



Resolving Power – Seeing is Understanding

Grade Level: High school

Subject area(s): Biology,
Physics,

Time required: 3-4 (50)
minute classes

Learning objectives:
understand how light and
electron microscopes
operate and the limitations
and advantages of each.

Summary: This lesson introduces students to light microscopes and their limitations in viewing small features and objects. In addition, they will learn about more powerful imaging tools by understanding the principles of operation of electron and atomic force microscopes. As a culminating activity, they will solve a mystery using evidence and microscopes. The lesson requires access to light microscopes. The lesson can be done with provided images or by remotely accessing instruments for high resolution analysis. Links to virtual electron and atomic force microscopes are provided as well as information on how to remotely access equipment for real time use.

Lesson Background: The performance of all imaging instruments (e.g., eyes, telescopes, microscopes, etc.) is measured by their ability to *resolve, or distinguish*, two similar adjacent objects. Think about the eyechart at the optometrist: If you can't distinguish an "F" from a "P" on the second line then your vision might be rated 20/100 meaning your eyes can resolve a difference from 20 feet away that an average eye could resolve from 100 feet away. The resolving power of optics is limited by two main factors: *aperture size*, which limits the amount and spread of information or light gathered; and *wavelength*, which limits the size of the smallest focal spot that the system can achieve. For both parameters smaller is better with smaller apertures and wavelengths allowing optical systems to better resolve smaller objects or finer details.

The light microscope has been a critical tool to allow researchers to see things at the microscopic level. It uses light beams and glass lenses to create a magnified image of an object. However, traditional light microscopes can't resolve objects smaller than about 500 nanometers (nm) or achieve magnifications more than ~10,000X. It's important to understand that these limitations are not due to flaws in the glass lenses or microscope design that could be corrected. Instead they are a fundamental outcome of optical physics – a result of the "size" of visible light (wavelength ~400 – 700 nm) and how it behaves as a wave.

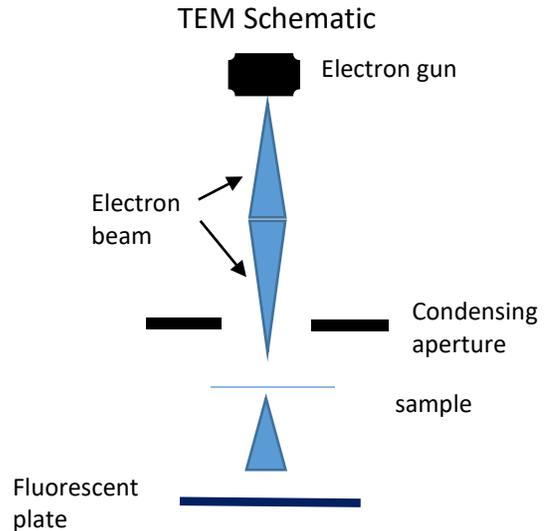
In 1924 a French physicist named Louis DeBroglie built on the understanding that light acts like both a wave and a particle to describe how matter – specifically electrons – might behave the same way. Previously, electrons were widely thought to act only like particles. According to his calculations, the wavelength of an electron could be 100,000 times shorter than that of visible light, which would allow a microscope using electrons to have much greater resolving power than a light microscope. By 1931, two Germans, Ernst Ruska and Maximillian Knoll, had used



