

Script for Power Point Slide Show***About This Activity***

This activity contains a series of images of very small things. Students will view the images, hear the information from a script, and make some observations and connections. They will see Scanning Electron Microscope (SEM) images and Transmission Electron Microscope (TEM) images of small living organisms and rock surfaces. Then, using their new perspective, they will view unidentified samples and speculate about the surfaces and the origins of the features in the images. The goal is to get the students to think about looking at very small objects and to realize that observations are a great way to start scientific research.

Learning Objective

In this lesson students will learn to use scale bars along with the shapes of microscopic specimens to determine whether an electron micrograph image possibly contains a microbe.

Slide 1: Title slide

Eyes are one of the most important tools any scientist uses; observation is one of the most important skills. The Scanning Electron Microscope (SEM) helps the scientists who are studying very small living things and rock surfaces. The SEM magnifies objects up to 50,000 times their actual size. Working at such phenomenal ranges, size and shape help the scientists to determine what they are seeing.

We'll begin by looking at something big-relatively.

Slide 2: Microscopic Fungi

In the microscopic world, fungi can be huge.

Slide 2: Inset 1 (top left)

Notice the scale bar at the bottom right. It measures 10 micrometers (or microns). Scale bars are used to help scientists keep size in mind when they are looking at images from any microscope. The adhesive loops in this image of a nematode-trapping fungus are about 20 microns across. Looking at the filament itself, we measure approximately 2 microns. The whole organism measures about 90 to 100 microns across.

Slide 2: Inset 2 (top right)

Here we are looking at the basidiospores of shiitake mushroom magnified 5,000 times. Notice the rounded shape. Remember that you are looking at only a part of this organism.

Slide 2: Inset 3 (bottom right)

The largest spores in this image of bread mold are about 2 microns. This image suggests the large number of spores and shows the chains. These are also round and part of a large organism.

Slide 2: Inset 4 (bottom left)

Here you see the bread mold again, but the magnification has increased.

So, from these pictures we can see that microscopic fungi are sometimes round, sometimes stalk-like or filament and we will see that they are large compared to other microscopic organisms

Slide 3: Bacteria

Bacteria are relatively small compared to most microscopic fungi. Most bacteria are about 1 to 2 microns across. Convenient for easy identification, all bacteria come in one of three basic shapes: round [called coccus(*kŏk'əs*), pl. cocci(*kŏk' cī*)], rod [called bacillus(*be sīl'əs*), pl. bacilli(*bə sīl' ī*)], and spiral [called spirochete(*spī' re kēt'*), pl. spirochetes].

Slide 3: Inset 1 (top left)

This bacillus or rod-shaped, elongated bacterium (Desulfovibro) is on an iron sulfide substrate where it feeds on sulfur. The scale bar in this picture measures 2 microns and indicates that these bacteria are about 3 microns long, which makes them about the size of the smallest bread mold spores we saw on the last slide.

Slide 3: Inset 2 (top right)

Here we see chains of spheres. This *Streptococcus sobrinus* (*strepto-* means chains) is a coccoid bacterium. These bacteria are about 2 microns across.

Slide 3: Inset 3 (bottom left)

This bacterium is strange looking. See the spiral corkscrew shaped structure that runs from the lower middle of the picture toward the top right corner? Bacteria with this shape are called spirochetes. The bacteria that cause the venereal disease syphilis are spirochetes. The scale bar in this picture is 1 micron long, so the spiral-shaped bacteria here are about 1.5 microns in length.

Slide 3: Inset 4 (bottom right)

Eschericia coli (or E. coli) is a famous bacterium that lives in all of our lower digestive tracts. Problems arise however when we ingest antibiotic resistant E. coli into our upper digestive tract. It can cause a deadly infection. These elongated cells, bacilli, are individual E. coli bacteria. The scale bar is missing from this picture, but the average cell e.coli is about 2 micrometers long.

From these pictures we can see that microscopic bacteria are sometimes round, sometimes rod-shaped, and sometimes spiral and they are in the size range of 2 to 3 microns.

Before we leave this look at the E.coli again. In this picture the small white dots are phage particles. These viral organisms attach themselves to the host cells and inject their genetic material. This is a great size comparison of bacteria to viruses.

