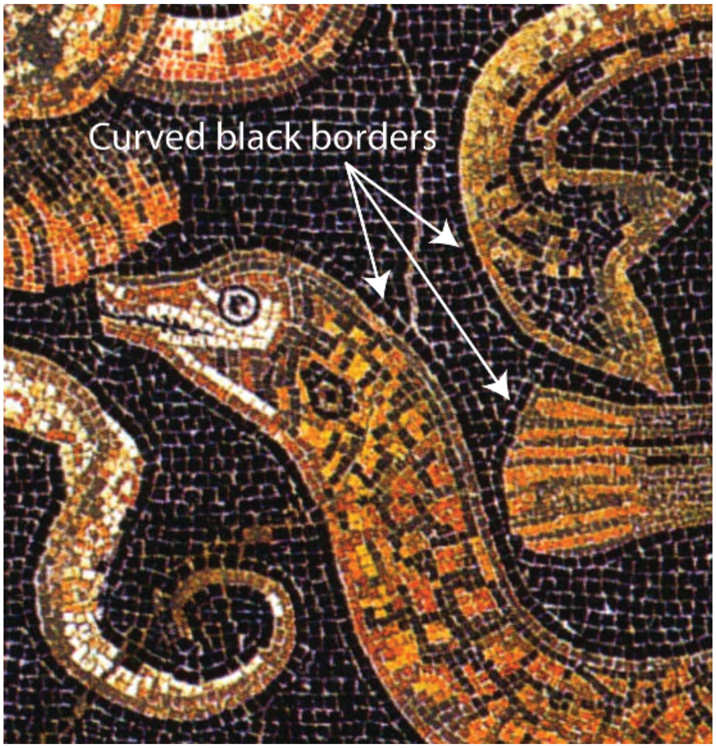


Figure 1.2 Detail from Figure 2.1. The line of black tiles around the curved borders of the eel and the fish are evidence that the mosaic employs object-order rendering.

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.

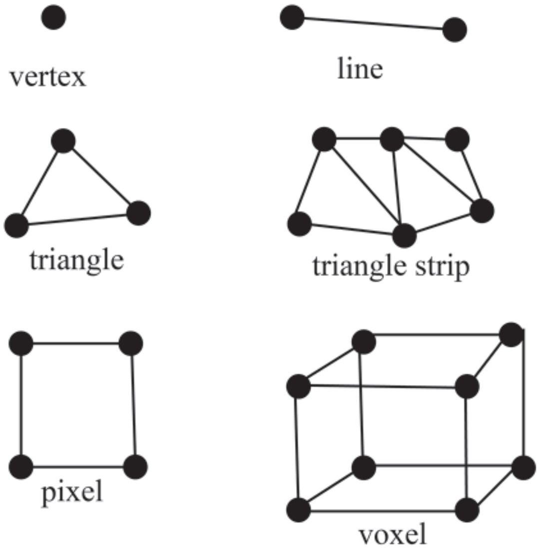
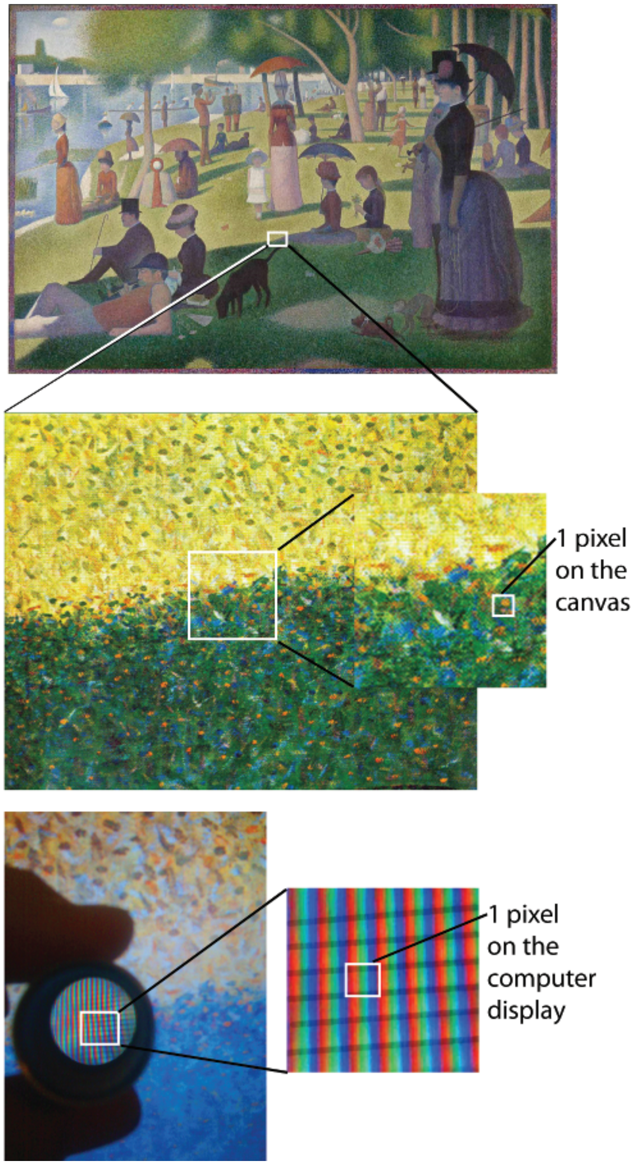


Figure 1.3 Cell types found in visualization systems that can handle two- and three-dimensional representation. Diagram by L. Griffing.