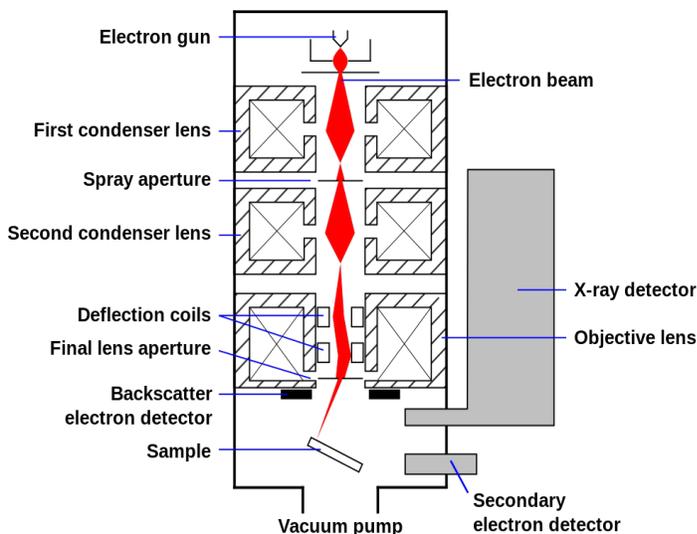
DeBroglie's theoretical insight to design an electron microscope (EM). Their microscope used an electromagnetic "lens" to control the path of electrons similar to the way that glass lenses focus light. Ruska's and Knoll's microscope was rudimentary as its magnification was only ~400X with a resolution of ~10 microns, but it was a significant breakthrough that led to further innovation. Later that decade, Canadian students made an effective electron microscope that could resolve features <100 nm. It was the first EM produced in North America.

There are three different versions of the electron microscope (EM): Transmission EM (TEM), Scanning EM (SEM), and Scanning TEM (STEM).

A **TEM** focuses a beam of electrons onto a very thin sample (100nm or less in thickness). The electrons pass through the sample and are projected onto a fluorescent screen to form an image. Visit: Transmission Electron Microscopy at: <https://www.youtube.com/watch?v=fQJYuTpK8Fs>.



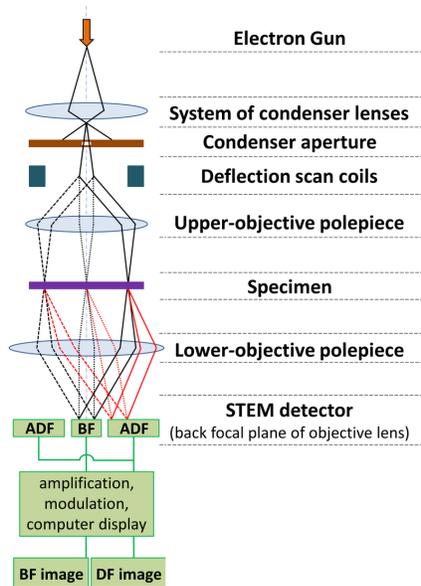
The **SEM** moves a focused beam of electrons across the surface of an object creating several types of signals. These signals include reflected and scattered electrons, light, and x-rays that result from what is called the electron-sample interactions. The various types of signal can provide information on the object's morphology (size and shape of surface features), elemental composition, and even crystal structure. Schematic of an SEM:



Schematic of an SEM. Source: [https://en.wikipedia.org/wiki/Scanning_electron_microscope#/media/File:Schema_MEB_\(en\).svg](https://en.wikipedia.org/wiki/Scanning_electron_microscope#/media/File:Schema_MEB_(en).svg)



STEM mode



Schematic of STEM. Source:
https://en.wikipedia.org/wiki/Scanning_transmission_electron_microscopy

The **STEM** is a hybrid of a TEM and an SEM. It uses very thin samples and transmits the electron beam through the sample as a TEM does. However, like in an SEM, the electrons are focused into a very narrow spot which is scanned across the sample providing both an image and spectroscopic data. Those simultaneous signals allow elemental or chemical information to be overlaid on the image the same way that Google maps superimposes live traffic data on a street map.

Electron microscopes are mainly used in research labs and universities. Although tabletop versions are available that are cheaper and easier to use and maintain, the larger more powerful research instruments require special low-noise, low-vibration locations to reach peak performance. Similarly, the more powerful the instrument, the more training is required to use it. Finally, samples to be analyzed need to be meticulously prepared to be able to tolerate the vacuum inside of an EM – the more detail needed, the more critical the preparation.

Light microscopes are still widely used especially for biology, and there are some new advanced optical techniques to “trick” light into resolving objects below the classical limits. However, it was the development of EMs that allowed scientists to begin to see and measure objects on the nanoscale for the first time.

The Scanning Tunneling Microscope (STM) was invented 1981 by Gerd Binnig and Heinrich Rohrer (IBM-Zurich) who won the Nobel Prize in Physics in 1986 for this achievement. If light and electron microscopes are like reading with a magnifying glass, STM and its close cousin Atomic Force Microscopy (AFM) are like reading by Braille.