Slide 4: Killer viruses

Viruses are much smaller than bacteria. In fact, one bacterium can be equal in size to 100 viruses stretched out side to side! Viruses take on many shapes, each one distinct from the other. Scientists identify viruses by their extremely small size and their complex shapes and structure. We will use the Transmission Electron Microscope (TEM) to look at some killer viruses.

Slide 4: Inset 1 (top left)

This is the Bacillus Killer Virus, so named because of its elongated shape- much like an elongated bacterium. But it is much, much smaller than any known bacteria.

Slide 4: Inset 2 (top middle)

These are many round viruses inside a dead bacterium. It might be easy to mistake these round viruses for coccoid bacteria if it weren't for the fact that they are so much smaller than bacteria. In this case, 7 or 8 viruses are lined up across the inside of the infected bacteria. I bet you didn't know that a bacterium could get sick from a virus just like you can.

Slide 4: Inset 3 (top right)

Here we have an image of the Bacillus Virus Theta 1. Can you tell why it's named "bacillus"? Remember, scientists are able to distinguish rod-shaped bacteria from rod-shaped viruses because of their size differences. Size and shape help us know what we're seeing under the microscope.

Slide 4: Inset 4 (bottom left)

Pseudo cowpox virus is a self-limiting disease that can be spread to humans with little effect, but it is related to the smallpox virus, which is deadly to humans. The pseudo cowpox virus is oval, or bacillus shaped, but it is much smaller than any known bacteria. Notice the crosshatching that makes it look like some types of cereal. Viruses consist of only a piece of nucleic acid with a protein coating. The crosshatching is the protein coat that protects the delicate viral nucleic acid until it enters the host cell, in this case a cell of a cow.

Slide 4: Inset 5 (bottom right)

Ebola Zaire is the most dreaded hemorrhagic fever known to man. The distinctive shape of this virus makes the most jaded scientist dizzy. The looping end is referred to as the shepherd's hook and is unique to this virus. Ebola spreads through direct body contact with an infected person and is 90% fatal within a few days. No one knows for sure how or from where humans get this virus nor is there any cure yet!

Viruses are the smallest microbes that we know, measuring in the .003-.05 micron range This is many times smaller than bacteria.

Slide 5: Living or Nonliving?

So, are all spheres, rods and spiral images microbial life? No. Here are some structures that could be confused with microbes if you only look at the shape. But size is important and must be considered.

Slide 5: Inset 1 (top left)

These are magnesium oxide crystals. They are round which means they look like the coccoid bacterium or a round virus. How do scientists know that they couldn't be either of these? The 10-micron scale bar indicates that these individual spheres are each about 8 to 9 microns across. That's 4 to 9 times too big to be a bacterium and way, way too big to be a virus.

Slide 5: Inset 2 (top right)

The green background is a leaf. See the round, bumpy reddish structure in the upper middle? Doesn't it look like the magnesium oxide crystals in the left picture? What about size? The 10-micron scale bar indicates that this structure on the leaf is about 15 microns across. Could it be a bacterium? No, remember bacteria are 1 to 2 microns across. This is a grain of pollen, the sperm of a flowering plant. Pollen grains come in all shapes and colors but are always bigger than bacteria.

Slide 5: Inset 3 (middle)

This is a stoma, a leaf structure that opens and closes to let out or keep in moisture. As you can see, this stoma is open. The scale bar on this picture is 10 microns. The stoma is quite large at approximately 20 microns across.

Slide 5: Inset 4 (bottom left)

This image is cotton fibers on the end of a Q-tip. The spiral nature of these fibers is reminiscent of a spirochete but each fiber is 20 microns wide, way too big to be a bacterium.

Slide 5: Inset 5 (bottom right)

This spiral is a magnified light bulb filament, the part of the bulb that glows brightly. The scale bar is 200 microns. The filament's coiled shape could be confused for a spirochete if it weren't for its huge size by comparison. This portion of the filament pictured here is more than 150 microns wide and more than 900 microns long.

Slide 6: Creature Feature

Here we can see some fun and interesting things under the microscope. These things are probably large enough to be seen with the naked eye, but not with much detail.

Slide 6: Inset 1 (top left)

This monstrous looking fellow is a tapeworm. In the larve stage here, the worm is looking for a host, probably a shark, and will use its four tentacles to attach to a mucous lining in the intestine. Notice the size.

Slide 6: Inset 2 (top middle)

This is the end of the stinger of a centipede. On the top toward the end we see an indentation – this is called the venom hole. This is where the venom comes out.

