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Illustrating biodiversity: The power of an image

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23.1 INTRODUCTION: THE POWER OF AN IMAGE

Our curious species has been trying to get a closer look at biominerals ever since the days when our earliest ancestors impulsively collected seashells from the beach. If an encounter with these intriguing natural objects is to be shared with others, we can do so with a picture, and we imbue ideas with special importance by preserving images—in antiquity through cave paintings and in modern times through social media.

For a scientist in the twenty-first century, for a picture to be worth its thousand *journal article words*, it will require quantification. The very fact that technology has allowed us to literally see further and deeper into the world around us is awe inspiring in itself. Reading accounts of the first glimpses of Saturn's rings or of microbes swimming in a water droplet, one realizes how astonishing it was for the inventors of the first telescopes and microscopes to see these things. With modern advances in imaging techniques, however, we often forget how difficult it once was to share these unique observations with an audience.

Photographic prints looked strange at first to people accustomed to viewing drawings that followed the perspective of the artists' eyes rather than a strict plane of projected rays. Perhaps, this is part of the charm of hand-drawn illustrations by naturalists of decades and centuries gone by. Views through a microscope had their own limitations that made drawings necessary. Obviously, it was not possible at first to photograph or print the view directly from an imaging lens. Adding to this problem was the simple fact that many research specimens of interest, being white or transparent, are notably difficult to image optically. We take it for granted now that a beautiful light micrograph can be made of pretty much anything, but decades of inventive optics and sample preparation methods have been called on to increase the contrast to useful levels in many systems.

Until relatively recently, scientists used to draw. Specifically, before those advances in optics were made, drawing was a necessary step to fill in detail from light microscopy (or for larger specimens, macroscale observations), which would not have been rendered by photographic reproduction. The artist—and one could say that in an age of hand-drawn scientific illustrations, every scientist was an artist—can do two special things to manage what their audience will see. First, they can *control* the detail shown, and second, they can *iconify* an image toward a generalization.

This chapter will first show how the field of biomineralization has been illuminated by hand tracings from the camera lucida, which in conjunction with optical microscopes of the time, enabled scientists to portray the scientifically pertinent levels of structural detail and make comparisons, for very small organisms with low optical imaging contrast. In addition, we will introduce the concept of the wide-field scanning electron microscopy (SEM), a modern method that can also simultaneously provide unexpected and valuable levels of detail and contrast from submicron through macroscopic length scales, with contrast unrelated to an object's optical properties.

Finally, we will address the path from image to generalization. The artist/scientist can iconify the representation of an organism, and this is a powerful step in the generalization that occurs when we apply a scientific theory to explain an observation. We know the difference between showing off the prettiest seashell in a collection, measuring that it is 5 cm across, and saying something useful about size distribution in the context of how it grows or functions as a hierarchical material. D'Arcy Wentworth

Thompson devoted the first chapter of *On Growth and Form* to emphasizing the watershed between, for example, observing the nautilus' spiral shell, and applying the test of whether that spiral follows a logarithmic geometry, and what that might mean. Thompson says, "The introduction of mathematical concepts into natural science has seemed to many men no mere stumbling-block, but a very parting of the ways." (Thompson 1917). Quantitative observations enabled by modern imaging techniques provide a tool for the reductionist approach to each data set, which in turn makes it possible to effectively discuss the diagnostic features or behaviors of a given species, not just the specimen in hand. In some instances, the generalization can itself be most powerfully conveyed by a drawing. Indeed, one finds that the iconic image of the spiral nautilus comes to represent collections of organisms or even an entire scientific field such as biomineralization. Such is the power of an image.

23.2 HISTORICAL ILLUSTRATIONS

23.2.1 CAMERA LUCIDA

First patented in 1807, the camera lucida (Figure 23.1) allowed early investigators to accurately image tiny objects at high levels of precision, through the sequential collection of a series of details at each focal plane, thus resulting in a highly accurate and focused image stack. In its simplest configuration, the setup typically consisted of a 45-degree tilted half-silvered mirror that allowed the observer to see both the image of an object through the microscope eyepiece as well as the paper on which the object was being traced. In more advanced versions, a prism was employed to correct for image reversal and reduced image intensity. Because of its simplicity in design and ease of use, the camera lucida has a long history in biological observation, spanning from the early nineteenth century well into the late twentieth century.

A modern, and significantly more advanced equivalent that can ultimately achieve a similar highly focused and low noise image stack either through photographic or digital image capture, is the confocal microscope. In confocal microscopy, point source illumination is employed, and only the light immediately scattered from the focal plane is collected and used for imaging. As with the camera lucida, separate images can be collected at each focal plane, which can then be combined to provide a realistic three-dimensional view of an object of interest. Figure 23.1 illustrates the similar types of images that can be obtained through the use of these two technologies.

23.2.2 PROBLEMATIC SPECIMENS FOR IMAGING STUDIES

In many instances, photography is simply not an option for collecting a highly detailed and representative view of a specimen or structure of interest. This issue is best illustrated using two specific examples, the polycystine radiolarians and the siphonophores. The polycystines are a group of marine protozoa that produce complex mineralized skeletal tests out of amorphous hydrated silica. Because their skeletons are often well-preserved in seafloor sediments, polycystines and other microfossils have been used extensively to infer historical information about the physical and chemical properties of seawater. Since each species is adapted to a specific set of environmental conditions, correct species identification is critical for performing accurate paleoclimatic reconstructions. Due to their small size and complex skeletal architectures, they are easily damaged, and as a result, "perfect" specimens are not always available for examination. In addition, the consistent mounting of specimens in identical orientations to permit a detailed direct comparison of closely related species can be extremely labor-intensive and oftentimes simply not possible. By examining multiple damaged specimens from a wide range of different orientations, it can be possible to construct highly accurate graphical depictions of a wide range of closely related species (Figure 23.2).