Both STM’s and AFM’s (collectively called Scanning Probe Microscopes – SPM’s) use a very sharp tip that is carefully brought close to the surface of the sample. In STM as the tip is moved across a small area, a voltage sends an electrical current through the tip. The small voltage would not typically result in a signal: Think of a light switch in your house: the power (voltage) is always present, but no current flows (light is off) when the switch is open. STM uses a quantum effect, called tunneling current, to “trick” nature into generating a signal even when the switch is still – mostly – open. This tunneling current is extremely sensitive to the distance between the tip and the sample allowing imaging at the atomic scale – resolution $<0.1\text{nm}$. In addition to “seeing” individual atoms, STM’s can also pick up and manipulate them. This has led to amazing advances in nanotechnology.

AFM is similar, but the tip actually touches the sample surface. The AFM measures the force between the probe tip and the sample to form images from the data. AFM’s are not quite as sensitive as STM’s but still have resolutions of a fraction of a nanometer. However, because the



AFM can work on all types of samples in both air and liquid, it has been absolutely revolutionary in exploring and manipulating materials at the nanoscale. New, fast growing fields have been created because of its invention. With the ability to see and manipulate tiny bits of matter, these instruments have helped create and develop the field of nanoscale science and engineering.

Resources:

- Scanning Electron Microscopy. nanoScience Instruments: <https://www.nanoscience.com/techniques/scanning-electron-microscopy/>
- Virtual SEM: <http://myscope-explore.org/virtualSEM.html>
<https://myscope.training/>
- Virtual SEM: <https://www.olympus-lifescience.com/en/microscope-resource/primer/java/electronmicroscopy/magnify1/>
- Virtual SEM: <https://www.microscopemaster.com/virtual-electron-microscope.html>
- Virtual SPM: http://virtual.itg.uiuc.edu/training/AFM_tutorial/
- University of Virginia Virtual Lab: AFM; SEM; STM
<https://wecanfigurethisout.org/VL/home.htm>
- SEM Activity - Getting to know all the part of an SEM. Hitachi:
<https://www.inspirestemeducation.us/teaching-tools/lesson-plans/>
- Hitachi's Inspire STEM Education has a variety of lessons on microscopes:
<https://www.inspirestemeducation.us/teaching-tools/>
- Molecular Expressions from Florida State University. Virtual SEM:
<https://micro.magnet.fsu.edu/primer/virtual/virtual.html> (plus several other pages on microscopy)
- Virtual microscope: <http://www1.udel.edu/biology/ketcham/microscope/scope.html> (requires Adobe Flash)

Other microscope links:

- <http://www.udel.edu/biology/ketcham/microscope/scope.html>
- <http://virtuallabs.nmsu.edu/micro.php>
- <http://www.exploratorium.edu/imaging-station/activities/classroom/classroom.php>
- <http://www.microscopy.info/Microscopy/IndexByCat/6>
- <http://www.101science.com/Microscope.htm#image>

Materials: A materials list is included in the pre-lab activity *Introduction to Microscopes*. The teacher may assemble the mystery clues according to what is available. A possible list of clues (evidence) is included as well as images of the objects. Light microscopes are needed for the pre-lab and the mystery activity.

PreLab: Have students complete the *Introduction to the Microscope Lab Activity*. A modified version of this is included with this lesson or access the full activity at: <https://www.rcsdk12.org/cms/lib04/NY01001156/Centricity/Domain/4824/IntroductiontotheMicroscopeLab.pdf>.

An additional version can be found at: <https://www.slideshare.net/jrt004/getting-to-know-the-microscope-lab> and a version with answers at:



https://brearleyhigh.kenilworthschools.com/UserFiles/Servers/Server_7985/File/microscope%20lab.pdf

Answers to the parts of the microscope:

1. eyepiece or ocular
2. body tube
3. fine adjustment knob
4. nose piece
5. high power objective
6. low power objective
7. diaphragm
8. mirror (many microscopes have a light instead)
9. base
10. coarse adjustment
11. stage clip
13. inclination joint.