Slide 6: Inset 3 (top right)

Slide 6: Inset 4 (bottom left) This is the head of a common maggot (fly larva). Cute isn't it?

This is a louse, or mite, on a wild zebra finch feather. This parasitic insect feeds on the feathers. Notice the scale bar-the louse is approximately the size of the tapeworm.

Slide 6: Inset 5 (bottom right)

Now here's that feather magnified again. We don't have the magnification but these vanes or webs are the ends and edges of each rachis or shaft coming off the quill.

Slide 7: Minerals and Glass

Now let's apply our size and shape awareness to geology.

Scientists are more likely to use the SEM to look at rocks and minerals than microbes. Just like living things, minerals have distinctive characteristics that aid in their identification. Pay attention to the rock and mineral textures revealed in the following images.

Slide 7: Inset 1 (top left)

Not surprisingly, glass looks fairly smooth even under the electron microscope. Glass is formed under high temperatures. Here the glass has vesicles (holes) created by gas bubbles trapped in the rapidly cooling molten rock. The scale bar, which measures 100 microns, shows that the smaller vesicle is about that size while the larger vesicle is twice that size.

Slide 7: Inset 2 (top right)

Minerals, like this one, have distinctive crystalline shapes. This is ilmenite (il'-mən-nīt), a mineral that forms a house-shaped crystal with smooth rectangular faces (sides). The 100-micron scale bar indicates this ilmenite crystal is more than 300 microns across.

Slide 7: Inset 3 (bottom left)

Rocks and minerals are weathered (eroded) by chemical and physical forces like acid solutions or ice movement. Here we see a sample of weathered soil taken from Antarctica. As ice, water, and chemicals interact with rocks on Antarctica, some parts of the minerals in the rock wear away in a patterned manner. These stalactite-looking structures are what are left of a mineral in an igneous rock after this slow weathering process. The 2-micron scale bar tells us just how small these "stalactite" structures are; each point is only about 2 microns from the next.

Slide 7: Inset 4 (bottom right)

Clay minerals are the product of weathering, too. Clay has this distinctive porous, "honeycomb" look. The 1-micron scale bars indicate the extremely small pores are only about 0.3 microns across! That's pretty small. No wonder clay seems so smooth and soft to touch.

Slide 8: Rocks

Rocks are made of bits and pieces of several kinds of minerals and sometimes have glass particles, too. Geologists study rocks under the electron microscope to get clues as to how and under what conditions they formed. With this type of information, geologists can draw conclusions about planetary conditions long ago.

Let's look at some typical rock surface textures.

Slide 8: Inset 1 (top left)

Look at the smooth areas on the bottom left part of the image. Don't they look like glass? See the facets (faces) of the mineral crystals sticking up like boulders in a valley? See the scratched areas in the middle right of the image? This looks like evidence of weathering. The 100-micron scale bar helps geologists relate the size of various features of a rock. Under much higher magnification, one might see better evidence of weathering.

Slide8: Inset 2 (top right)

This is Columbia River Basalt. This kind of volcanic rock (basalt) can be found on earth, the moon, and probably on Mars and other rocky planets. This scale bar is quite small and indicates only 10 microns. Do you see evidence of weathering? There is some. Should rock from deep under ground be extensively weathered? Yes, it should.

Slide 8: Inset 3 (bottom left)

This rock shows crystalline features except in the vicinity of the micro-crater where it is very smooth like glass. The 10-micron scale bar indicates the micro-crater is about 80 microns across.

Slide 8: Inset 4 (bottom right)

This is dolomite- calcium magnesium carbonate $[CaMg(CO_3)_2]$ -from the subsurface of Kansas. Magnified 1000 times, we see tabular to boxy dolomite crystals that are being coated by small prismatic quartz crystals. Minor platy clay crystals are also seen. The scale bar is 50 microns. Use it to determine the size of the crystals.

Slide 9: Life in Earth's Extreme Environments

Recent discoveries have pushed the boundaries of our understanding about where life can exist on earth. We have found life flourishing in some very extreme environments, places that had previously been thought inhospitable to life. Using SEM images, these become *extremely* interesting places to explore.

Slide 9: Inset 1 (top left)

Microbes have been found living on rock and water only several kilometers below the ground in ancient basalt flows (volcanic rock layers). Columbia River Basalt is from the ancient volcanic bedrock deep under Washington State. Since 1996, scientists have been studying these rocks to see how bacteria can live deep below the Earth's surface. Here's what they've discovered.