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

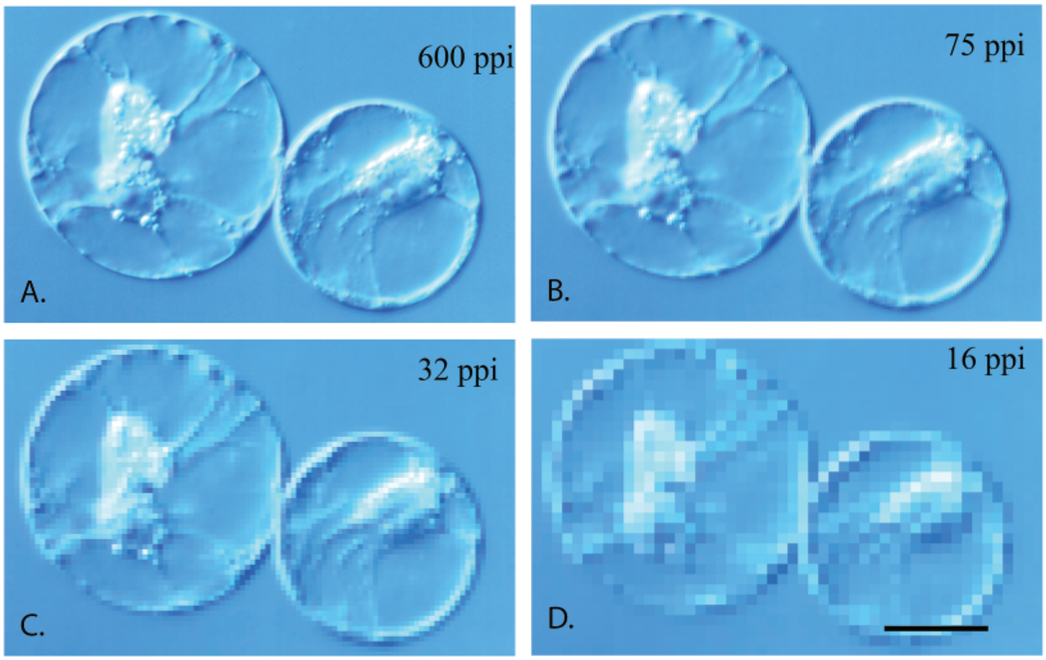


Figure 1.5 Soybean protoplasts (cells with their cell walls digested away with enzymes) imaged with differential interference contrast microscopy and displayed at different resolving powers. The scale bar is 10 μm long. The mosaic pixelation filter in Photoshop generated these images. This filter divides 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 (×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

Figure 1.6 (A) A photograph of a moose at 600 ppi. (B) When A is enlarged sixfold by keeping the same information and dropping the resolving power to 100 ppi, two calves become clear (and a spotted rump on the female). (C) Further magnification of 1.6× produces pixelation and blur. (D) Even further magnification of 2× produces empty magnification. Photo by L. Griffing.

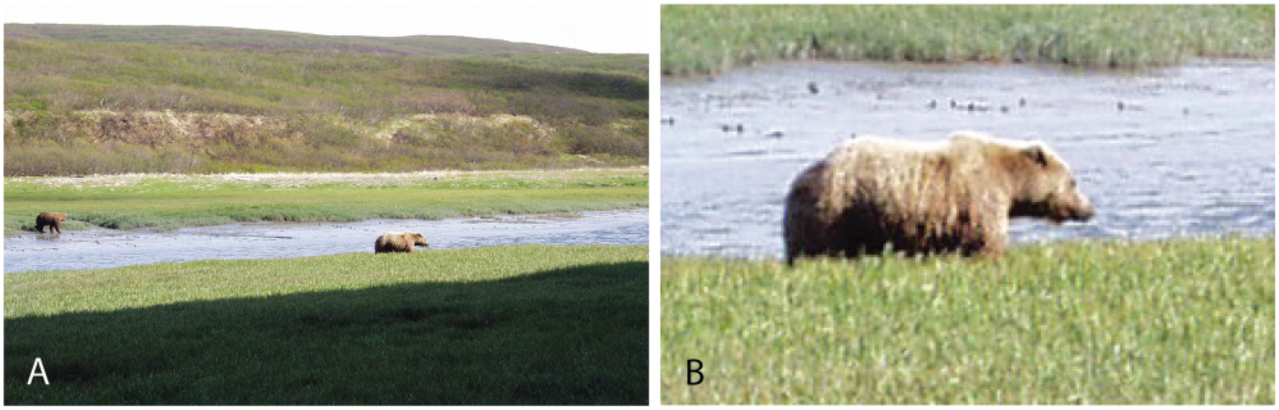


Figure 1.7 (A) A 600-ppi view of two grizzlies in Alaska shows the terrain and the distance between the two grizzlies. Hence, even though the grizzlies themselves are not clear, the information about the distance between them is clear. (B) A cropped 100-ppi enlargement of A that shows a clearly identifiable grizzly, which fills the frame. Although the enlargement latitude is acceptable, resizing for journal publication to 600 ppi would use pixel interpolation. Photo by L. Griffing.

on who is being selective for their mate, the male or the female). The foliage in the alders on the hillside shows that it is spring. Therefore, showing both the bears and the time of year requires most of the field of view in Figure 1.7A as the ROI. On the other hand, getting a more detailed view of the behavior of the female responding to the presence of the male requires the magnified image in Figure 1.7B. Here, the position of the jaw (closed) and ears (back) are clear, but they were not in the original image. This digital zoom is at the limit of pixelation. If a journal were to want a 600 ppi image of the female, it would be necessary to resize the 100 ppi image by increasing the spatial frequency to 600 ppi using interpolation (see Section 1.7).