Advance Preparation: For the Mystery activity, the teacher has two options: 1. to use the images of objects provided with the activity; or 2. to schedule a remote session with an electron microscope. Such equipment can be accessed through the Remotely Access Instrumentation for Nanotechnology (RAIN) network (<http://nano4me.org/remotearchive>). This network allows schools to access and interact with SEM, AFM and optical microscopes along with the professionals that operate them. Hitachi, HTA provides a loaner tabletop SEM to classrooms for up to two weeks. The application form can be found here:

<https://www.inspirestemeducation.us/teaching-tools/>. Also, check with your local research university to see if they can provide access. If using the remote access option, gather samples for submission to the site. RAIN sites provide information on sample preparation.

The <http://www.graphene-battery.net/graphene.htm> link describes several methods for making graphene, but the procedure involving *making graphite oxide* is **not** safe. So, Method 1 was used by this lesson's author.

Directions for the Activity:

Part 1. Resolving Power

1. Administer pretest to determine what students know about the mechanisms and resolving power of microscopes.
2. Students read the page on resolving power and discuss the methods and abilities of the various scopes: <https://www.sciencelearn.org.nz/resources/495-magnification-and-resolution>.
3. Students access the virtual SEM and AFM (links in Resources above).
4. Class discussion about resolving power and the capabilities of the scopes.



National Nanotechnology Coordinated Infrastructure

www.nnci.net

This work is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

Development and distribution funded by the **National Science Foundation**

Pre-quiz answers:

1 – C; 2 – A; 3 – B; 4 – A; 5 – D; 6 – A

7 - There are five common aberrations: spherical, chromatic, curvature of field, comatic, and astigmatic. An extension to this question would be to have students explain in more detail what causes the aberration and how does it affect the image.

8 - The advances in electron microscopy and atomic probe microscopy has allowed scientists and engineers to see nanoscale objects that play an important role in materials that surround us as well as compose us. These tools are critically in the advancement of nanotechnology and have been used to characterize new materials and devices in a variety of fields including solar cells, transistors in computers, biomedical devices and drugs, environmental remediation, clothing, among many others. Students should pick one advance and explain how and why instrumentation was important in its development.

Part 2. Solving the mystery: “Who Kidnapped Abigail?”

As noted above, this part can be done in two ways: using the images provided with the lesson or remotely accessing the SEM and AFM. Below is the list for using evidence provided in the lesson. The teacher has the option to create a list of clues based on materials available to him/her. If doing remote access, it is suggested to have the entire class be involved in determining the guilty party. Students will have prepared samples to view or to send to remote scopes for imaging. They will capture and draw images with the scopes, discuss the models, and see the strengths of the various instruments.

1. Students will gather their evidence and determine which objects/materials need to be observed using either a light microscope, SEM, or AFM.
2. Using the table provided they will enter the evidence and discuss the pros and cons for the suspects.
3. Students will determine who they think the perpetrator of the crime is and defend their conclusion.
4. As a class, discuss the results.

Student Guide (answers in red)

Resolving Power – Seeing is Understanding

Task

Your favorite NCIS forensic specialist has gone missing. To help rescue Ms. Abigail, you need to identify several samples, using the tools in her lab. Some of these samples are identified with only one suspect, while others may implicate more than one suspect. Your task is to narrow the field of suspects and try to predict who may know the location of Abigail.

Tools: Optical microscopes, electron microscope, atomic force microscope

Prefix Review



National Nanotechnology Coordinated Infrastructure

www.nnci.net

This work is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

Development and distribution funded by the **National Science Foundation**

1 meter	= 1. m	$1 \times 10^0\text{m}$
1 millimeter	= 0.001 m	$1 \times 10^{-3}\text{m}$
1 micrometer	= 0.000001 m	$1 \times 10^{-6}\text{m}$
1 nanometer	= 0.000000001 m	$1 \times 10^{-9}\text{m}$

Can we go smaller? A hydrogen atom is 1/10 of a nanometer. **Think pico to femto.**

In Part 1, you explored resolving power and the capabilities of the microscopes that are the tools at your disposal. It will be up to you to decide which tool to use to examine the evidence.