Another group of problematic species are those that are simply impossible to photograph at any reasonable level of detail, of which the siphonophores are an excellent example. The siphonophores are a large group of colonial gelatinous zooplankton, which exhibit extensive structural polymorphism. Exhibiting the most complex body plans of all cnidarians (the phylum that includes the jellyfish, corals, hydroids, and their allies), they can reach incredibly large sizes, with sweeping curtains of largely transparent tentacles extending for tens of meters, often in multiple directions. In addition to their large

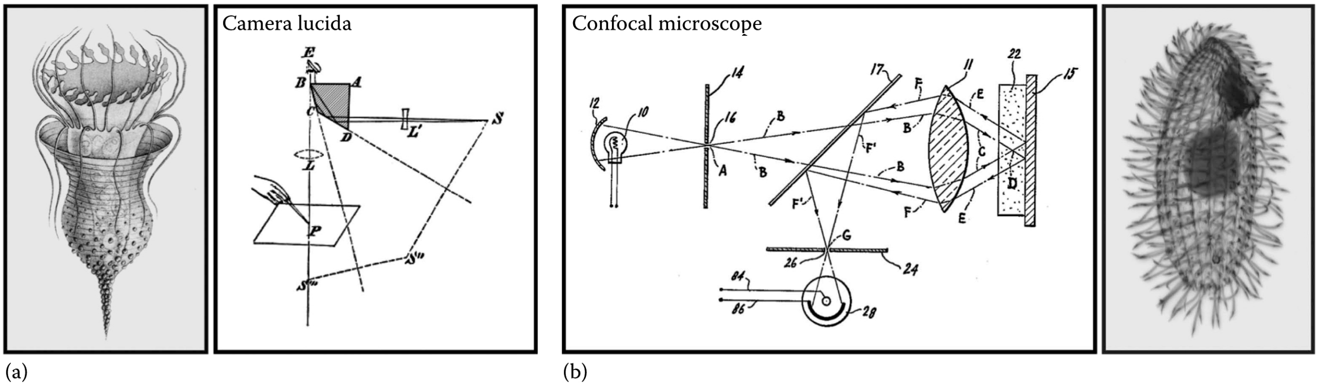
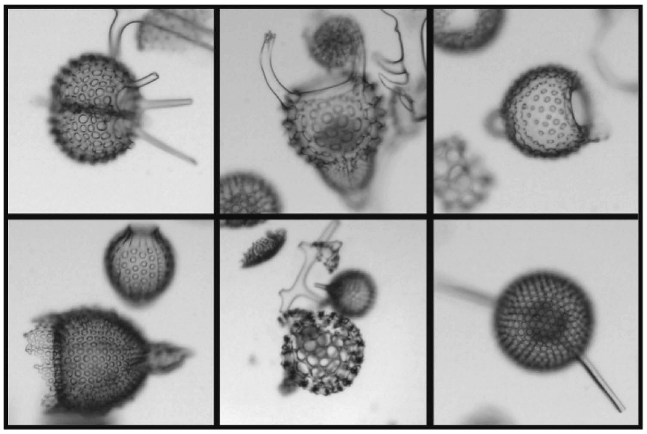


Figure 23.1 Schematic comparisons of the camera lucida (a, right: From Bartlett, W.H.C., *Elements of Natural Philosophy*, A. S. Barnes and Company, New York, 1852) and the confocal microscope (b, left: From Minsky, M., US Patent 3.013.467, 1957), showing the various optical elements and the corresponding ray tracings through the system and the types of representative images that can be generated through the use of each technique. While the design elements of both systems differ dramatically, both are capable of producing highly detailed three-dimensional representations of an object or structure of interest, in this case, a tintinnid ciliate (a, left: From Haeckel, E., *Kunstformen der Natur*, Bibliographischen Instituts, Liepzig, Germany, 1904.) and *Tetrahymena sp.* (b, right: From Robinson, R., *PLoS Biol.*, 4, e304, 2006). (Images from Wikimedia Commons.)


(a)

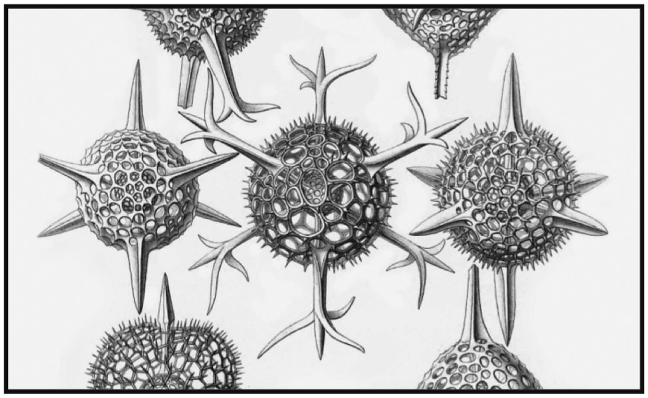

(b)

Figure 23.2 Radiolarian species diversity. Compared to photographs (a), illustrations (b) often provide a much clearer avenue for the detailed comparisons of closely related species as they permit the artist complete control over specimen orientation and the generation of a representative image from several damaged specimens. For an historical perspective, it should be noted that the photographs in (a) were published more than a 100 years after the illustrations in (b) were produced. (a: Acquired by Luis Fernández Garcia; b: From Haeckel, E., Report on the scientific results of the voyage of H.M.S. challenger during the years 1873–1876, Zoology, Volume XVIII, Report on the Radiolaria collected by H.M.S. Challenger, Eyre and Spottiswoode, London, U.K.; Adam & Charles Black, Edinburgh, Scotland; Hodges, Figgies, & Co., Dublin, Ireland, 1887.) Field diameter in (b): 0.5 mm.

size and structural complexity, because of their high water content, they are virtually impossible to collect intact using standard marine specimen sampling gear. Despite recent advances in the use of remotely operated vehicles for the photographic documentation of siphonophores in their natural habitat, they remain one of the most challenging groups to study from an anatomical perspective (Figure 23.3). As a result, even modern investigators rely heavily on the use of intricate drawings in their descriptions of these fascinating creatures.

23.3 WIDE-FIELD SCANNING ELECTRON MICROSCOPY

Until the mid-twentieth century, scientists were severely limited in the types of imaging techniques, which could be employed in their studies, and thus still heavily relied on illustrations to fill the technological void. While modern advances in optical, scanning probe, and electron microscopies have played a critical role in increasing the macro-, micro-, and nanoscale understanding of the natural world, the artistic challenges of accurately documenting a species or structure of interest in a clear and concise biologically relevant context remain. Scientists need to therefore learn to adapt these new techniques to describe their systems of study in new and creative ways. The merits of SEM, for example, are widely recognized: they permit the high-resolution imaging of submicron-scale features and are ideally suited for specimens that are highly reflective, transparent, or otherwise exhibit low surface contrast when imaged optically. Until recently, the *maximum* accessible feature sizes that one could typically image with a SEM were of order of a few millimeters, and it would have been inconceivable to imagine attempting to image larger objects, or even dream up a reason to try. Advances in SEM column design, exemplified by the unique four-lens electron-optical system, first introduced in the VEGA line of SEMs (Tescan, Czech Republic), however, have radically changed this imaging landscape (Weaver 2010).