1.5 Technology Determines Scale and Resolution

To record objects within vast or small spaces, changing over long or very short times, requires technology that aids the eye (Figure 1.8). Limits of resolution set the boundaries of scale intrinsic to the eye (see Section 4.1) or any sensing device. The **spatial resolution limit** is the shortest distance between two discrete points or lines. To extend the spatial resolution of the eye, these devices provide images that resolve distances less than 0.1 mm apart at near point (25 cm) or angles of separation less than 1 arc-minute (objects farther away have smaller angles of separation). The **temporal resolution limit** is the shortest time between two separate events. To extend the temporal resolution of the eye, devices detect changes that are faster than about one 20th of a second.

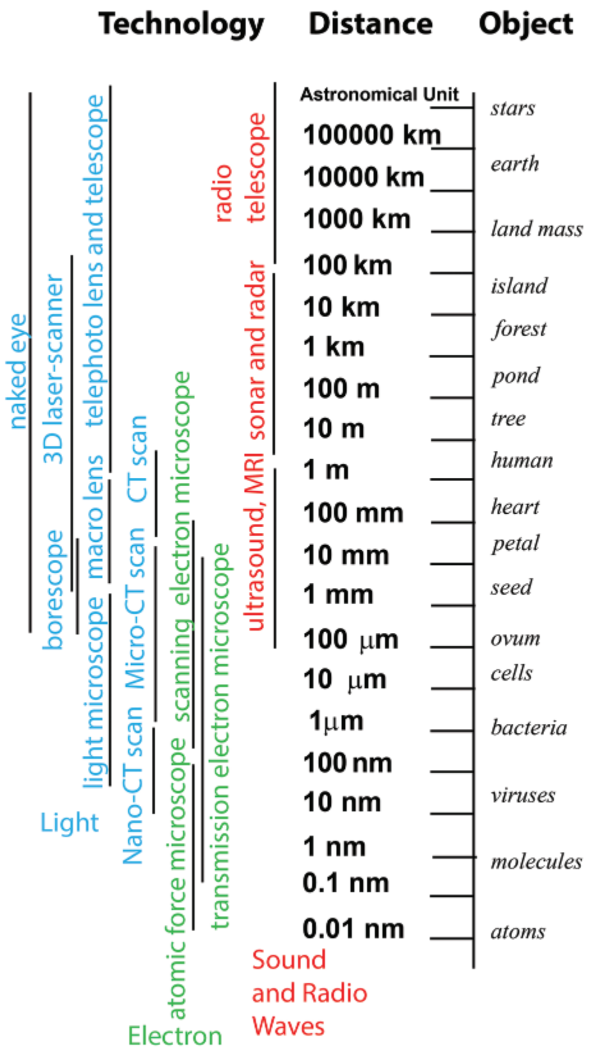


Figure 1.8 Useful range for imaging technologies. 3D, three dimensional; CT, computed tomography. Diagram by L. Griffing.

The devices that extend our spatial resolution limit include a variety of lens and scanning systems based on light, electrons, or sound and magnetic pulses (see Figure 1.8), described elsewhere in this book. In all of these technologies, to resolve an object, the acquisition system must have a resolving power that is double the spatial frequency of the smallest objects to be resolved. The technologies provide magnification that lowers the spatial frequency of these objects to half (or less) that of the spatial frequency of the recording medium. Likewise, to record temporally resolved signals, the recording medium has to run a timed frequency that is twice (or more) the speed of the fastest recordable event. Both of these rules are a consequence of the Nyquist criterion.

1.6 The Nyquist Criterion: Capture at Twice the Spatial Frequency of the Smallest Object Imaged

In taking an image of living cells (see Figure 1.5), there are several components of the imaging chain: the microscope lenses and image modifiers (the polarizers, analyzers, and prisms for differential interference contrast), the lens that projects the image onto the camera (the projection lens), the camera chip, and the print from the camera. Each one of these links in the image chain has a certain resolving power. The lenses are particularly interesting because they magnify (i.e., reduce the spatial frequency). They detect a high spatial frequency and produce a lower one over a larger area. Our eyes can then see these small features.