The 100-nanometer scale bar (which is the same as a 0.1-micron scale bar) indicates that the structure in the middle of the screen could be a 1.5 micron bacillum.

Slide 9: Inset 2 (top right)

The spiral-shaped structure in the middle of the image is in the size range of a spirochete. A bacillus-shaped feature is seen on the right bottom of the image. It, too, is in the correct size range to be a bacterium. These are good candidates for bacteria that can live in the extreme conditions found deep below ground.

Slide 9: Inset 3 (bottom left)

The cocci-shaped features are obvious in the middle of the image. Although they are a little smaller than 1 micron each, it is possible that they became dehydrated and therefore shrank.

Slide 9: Inset 4 (bottom right)

Here we have a bacteria chain, in the size range that we would expect. However, these microbes are living in Jemez Springs, a hot sulphur spring deep underground. That's an extreme environment.

If we are finding living microbes in these extreme environments, can we find evidence of past life-fossils-using the SEM technology?

Slide 10: Fossilized Microbes in Terrestrial Rock

These are images of fossilized microbes. The sizes and shapes continue to help us understand what we are seeing.

Slide 10: Inset 1(top left)

This is an example of a biogeochemical relationship in the desert. Desert varnish is a thin, black or reddish-brown coating formed on rocks, boulders, and entire mountain ranges by microscopic bacteria living on the rock surface for thousand of years. The bacteria take in manganese and iron and give it back as black-manganese oxide or red-iron oxide. There are cemented clay particles that protect the bacteria and help preserve the coating. The inset shows desert varnish and the underlying rock artfully exposed for us by ancient North American Indians. The SEM image shows cells of the bacteria.

Slide 10: Inset 2(top right)

This sample came from deep below the surface. Remember, it is only recently that researchers have known conclusively that microbes live there. These fossils tell us that they have been there over a long period of time. The oval features are in the size range of a bacillus.

Slide 10: Inset 3(bottom left)

This is an image from a stromatolite in the Bahamas. A stromatolite is an ancient rock formation that formed in the presence of microbes and water. The 1-micron scale bar gives us a reference for searching for the bacteria that participated in the formation of this stromatolite. The sectional tube-like structure running across the image from left to right may be the fossilized remains of strepto-bacillus bacteria. Each section of the chain is about 2 microns long, the right size to be a bacterium. The spherical structures are too small to be coccoid bacteria and so are likely mineral deposits.

Slide 10: Inset 4(bottom right)

This is the same image with increased magnification.

Slide 11: Unknowns

Let's use our newly gained experience to make observations and make inferences about some unlabeled samples with scale bars.

Slide 11: Inset 1 (top left)

This sample has a 1-micron scale bar. What do you see? A relatively smooth background with raised worm-shaped structures. How big are the “worms”? They appear to be about 1 micron long. That’s in the range of a bacillus-shaped bacterium. Interesting!

Slide 11: Inset 2 (bottom left)

This sample has a 2-micron scale bar. What do you see? A relatively smooth substrate that looks like glass; it has a crack in it that runs up and down along the left side of the image. What about the spheres sitting on the smooth surface? How big are they? They look about 1 micron across. That’s the size of a coccoid bacterium. Hmmm!

Slide 11: Inset 3 (right middle)

This image has a 1-micron scale bar. I see a background that looks like crystalline facets of some sort of mineral. What is that big feature in the middle? It looks to be about 2 microns long. How does it resemble previous images? Is the size comparable to other objects in previous images? Could it be a fossilized rod-shaped bacterium?

Slide 11: Revealed

At this point you have probably concluded that each of the images looks like some sort of rock with fossilized bacteria on it. These images are of pieces of Mars rocks! Researchers have determined that these rocks are definitely pieces of Mars that fell to Earth as meteorites. The upper left is Allen Hills 84001, to the right Nakla, below is Shergotty. Like most meteorites their names tell something about where they were found. Scientists have extensively analyzed these rocks. SEM images were essential in the research. Scientists are astounded and intrigued to think that they may be looking at fossils of “Martians” with their electron microscopes!

Maybe, **just maybe** . . . there was (is) life on Mars and this is the first evidence.

These images and their questions have sparked a scientific discussion that continues today. It has increased the research of extreme environments here on earth and encouraged a previously existing interest in going to Mars. There is still much research to do.