The four-lens column design is illustrated in Figure 23.4a, which schematically locates the doublet condenser lens system (C1 and C2), an intermediate lens (IML) with its own electromagnetic centering system (distinct from that just below the gun and anode), and a low-aberration conical objective lens (OB) with integrated scanning and stigmator coils (SC). In the familiar resolution-imaging mode, the condenser lenses are controlled as a zoom condenser, the intermediate lens is off, and the objective lens projects the focused electron beam onto the specimen with a minimum spot size. The results are high spatial resolution, but at the expense of a limited field diameter, and a reduced depth of field (Figure 23.4b).

In the wide-field imaging mode, the electron beam is focused on the specimen using the intermediate lens. The objective lens is operated at maximum excitation, and the scan coils are adjusted so as to utilize the entire area of the final lens bore. In this configuration, a very high deflection angle and an extra large field of view are obtained for a given working distance (Figure 23.4c). Since the angular aperture is very small, the result is an exceptionally high depth of field, and the image is focused in all accessible positions of the specimen stage.

The significantly increased depth of field is an essential benefit of the wide-field imaging mode, because it preserves the capability of the SEM to image an enormous amount of detail across a macroscopic field—in many cases, with sufficient resolution so as to make additional images at higher magnification unnecessary. During imaging, the electron beam is focused at a single distance from the pole piece of the objective lens in a convergent geometry onto a spot on the specimen surface. Where the sample surface is some distance away from this focal plane, the spot enlarges into a circle of confusion. Eventually, this circle of confusion becomes large enough, such that information from adjacent pixels overlaps and the image blurs. The extent above and below the focal plane that is considered to be in acceptable focus defines the depth of field of the image.

Depth of field in a conventional SEM is typically controlled by changing the size of the final aperture and increasing the working distance to the sample surface. Both of these techniques, however, have limitations. Changing apertures requires mechanical realignment, both at the new aperture position and when returning to the original aperture. In addition, many SEMs

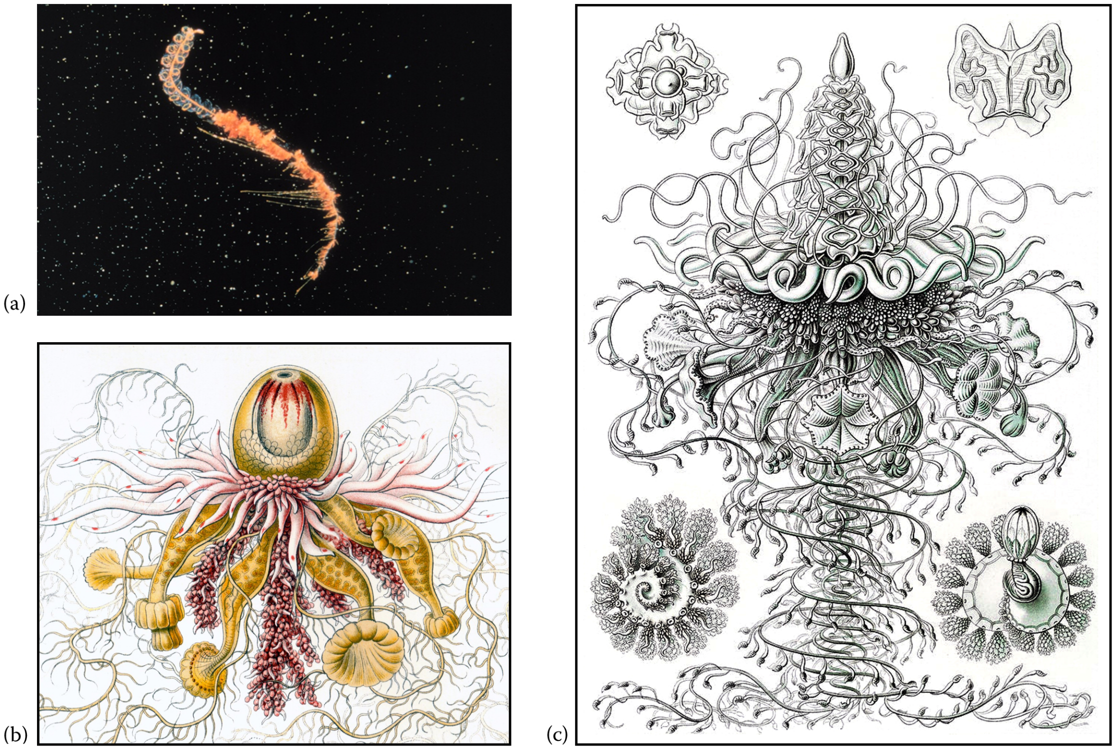


Figure 23.3 Anatomical documentation of siphonophores. The siphonophores rank among some of the most challenging organisms to photograph. A barely decipherable photo (a) is accompanied by two highly detailed illustrations (b and c) of related species. As with the illustrations shown in Figure 23.2, the much easier to interpret drawings in (b) and (c) were published more than a 100 years before the photograph in (a) was acquired. (a: Courtesy of NOAA, Silver Spring, MD; b and c: Adapted from Haeckel, E., *Kunstformen der Natur*, Bibliographischen Instituts, Liepzig, Germany, 1904.)