We use still more powerful cameras to detect these lowered spatial frequencies. The diameter of small organelles, such as mitochondria, is about half of a micrometer, not far from the **diffraction limit of resolution** with light microscopy (see Sections 5.14, 8.4, and 18.3), about a fifth of a micrometer. To resolve mitochondria with a camera that has a resolving power of 4618 ppi (5.5-µm pixels, Orca Lightning; see Section 5.3, Table 5.1), the spatial frequency of the mitochondrial diameter

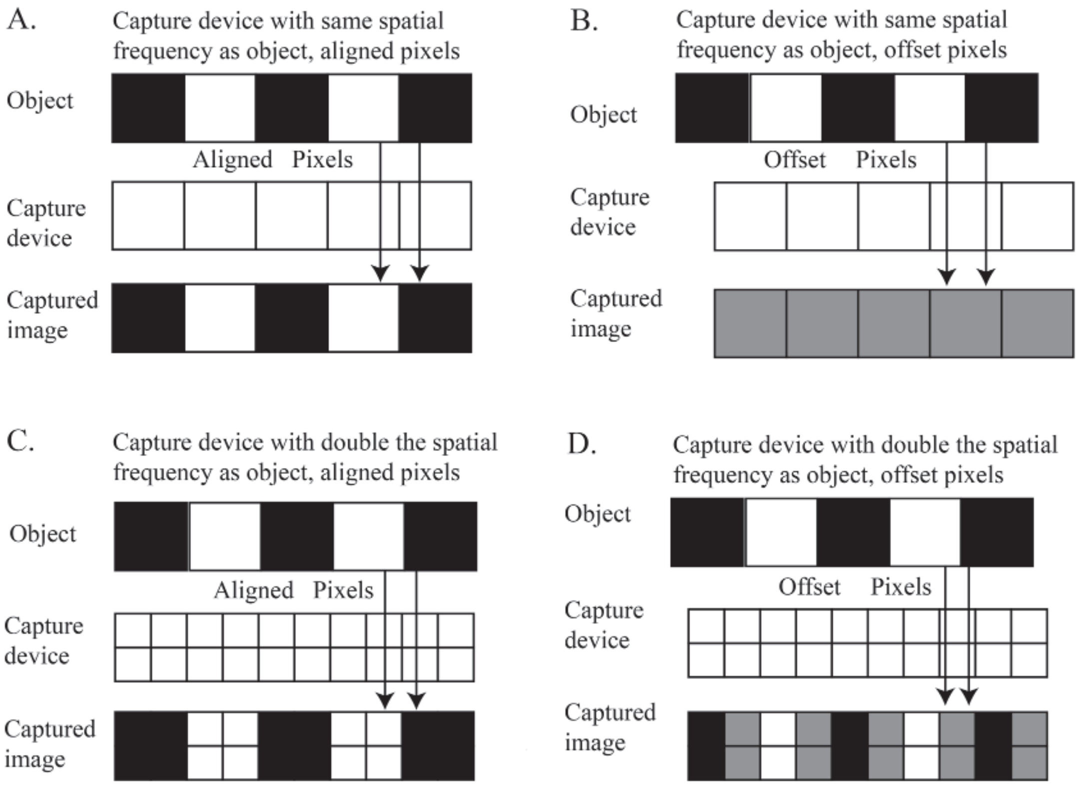


Figure 1.9 (A) and (B) Capture when the resolving power of the capture device is equal to the spatial frequency of the object pixels. (A) When pixels of the camera and the object align, the object is resolved. (B) When the pixels are offset, the object "disappears." (C) and (D) Doubling the resolving power of the capture device resolves the stripe pattern of the object even when the pixels are offset. (C) Aligned pixels completely reproduce the object. (D) Offset pixels still reproduce the alternating pattern, with peaks (white) at the same spatial frequency as the object. Diagram by L. Griffing.

(127,000 ppi for a 0.5-µm object) has to be reduced by at least a factor of 40 (3175 ppi) to capture the object pixel by pixel. Ah ha! To do this, we use a 40× magnification objective. However, we have to go up even further in magnification because the resolving power of the capture device needs to be double the new magnified image spatial frequency.

To see why the **resolving power** of the capture device has to be double the spatial frequency of the object or image it captures, examine Figure 1.9. In panel A, the object is a series of alternating dark and bright pixels. If the capture device has the same resolving power as the spatial frequency of the object *and* its pixels align with the object pixels, the alternating pixels are visible in the captured image. However, if the pixels of the camera are not aligned with the pixels of the object, then the white and black pixels combine to make gray in each capture device pixel (bright + dark = gray), and the object pattern disappears! If we double the resolving power of the capture device, as in panel B, the alternating pixel pattern is very sharp when the pixels align, as in panel A. However, even if the capture device pixels do not align with object pixels, an alternating pattern of light and dark pixels is still visible; it is still not perfect, with gray between the dark and bright pixels.

To resolve the alternating pattern of the object pixels, the camera has to sample the object at twice its spatial frequency. This level of sampling uses the **Nyquist criterion** and comes from statistical sampling theory. If the camera has double the spatial frequency of the smallest object in the field, the camera faithfully captures the image details. In terms of **resolution**, the inverse of resolving power, the pixel size in the capturing device should be half the size of the pixel size, or the smallest resolvable feature, of the object. The camera needs finer resolution than the projected image of the object.

Figure 1.10 An image of a coat captured with color camera showing regions of moiré patterns. Image from Paul Roth. Used with permission.