Miss Abigail was last seen on the central staircase of NCIS, an area cleaned not long before, but frequented by many people. The NCIS forensic team has gathered evidence on the staircase. You have a list of suspects and clues (objects/materials) that are linked to each suspect. It is your job to examine these and decide who is guilty of kidnapping Ms. Abigail.

The teacher will provide evidence to the students that will point to 1 or 2 of the suspects. An example for the intended “culprits” Director Jene and Hailey Berrie, who have taken Abigail dancing/bowling and not really kidnapped her. The blond hair could implicate Duchess Sarah as well, but if dyed red hair is found, that’s a singular clue for Director Jene. Jeans could also point to the director. If graphene is available, that’s also supporting Hailey. CDs (music and dancing) are easy to prepare and useful to image on the AFM. Add to the “key” other samples from suspects to make students determine which sample goes with which suspect. For example, a white powder could be talc or flour until they are distinguished using the SEM. The teacher is at liberty to create the evidence to meet what materials are easily available if the remote access is to be used.

Procedure:

1. Examine and draw all the samples your group is given, and compare them to the clues from the AFM and SEM which have been provided as images.
2. Are any of the samples that require an optical microscope to examine useful? Are there some samples that can’t be used on the other scopes?

Students may answer that the insects can be viewed with the eye but better with a light microscope. Sandpaper cannot be imaged with an AFM because it is too rough. Pollen would be best with the SEM while graphene and CDs with the AFM.

Based on the clues found where Abigail was last seen, who do you think might have information about her whereabouts. Defend your conclusion.

Answers will vary based on clues presented by the teacher. They must defend their answer based on the evidence and use of the tools to examine the evidence.

3. What are the different strengths of the various instruments? The answers should discuss resolution of the three scopes. Students should state that the light microscope is very useful for seeing micron sized objects and details of larger objects. They should indicate that light scopes are limited by the wavelength of light and therefore objects or details below 500nm cannot be seen. The SEM uses electrons so can view objects and details on the nanoscale. The AFM has capabilities to provide information with resolution of a fraction of a nanometer. It can actually move atoms.



4. What other samples or substances would you sample if given the chance?

Answers will vary.

Optional: Research how high tech imaging systems such as STM, AFM, SEM, and STEM have and are advancing the field of nanotechnology and/or forensic science. Prepare a short PowerPoint to present your results including what may occur in the future using these tools.

Extension:

The author built two model microscopes to demonstrate the principles (cantilever vs. tunneling) of newer microscopes. Having students build these would make excellent extension projects. One Lego® model AFM is described in

<http://umassk12.net/nano/materials/AFMlego.pdf>

Another microscope model is described by Paninsic and Kovac in Physics Education (2008).

<http://iopscience.iop.org/0031-9120/43/1/002>. Also at:

<https://pdfs.semanticscholar.org/9a09/096b96ada2aeccad3ac798f2e4729e98a7f4.pdf>

Although they describe it as an AFM model, this writer believes the magnetic forces might serve better as an analogy to the STM.

Other LEGO AFM links:

https://users.physics.unc.edu/~falvo/NUE/LEGO_AFM_WEBPAGES/web_files/nanoworld.html

https://www.researchgate.net/publication/269666768_A_Conceptual_Atomic_Force_Microscope_using_LEGO_for_Nanoscience_Education

Assessment: Answers to the pre-test should allow the teacher to know the student's understanding of microscopes. It can also be used as a post-test to see if students gained knowledge. Accuracy and completeness of answers for the six objectives in the *Introduction to the Microscope Activity*. Accuracy and completeness of answers for the Mystery activity.

Next Generation Science Standards:

Influence of Science, Engineering, and Technology on Society and the Natural World

Contributors: Edward Lawrence, RET at Georgia Institute of Technology with special thanks to Dr. Walter Henderson, Materials Characterization Facility at Georgia Institute of Technology

Supporting Programs: RET site at Georgia Institute of Technology: NSF EEC 1200925 and National Nanotechnology Coordinated Infrastructure NSF ECCS 1626153