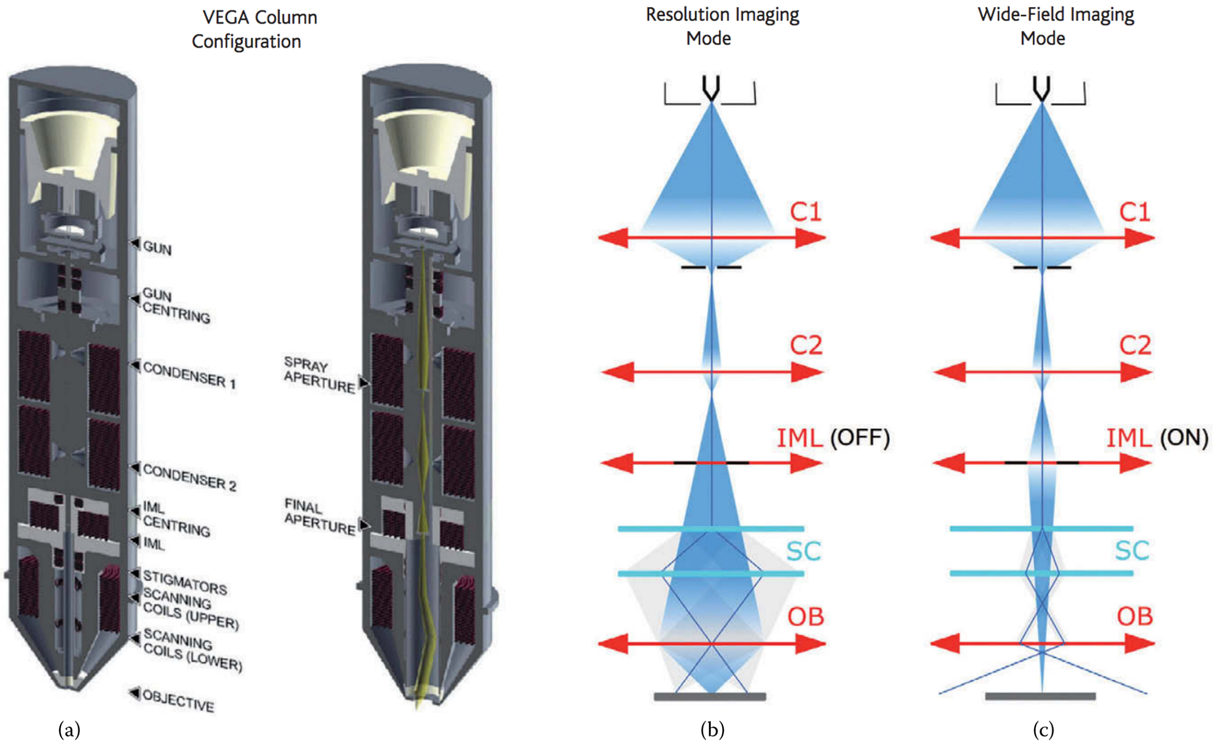


Figure 23.4 (a) Schematic of the four-lens column of the Tescan VEGA SEM, with the depiction of electron beam paths and divergences in (b) resolution and (c) wide-field imaging modes. C1, C2—condenser lenses; IML—intermediate lens; SC—scanning and stigmator coils; OB—objective lens. (Reprinted from *Mater. Today*, 13, Weaver, J.C., Mershon, W., Zadrazil, M., Kooser, M., and Kisailus, D., Wide-field SEM of semiconducting materials, 46–53, Copyright 2010 with permission from Elsevier.)

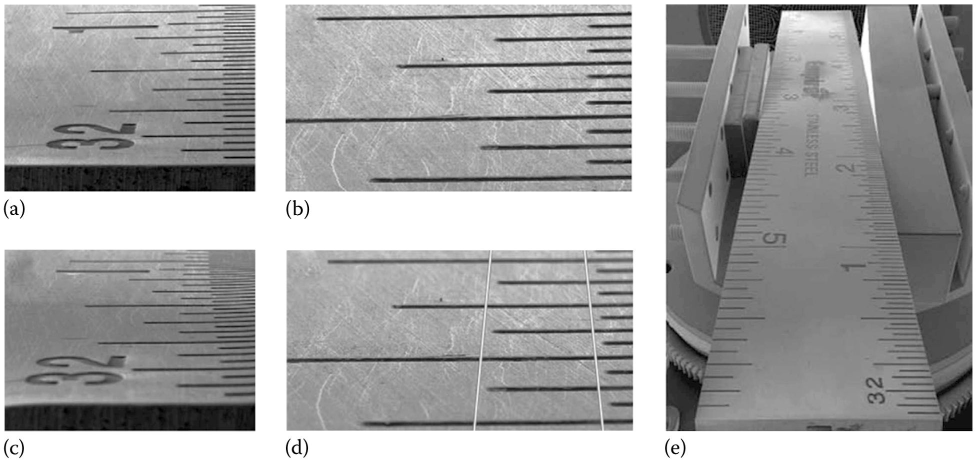


Figure 23.5 Comparison between wide-field (a and b) and resolution (c and d) modes in the VEGA SEM using a 6-in. scale tilted ca. 85° from the horizontal. White lines in (d) are guides for the eye and indicate the convergent image distortion in the resolution mode. (e) The same ruler at a 55° angle to the horizontal, imaged in its entirety. (Reprinted from *Mater. Today*, 13, Weaver, J.C., Mershon, W., Zadrazil, M., Kooser, M., and Kisailus, D., Wide-field SEM of semiconducting materials, 46–53, Copyright 2010 with permission from Elsevier.)

have limited z-axis movement of the stage and may not be able to achieve a long enough working distance to increase the depth of field to any useful degree when large samples are examined. This, coupled with the significant loss of secondary and backscattered electron signal collection efficiency with increasing working distance, can make the process of obtaining micrographs exhibiting high signal-to-noise ratios prohibitive.

Figure 23.5 uses SEM images of a 6-in. metal ruler with 16ths and 32nds of an inch subdivisions, to illustrate the dramatically enhanced depth of field of the wide-field SEM. In panels (a) through (d), the ruler is inclined 85° from the horizontal. Figure 23.5a and b show the wide-field image, while Figure 23.5c and d show the same in resolution mode. In both modes, the sample is imaged at 10 keV with a 26 mm working distance, focused at the 16/32 rule marking, with 2048 × 1536 pixel images of a 5.8 × 4.35 field of view. Pixel sizes are comparable, 2.88 and 2.83 μm, for the resolution and wide-field mode images, respectively. The perceived depth of field in images (a) and (c) may be assessed by observing that the 17/32 rule line is not clearly resolved in the resolution mode image, while the entire wide-field mode image is acceptably in focus.