Getting back to the magnification needed to resolve a 0.5-µm mitochondrion with a 4618-ppi camera, a 40× lens produces an image with mitochondria at a spatial frequency of about 3175 ppi. To reduce the spatial frequency in the image even more, a projection lens magnifies the image 2.5 times, projecting an enlarged image onto the camera chip. This is the standard magnification of projection lenses, which are a basic part of compound photomicroscopes. The projection lens produces an image of mitochondria at a spatial frequency of 1270 ppi, well below the sampling frequency of 2309 ppi needed to exceed the Nyquist criterion for the 4618-ppi camera.

Sampling at the Nyquist criterion reduces aliasing, in which the image pixel value changes depending on the alignment of the camera with the object. Aliasing produces **moiré patterns**. In Figure 1.10, the woven pattern in the sports coat generates wavy moiré patterns when the spatial frequency of the woven pattern matches or exceeds the spatial frequency of the camera and its lens. A higher magnification lens can eliminate moiré patterns by reducing the spatial frequency of the weave. In addition, reducing the aperture of the lens will eliminate the pattern by only collecting lower spatial frequencies (see Sections 5.14 and 8.3).

The presence of moiré patterns in microscopes reveals that there are higher spatial frequencies to capture. Capturing higher frequencies than the diffraction limit (moiré patterns) by illuminating the sample with structured light (see Section 18.7) produces a form of superresolution microscopy, structured illumination microscopy.

1.7 Archival Time, Storage Limits, and the Resolution of the Display Medium Influence Capture and Scan Resolving Power

Flatbed scanners archive photographs, slides, gels, and radiograms (see Sections 6.1 and 6.2). Copying with scanners should use the Nyquist criterion. For example, most consumer-grade electronic scanners for printed material now come with a 1200 × 1200 dpi resolving power because half this spatial frequency, 600 ppi, is optimal for printed color photographs (see Table 1.4). For slide scanners, the highest resolving power should be 1500 to 3000 dpi, 1500 dpi for black and white and 3000 dpi for color slides (see Table 1.4).

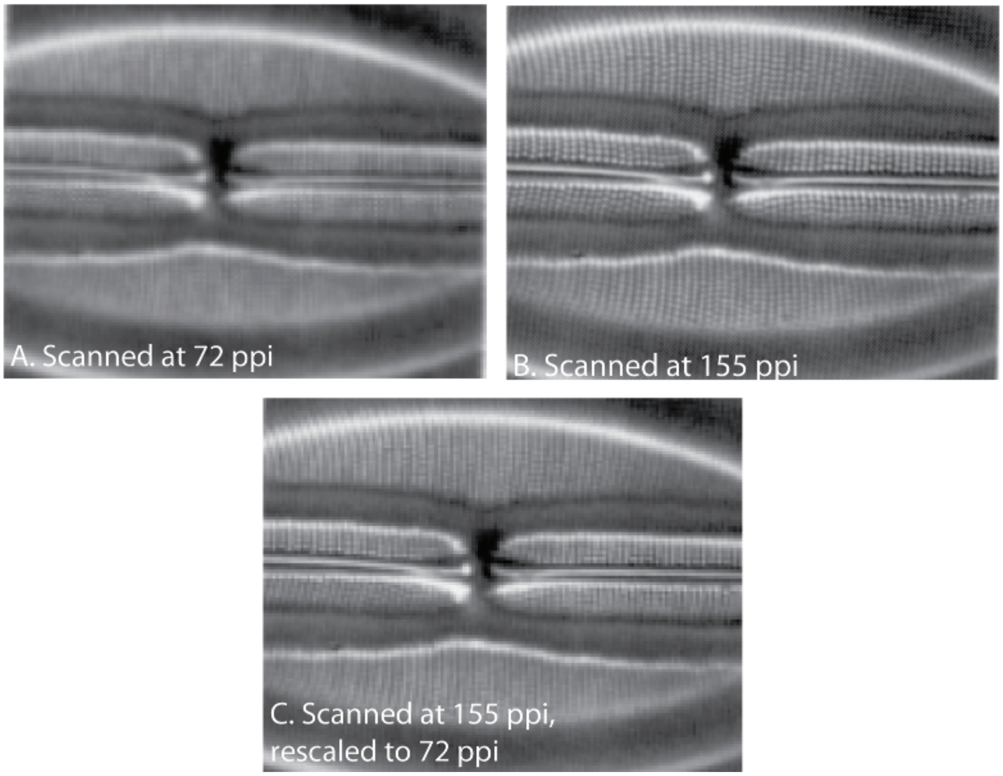


Figure 1.11 (A) Image of the central region of a diatom scanned at 72ppi. The vertical stripes, the striae, on the shell of the diatom are prominent, but the bumps, or spicules, within the striae are not. (B) Scanning the images at 155 ppi reveals the spicules. However, this may be too large for web presentation. (C) Resizing the image using interpolation (bicubic) to 72 ppi maintains the view of the spicules and is therefore better than the original scan at 72 ppi. This is a scan of an image in Inoue, S. and Spring, K. 1997. *Video Microscopy.* Second Edition. Plenum Press New York, NY. p. 528.

When setting scan resolving power in dots per inch, consider the final display medium of the copy. For web display, the final ppi of the image is 72. However, the scan should meet or exceed the Nyquist criterion of 144 ppi. In the example shown in Figure 1.11, there is a clear advantage to using a higher resolving power, 155 ppi, in the original scan even when software rescales the image to 72 ppi.