The calculated depth of field, defined from the spreading of the beam spot into a circle of confusion twice the pixel size, is naturally dependent on the electron beam convergence angles. Panels (b) and (d) in Figure 23.5 are cropped from (a) and (c), respectively. The greater convergence of lines in the resolution mode case (2.55 vs. 0.15 in. wide-field mode) is clearly shown (white lines are guides for the eye in Figure 23.5d). The electron beam convergence angles under these conditions are 2.55 mrad for the resolution mode and 0.15 mrad for the wide-field mode. Not only is the depth of field much larger for the wide-field mode image, 18.5 mm versus 1.1 mm for the resolution mode, but the perspective distortion is greatly minimized. The resolution mode image shows the 1/32 in. rule marks converging to the center of the image as the surface of the sample recedes from the pole piece. The wide-field mode image shows almost none of this effect. For large samples exhibiting extremes in surface topography, this angular distortion in traditional SEM imaging can make the tiling of multiple images to form a large mosaic problematic and oftentimes logistically impossible, highlighting the need for wide-field SEM imaging techniques. Figure 23.5e shows the same ruler imaged in its entirety with the wide-field mode. The ruler is tilted approximately 55° from the horizontal. The calculated depth-of-field for this image is 104 mm, and the entire ruler is in focus.

This capability implies changes for sample preparation. A typical SEM techniques chapter may require discussion of fixing tiny specimens to stubs, or for some experiments, sectioning and polishing techniques that reduce variations in the surface topography, which would prevent it from remaining in focus. With wide-field SEM, the considerations may be more along the lines of how to clean an entire animal skull with dermestid beetles, and how to select a specimen just small enough to fit through the microscope load lock.

23.4 WIDE-FIELD SEM CASE STUDIES IN BIOMINERALIZATION

23.4.1 HIERARCHICAL BIOMINERAL STRUCTURES IN INVERTEBRATES

Many biominerals exhibit a distinct architectural hierarchy with various levels of structural complexity, spanning the size range from tens of nanometers to tens of millimeters. In order to convey how these different features relate to one another, clear images at progressively higher and higher magnifications are required. To avoid ambiguities, the magnification difference from each subsequent image should be small enough as to always include an internal point of reference—the same feature should be clearly visible in two subsequent images. Three illustrations of this point are shown in Figure 23.6: a sea urchin test, the glassy skeletal system of a marine sponge, and a colonial coral skeleton. The upper three images (a, b, and c) are all wide-field SEMs, and their magnifications were chosen such that the individual building blocks of the skeletal systems are clearly visible (the ossicle plates of the urchin, the six-rayed hexactine spicules in the marine sponge, and an individual corallite in the coral skeleton). Following this general theme, a third set of images could have shown a small cluster of pores in a single ossicle, the consolidated silica

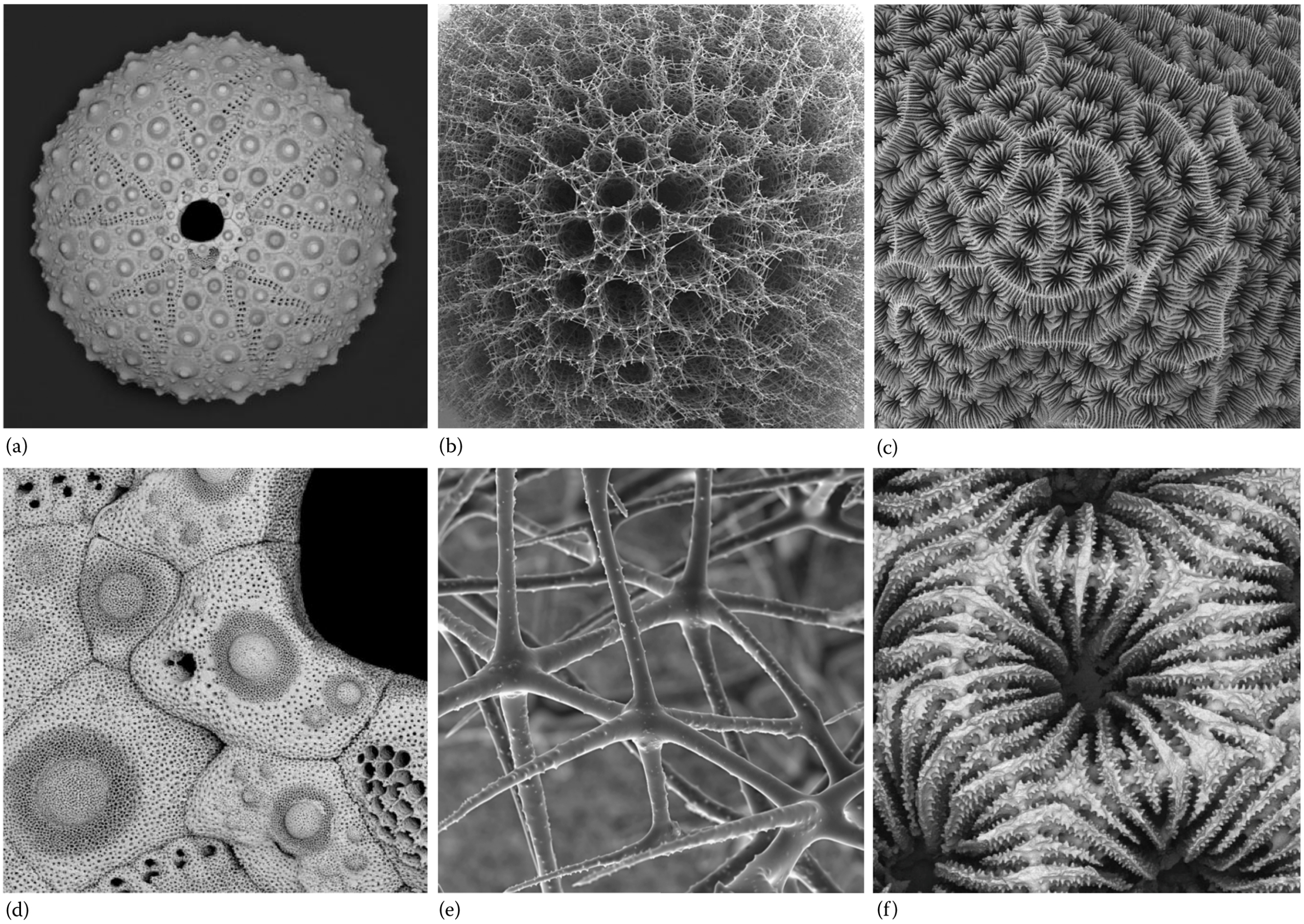


Figure 23.6 Wide-field SEM (upper) and subsequent higher magnification SEM images of the individual skeletal building blocks (lower) of the purple sea urchin, *Strongylocentrotus purpuratus* (a and d), the cloud sponge, *Aphrocallistes vastus* (b and e), and the lettuce coral, *Agaricia humilis* (c and f). Field diameters: (a) 2 cm, (b) 2 cm, and (c) 3 cm.

nanoparticles that form one of the spicule's spines, and the fused aragonitic spherulites in each septa of the corallite. At the other end of the length scale, we could have also included photos of the living animals, which too would have been chosen such that at least some of the features in the wide-field SEM images were clearly visible. Too frequently, however, these intermediate magnifications are omitted, which makes it nearly impossible for a reader to understand the complexities of these remarkable structures.

In addition to the high level of contrast detail that can be achieved in wide-field electron micrographs, the greatly enhanced depth of field means that in many cases, it is possible to obtain images of the entire research specimen. When only a zoomed-in image of a periodic structure is shown (like a sponge or coral skeleton), it raises questions as to how representative the features are and what is the natural variability in structural complexity or size from one region to another. When the whole specimen is shown in its entirety, all of the information is conveyed in a single image, often revealing large-scale patterns of ordering that might otherwise have been missed.

23.4.2 WHOLE SKULL IMAGING IN THE SEM

One of the other advantages of wide-field SEM is that it allows the researcher to expand the range of suitable specimens for imaging into realms, which they never before dreamed possible. As a result, one can capture a single image of an object measuring several centimeters across while simultaneously maintaining the entire field of view of interest in focus, without the accompanying perspective distortions that can make image tiling over large areas problematic. To best illustrate this point, we have chosen a group of test specimens, in this case, the skulls of various mammal species, which due to their highly reflective nature are not only difficult to photograph, but also exhibit sample height differences across a single specimen that are in the centimeter-scale range. As illustrated in Figure 23.7, not only are the entire specimens in focus, but these images also clearly reveal some intriguing features, such as the differences in electron density of the teeth (a), the details of the nasal turbinates (b), and the vastly different dentitions between two related species of rodents (e and f). In all of these images, even the smallest organizational details of the suture joints are clearly visible, thus permitting the accurate description of these important diagnostic features.

23.5 *THE BIRD WORLD*: FROM AN IMAGE TO A GENERALIZATION

Despite recent advantages in new imaging technologies, detailed illustrations still provide several key advantages over a photograph. For example, like the iconic paintings in the Audubon bird books, the illustrations of the tintinnid ciliates in Figure 23.1, the radiolarians in Figure 23.2, and the siphonophores in Figure 23.3 are not intended to represent

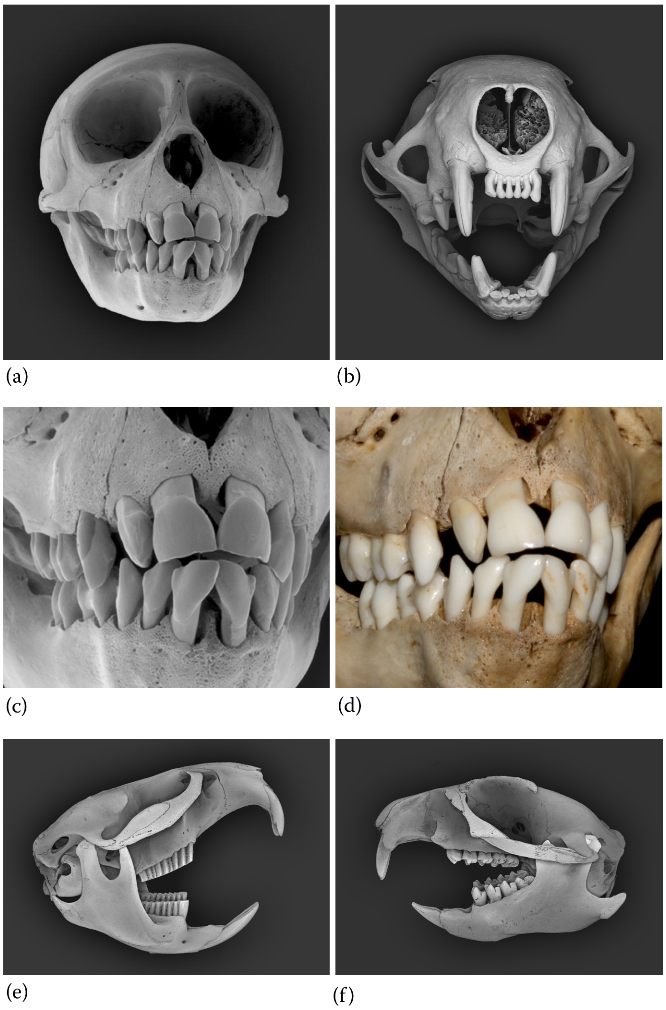


Figure 23.7 Wide-field SEM of mammal crania. These images of macaque (a and c), weasel (b), muskrat (e), and squirrel (f) skulls clearly illustrate the power of this technique for the generation of incredibly detailed images of large mineralized structures of an intrinsically low optical contrast material (like bone). Image (c) is a cropped portion of the image shown in (a), compared side by side with the same region photographed with a digital camera (d). In addition to the fact that the entire SEM image is in focus, local variations in tooth electron density (most notable in the canines) are clearly visible. Because of the large sample size, the entire motorized stage assembly of the SEM had to be removed to accommodate these specimens. Field diameters: (a) 7 cm, (b) 3 cm, (e) 7 cm, and (f) 6 cm. (Sample skulls were kindly provided by Skulls Unlimited, Oklahoma City, Oklahoma.)

a specific individual, but rather, through the naturalist's examination of multiple individuals, provide an accurate visualization of what a representative of a given species would look like. These illustrations can also draw the viewer's attention to specific diagnostic features of a given species and thus function as an accurate identification guide.

The catalogs of birds that have been produced through the past two centuries are testaments to the habits and philosophies of their times on this account. Immobilization of a bird for a painting was typically done by hunting followed by taxidermy, at the time John James Audubon (1875–1851) began his ornithological career. Audubon's uncounted hours spent in the field observing the wildlife he loved began in his childhood, with his father's encouragement to observe nature's details and seasonal ebb and flow. Biographer Richard Rhodes relates the young Audubon's introduction to illustration (Rhodes 2004):

> With a child's natural avarice he came to wish to possess birds totally. That wish was inevitably frustrated, he wrote, because "the moment a bird was dead, however beautiful it had been when in life, the pleasure arising from the possession of it became blunted." Whatever effort he gave to preservation, "I looked upon its vesture as more than sullied….I turned to my father, and made known to him my disappointment and anxiety. He produced a book of *illustrations*. A new life ran in my veins."