If the output resolution can only accommodate a digital image of low resolving power, then saving the image as a low-resolving-power image will conserve computer disk space. However, if scanning time and storage limits allow, it is always best to save the original scan that used the Nyquist criterion. This fine-resolution image is then available for analysis and display on devices with higher resolving powers.

1.8 Digital Image Resizing or Scaling Match the Captured Image Resolution to the Output Resolution

If the final output resolution is a print, there are varieties of printing methods, each with its own resolving power. Laser prints with a resolving power of 300 dpi produce high-quality images of black and white text with excellent legibility, as would be expected from Table 1.1. However, in printers that report their dpi to include the dots inside half-tone cells (Figure 1.12), which are the pixels of the image, the dpi set for the scan needs to be much higher than the value listed in Table 1.4. Printers used by printing presses have the size of their half-tone screens pre-set. The resolution of these printers is in lines per inch or lines per millimeter, each line being a row of half-tone cells. For these printers, half-tone images of the highest quality come from a captured image resolving power (ppi) that is two times (i.e., the Nyquist criterion) the printer half-tone screen frequency. Typical screen frequencies are 65 lpi (grocery coupons), 85 lpi (newsprint), 133 lpi (magazines), and 177 lpi (art books).

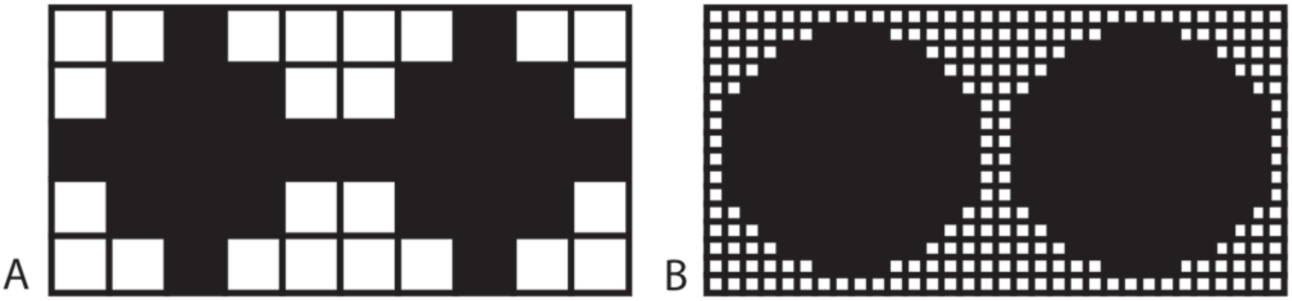


Figure 1.12 Half-tone cells for inkjet and laser printers. (A) Two half-tone cells composed of a 5 × 5 grid of dots. A 300-dpi printer with 5 × 5 half-tone cells would print at 300/5 or 60 cells per inch (60 ppi). This is lower resolution than all computer screens. These cells could represent 26 shades of gray. (B) Two half-tone cells composed of a 16 × 16 grid of dots. A 1440-dpi printer with 16 × 16 half-tone cells would print at 90 cells per inch. This is good legibility but not excellent. These cells (90 ppi) could represent 256 shades of gray. Diagram by L. Griffing.

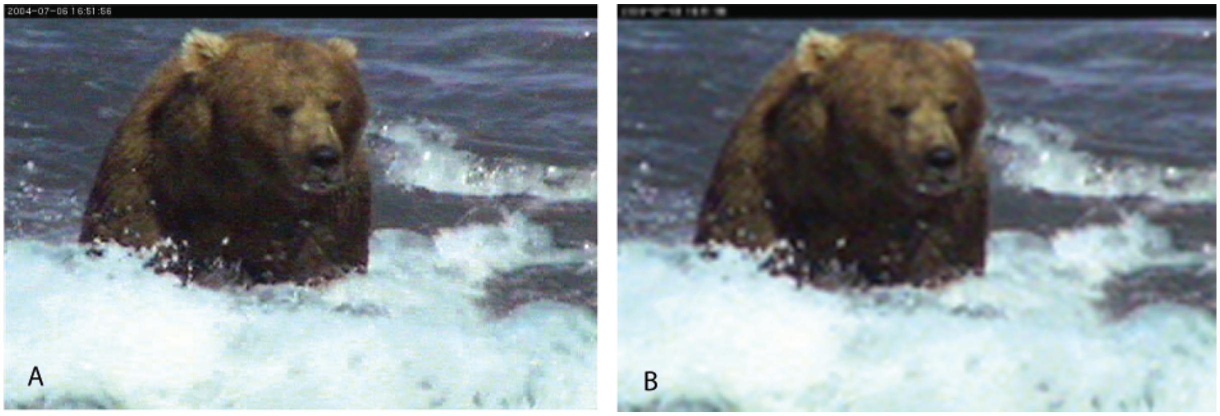


Figure 1.13 Information loss during resizing. (A) The original image (2.3 inches ×1.6 inches). (B) The result achieved after reducing A about fourfold (0.5 inches in width) and re-enlarging using interpolation during both shrinking and enlarging. Note the complete blurring of fine details and the text in the header. Photo by L. Griffing.

For image display at the same size in both a web browser and printed presentation, scan it at the resolution needed for printing and then rescale it for display on the web. In other words, always acquire images at the resolving power required for the display with the higher resolving power and rescale it for the lower resolving power display (see Figure 1.11).