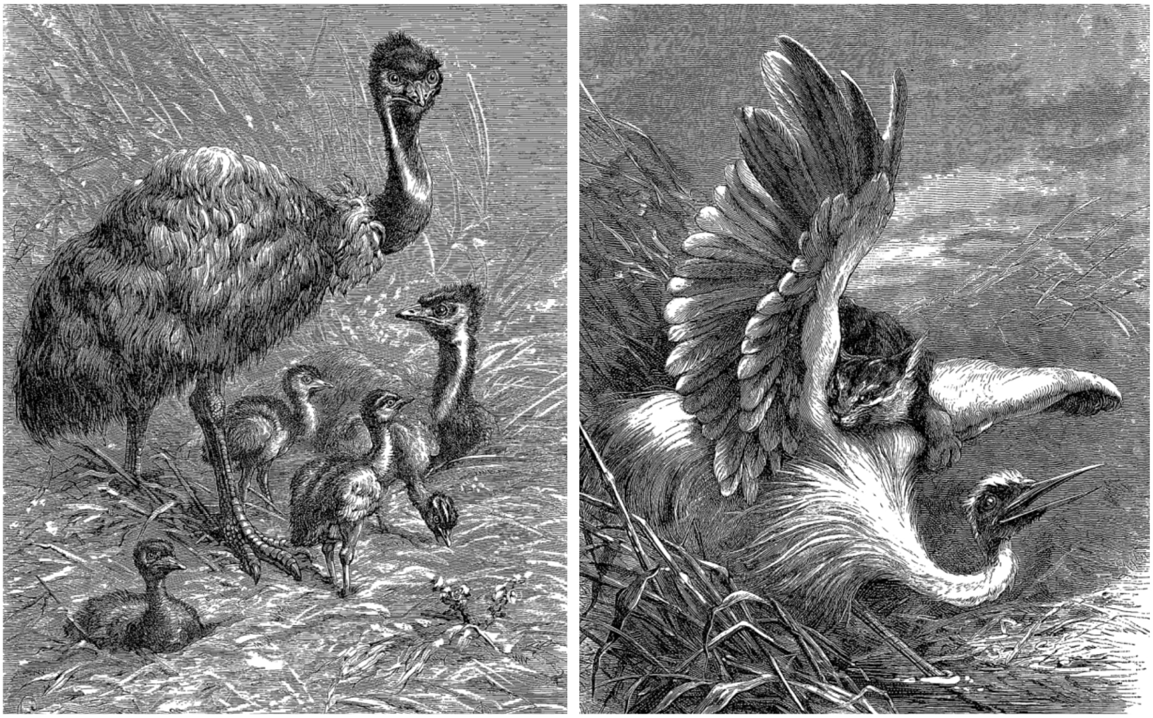
Thus began Audubon's lifelong dedication to improving his drawing and painting skills. Furthermore, Audubon was noted for new techniques in assembling his models. Rather than stuffing the birds into stiff, symmetrical poses, Audubon killed birds with light shots, used wires to prop them into natural positions, and might spend four long days with studies and sketches before opening the paintbox. Basing his paintings on such extensive field observations, Audubon could portray his subjects as if they were caught in motion.

As if in homage to these roots, the contributors to field guides through the twentieth century have employed illustrations long after photographs were available and ubiquitous, again acknowledging that the distinctive marks and habitual postures of a species are not always best brought out by a photo of a specific individual. Perusing currently available field guides, one finds a combination of illustrations and photos, but always with accolades and respect awarded to artists who combine their powers; for example, contemporary ornithologist Kenn Kaufman, whose press kit describes how his "innovative technique of combining the best features of photographs and paintings results in the most accurate and helpful images ever to appear in any field guide…. The photographs… are digitally enhanced to illustrate the field marks necessary for quick and easy identification."

In other words, with the eye of an illustrator, the photo is edited to create the desired image of the representative individual. Field guide author John Muir Laws (2012) makes a related point about the relationship between observing in the field and preparing a drawing for a guide:

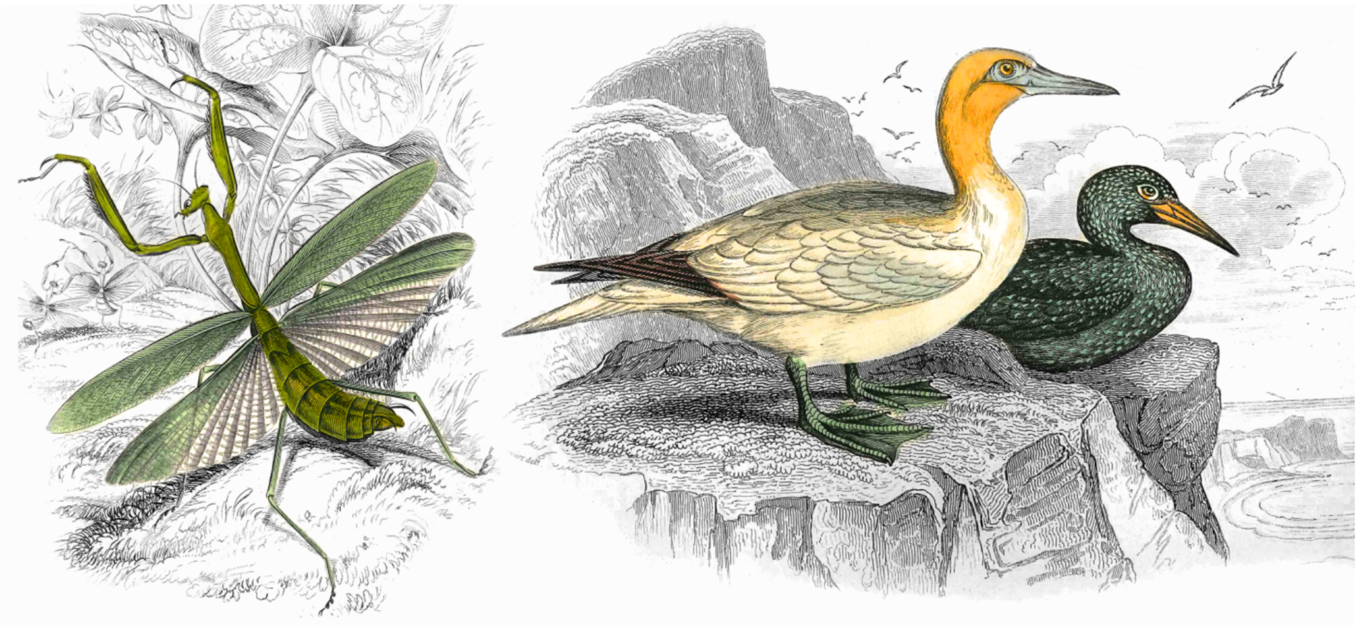
> Those drawings that are in the bird book—the artist isn't walking in the field, seeing a Lincoln Sparrow, and putting that Lincoln Sparrow into the field guide. They're walking out there in the field, they see the bird, they make tons of sketches in the field, they come back to their studio, they put all their sketches around their easel, they get a dead bird from the science museum, they get out all the photographs they can find, they put all this stuff together, and they make that picture which they then put into the field guide.