Digital image resolving power diminishes when resizing or scaling produces fewer pixels in the image. Reducing the image to half size could just remove every other pixel. However, this does not result in a satisfactory image because the image leaves out a large part of the information in the scene that it could otherwise incorporate. A more satisfactory way is to group several pixels together and make a single new pixel from them. The value assigned to the new pixel comes from the values of the grouped pixels. However, even with this form of reduction, there is, of course, lost resolving power (compare Figure 1.11C with 1.11B and Figure 1.13B with 1.13A). Computational resizing and rescaling a fine resolution image (Figure 1.11C) is better than capturing the image at lower resolving power (Figure 1.11A).

Enlarging an image can either make the pixels bigger or interpolate new pixels between the old pixels. The accuracy of **interpolation** depends on the sample and the process used. Three approaches for interpolating new pixel values in order of increasing accuracy and processing time are the **near-neighbor process**, the **bilinear process**, and the **bicubic process** (see also Section 11.3 for 3D objects). Generating new pixels might result in a higher pixels per inch, but all of the information necessary to generate the scene resides in the original smaller image. True resolving power is not improved; in fact, some information might be lost. Even simply reducing the image is problematic because shrinking the image by the process described earlier using groups of pixels changes information content of the image.

1.9 Metadata Describes Image Content, Structure, and Conditions of Acquisition

Recording the settings for acquiring an image in scientific work (pixels per inch of acquisition device, lenses, exposure, date and time of acquisition, and so on) is very important. Sometimes this **metadata** is in the image file itself (Figures 1.13 and 1.14). In the picture of the bear (Figure 1.13), the metadata is a header stating the time and date of image acquisition. In the picture of the plant meristem (Figure 1.14), the metadata is a footer stating the voltage of the scanning electron microscope, the magnification, a scale bar, and a unique numbered identifier. Including the metadata as part of the image has advantages. A major advantage is that an internal scale bar provides accurate calibration of the image upon reduction or rescaling. A major disadvantage is that resizing the image can make the metadata unreadable as the resolving power of the image decreases (Figure 1.13B, header). Because digital imaging can rescale the x and y dimensions differently (without a specific command such as holding down the shift key), a 2D internal scale bar would be best, but this is rare.

For digital camera and recording systems, the image file stores the metadata separately from the image pixel information. The standard metadata format is **EXIF (Exchangeable Image File) format**. Table 1.5 provides an example of some of the recorded metadata from a consumer-grade digital camera. However, not all imaging software recognizes and uses the same codes for metadata. Therefore, the software that comes with the camera can read all of the metadata codes from that camera, but other more general image processing software may not. This makes metadata somewhat volatile because just opening and saving images in a new software package can remove it.

Several images may share metadata. Image scaling (changing the pixels per inch) is a common operation in image processing, making it very important that there be internal measurement calibration on digital scientific images. **Fiducial markers** are calibration standards of known size contained within the image, such as a ruler or coin (for macro work), a stage micrometer (for microscopy), or gold beads (fine resolution electron microscopy). However, their inclusion as an internal standard is not always possible. A separate picture of such calibration standards taken under identical conditions as the picture of the object produces a **fiducial image**, and metadata can refer to the fiducial image for scaling information of the object of interest.

Image databases use metadata. A uniform EXIF format facilitates integration of this information into databases. There are emerging standards for the integration of metadata into databases, but for now, many different standards exist. For example, medical imaging metadata standards are different from the standards used for basic cell biology research. Hence, the databases for these professions are different. However, in both these professions, it is important to record the conditions of image acquisition in automatically generated EXIF files or in lab, field, and clinical notes.

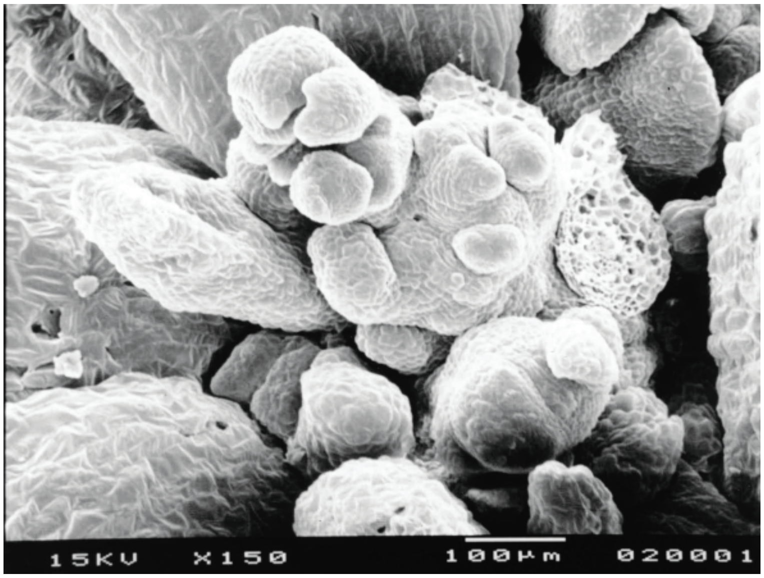


Figure 1.14 Scanning electron micrograph with an internal scale bar and other metadata. This is an image of a telomerase-minus mutant of *Arabidopsis thaliana*. The accelerating voltage (15 kV), the magnification (×150), a scale bar (100 µm), and a negative number are included as an information strip below the captured image. Photo by L. Griffing.