That being said, illustrations can certainly enhance the individuality of the subject matter, and the author as well. An illustration can dramatically portray an event that might have been missed by the photographer or reduce the complexity of a background in order to emphasize the actions taking place. For example, the illustrations shown in Figure 23.8 that have been adapted from Jules Michelet's *The Bird World* published in 1885 provide two such examples: the first of emu parents watching after their young, and the second of an egret being attacked by a lynx. Not only do these two images illustrate important events in the lives of these birds, but they also provide critical insights into the



(a) (b)

Figure 23.8 Illustrations from *The Bird World*. (From Michelet, J., *The Bird World*, Thomas Nelson & Sons, London, U.K., 1885.) (a) Emu parents with their young. The watchful (arguably anthropomorphic) expression on the standing bird is ironic testimony to the fact that early American ornithological societies were as likely to collect species to extinction as to agitate for their protection. (b) A white egret is attacked by a lynx. Notice how the high contrast lines in the drawing's subjects and foreground add to the drama, while the muted background also highlights the animals' postures in sharp relief. Nothing of the artist's intent is left to imagination.



(a) (b)

Figure 23.9 Using a highly reduced color palette, the illustrator can dramatically draw attention to the species of interest, while simultaneously maintaining a high level of detail in the image background. Following the printing of each metal engraving in black ink, a small army of artists would individually hand-color each illustration. (a) European Mantis; (b) The Solan Goose (young and old plumage). (Images adapted from Jardine, W., *The Naturalist's Library. Introduction to Entomology I: 1840; Birds of Great Britain and Ireland IV: 1860*, H.W. Lizars, Edinburgh, Scotland, 1833–1860.)

mind of the naturalist who drew them. A large number of images in Michelet's book deliberately demonstrate the sinister and violent lives of birds, whether they be of mews foraging on a drowned corpse, a falcon attacking a group of small song birds, an eagle and a fish hawk engaged in battle on a rocky shore, or a secretary bird posing with a dead snake. Perhaps, it is the author's goal to emphasize the notion that in addition to their beauty and splendor, birds also possess many violent behavioral traits to which we humans can so intimately relate. These images elegantly accomplish this in a way that photography would not so easily portray.

In an effort to simultaneously illustrate the detail of a species of interest and the habitat in which it resides, the precise use of color was often employed to reduce confusion for the reader (Figure 23.9). It should be noted that this technique was first employed before the development of color-printing processes, and each of the individually printed steel or copper engravings in each published volume was colored by hand. The results were truly stunning and to this day represent some of the most elegant scientific illustrations ever generated.

23.6 OUTLOOK

The biomineralization research community, more than ever before, finds itself in an abundance of riches with respect to analytical tools. Most of them, the SEM, for example, see new orders of magnitude each decade in figures of merit like obtainable resolution, contrast and focus modes, chemical sensitivity via spectroscopy, and more, thus opening the door for increasingly detailed investigations into the composition and structural complexity of mineralized tissues. The wide-field SEM, for example, is poised to set new standards for the high-throughput survey of macroscale samples via x-ray microanalysis (EDS). At the same time, we can be aware that a terabyte of imaging data arising from a single interrogated sample provides a new challenge to our sense of what may be generally true of a fundamental reaction, a growth process, a tissue, an organ, or a species. To achieve these goals and tackle this exciting challenge, we as scientists must discover new and innovative methods by which we can reconnect with our artistic predecessors as we continue to explore the wonders of our natural world.

ACKNOWLEDGMENTS

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REFERENCES

Bartlett, W.H.C. 1852. *Elements of Natural Philosophy*. New York: A. S. Barnes and Company.

Haeckel, E. 1887. Report on the scientific results of the voyage of H.M.S. challenger during the years 1873–1876. Zoology, Volume XVIII. Report on the Radiolaria collected by H.M.S. Challenger. London, U.K.: Eyre and Spottiswoode; Edinburgh, Scotland: Adam & Charles Black; Dublin, Ireland: Hodges, Figgies, & Co.

Haeckel, E. 1904. *Kunstformen der Natur*. Liepzig, Germany: Bibliographischen Instituts.

Jardine, W. 1833–1860. *The Naturalist's Library. Introduction to Entomology I: 1840; Birds of Great Britain and Ireland IV: 1860.* Edinburgh, Scotland: H.W. Lizars.

Laws, J.M. 2012. *The Laws Guide to Drawing Birds*. Berkeley, CA: Heyday.

Michelet, J. 1885. *The Bird World*. London, U.K.: Thomas Nelson and Sons.

Minsky, M. 1957. US Patent 3.013.467.

Rhodes, R. 2004. *John James Audubon: The Making of an American*. New York: Random House.

Robinson, R. 2006. Ciliate genome sequence reveals unique features of a model eukaryote. *PLoS Biology* 4:e304.

Thompson, D.W. 1917. *On Growth and Form*, Abridged edition, ed. J.T. Bonner. 1966. London, U.K.: Cambridge University Press.

Weaver, J.C., W. Mershon, M. Zadrazil, M. Kooser, and D. Kisailus. 2010. Wide-field SEM of semiconducting materials. *Materials Today* 13:46–53.