Table 1.5 Partial Exchangeable Image File Information for an Image from a Canon Rebel.

Title	**IMG_6505**
Image description	Icelandic buttercups
Make	Canon
Model	Canon EOS DIGITAL REBEL XT
Orientation	Left side, bottom
X resolution	72 dpi
Y resolution	72 dpi
Resolution unit	Inch
Date/time	2008:06:15 01:42:00
YCbCr positioning	Datum point
Exposure time	1/500 sec
F-number	F5.6
Exposure program	Program action (high-speed program)
ISO speed ratings	400
Exif version	2.21
Date/time original	2008:06:15 01:42:00
Date/time digitized	2008:06:15 01:42:00
Components configuration	YCbCr
Shutter speed value	1/256 sec
Aperture value	F5.6
Exposure Bias value	0
Metering mode	Multi-segment
Flash	Unknown (16)
Focal length	44.0 mm
User comment	
FlashPix version	1
Color space	sRGB
EXIF image width	3456 pixels
EXIF image height	2304 pixels
Focal plane X resolution	437/1728000 inches
Focal plane Y resolution	291/1152000 inches
Focal plane resolution unit	Inches
Compression	JPEG compression
Thumbnail offset	9716 bytes
Thumbnail length	12,493 bytes
Thumbnail data	12,493 bytes of thumbnail data
Macro mode	Normal
Self-timer delay	Self-timer not used
Unknown tag (0xc103)	3
Flash mode	Auto and red-eye reduction
Continuous drive mode	Continuous

(Continued)

Table 1.5 (Continued)

Title	IMG_6505
Focus mode	AI Servo
Image size	Large
Easy shooting mode	Sports
Contrast	High
Saturation	High
Sharpness	High

Annotated Images, Video, Web Sites, and References

1.1 The Pixel Is the Smallest Discrete Unit of a Picture

The mosaic in Figures 1.1 and 1.2 resides in the Museo Archeologico Nazionale (Naples).

For image-order and object-order rendering, see Schroder, W., Martin, K., and Lorensen, B. 2002. *The Visualization Toolkit*. Third Edition. Kitware Inc. p. 35–36.

For a complete list of the different cell types, see Schroder, W., Martin, K., and Lorensen, B. 2002. *The Visualization Toolkit*. Third Edition. Kitware Inc. p. 115.

The original painting in Figure 1.4 resides at the Art Institute of Chicago.

More discussion of subpixel color is in Russ, J. 2007. *The Image Processing Handbook*. CRC Taylor and Francis, Boca Raton, FL. p. 136.

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

The reciprocal relationship between resolving power and resolution is key to understanding the measurement of the fidelity of optical systems. The concept of spatial frequency, also called reciprocal space or k space, is necessary for the future treatments in this book of Fourier optics, found in Chapters 8 and 14–19.

For more on video display standards, see https://en.wikipedia.org/wiki/List_of_common_resolutions.

Appropriate viewing distance is in Anshel, J. 2005. *Visual Ergonomics Handbook*. CRC Press, Taylor and Francis Group, Boca Raton, FL.

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

Williams, J. B. 1990. *Image Clarity: High Resolution Photography*. Focal Press, Boston, MA. p 56, further develops the information in Table 1.3.

Publication guidelines in journals are the basis for the stated resolving power for different media. For camera resolving powers, see Section 5.3 and Table 5.1.

1.4 Magnification Reduces Spatial Frequencies While Making Bigger Images

More discussion of the concept of enlargement latitude is in Williams, J.B. 1990. *Image Clarity: High Resolution Photography*. Focal Press, Boston, MA. p 57.

1.5 Technology Determines Scale and Resolution

Chapters 8 and 14–19 discuss the resolution criteria for each imaging modality.

1.6 The Nyquist Criterion: Capture at Twice the Spatial Frequency of the Smallest Object Imaged

The Nyquist criterion is from Shannon, C. 1949. Communication in the presence of noise. *Proceedings of the Institute of Radio Engineers* 37:10–21. and Nyquist, H. 1928. Certain topics in telegraph transmission theory. *Transactions of the American Institute of Electrical Engineers* 47:617–644.

1.7 Archival Time, Storage Limits, and the Resolution of the Display Medium Influence Capture and Scan Resolving Power

Figure 1.10 is a scan of diatom images in Inoue, S. and Spring, K. 1997. *Video Microscopy*. Second Edition. Plenum Press, New York, NY. p. 528.

1.8 Digital Image Resizing or Scaling Match the Captured Image Resolution to the Output Resolution

See the half-tone cell discussion in Russ, J. 2007. *The Image Processing Handbook*. CRC Taylor and Francis, Boca Raton, FL. p. 137

Printer technology is now at the level where standard desk jet printers are satisfactory for most printing needs.

1.9 Metadata Describes Image Content, Structure, and Conditions of Acquisition

Figure 1.12 is from the study reported in Riha, K., McKnight, T., Griffing, L., and Shippen, D. 2001. Living with genome instability: Plant responses to telomere dysfunction. *Science* 291: 1797–1800.

For a discussion of metadata and databases, see Chapter 7 on measurement.