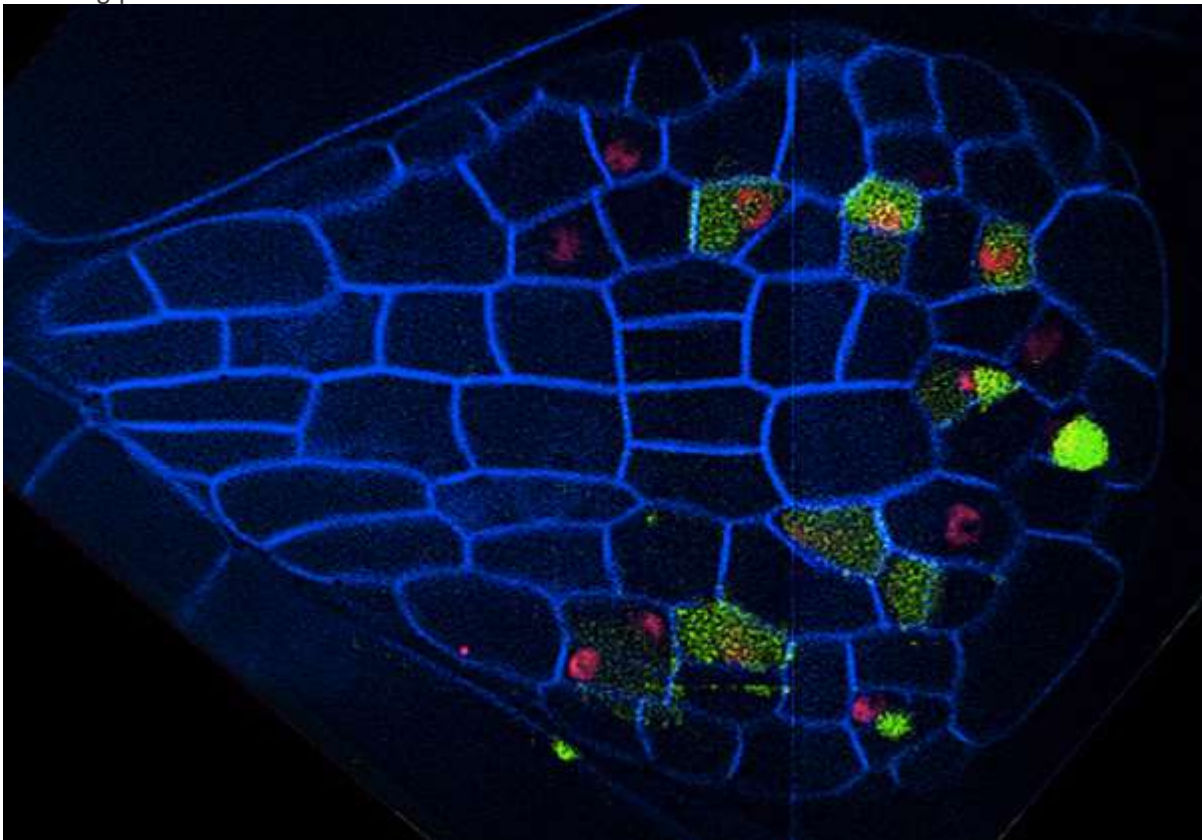


Microscopy

Introduction to microscopes and how they work. Covers bright field microscopy, fluorescence microscopy, and electron microscopy.

Introduction

If you meet some cell biologists and get them talking about what they enjoy most in their work, you may find it comes down to one thing: secretly, they're all microscope freaks. At the end of the day, what they really love is the chance to sit in a small, dark room for hours on end, communing with their favourite cell type through the lens of a beautiful microscope. That may seem odd, but the truth is, cells can be pretty gorgeous, like living stained glass. One of my favourite examples of this is the picture below, which shows cells in a very young leaf of thale cress, a small flowering plant related to mustard.



Confocal microscopy image of a young leaf of thale cress, with one marker outlining the cells and other markers indicating young cells of the stomatal lineage (cells that will ultimately give rise to stomata, cellular valves used for gas exchange).

Image credit: Carrie Metzinger Northover, Bergmann Lab, Stanford University.

This picture isn't a plain light micrograph; it's a fluorescent image of a specially prepared plant where various parts of the cell were labelled with tags to make them glow. However, this kind of cellular complexity and beauty is all around us, whether we can see it or not.

You could find cells just as intricately patterned and beautifully formed in any plant you looked at – from the rose in your backyard, to the grass growing up through the sidewalk, to the carrots you ate for a snack. Let's not limit it to plants, either: exquisite layers of cells can be found in your skin, in an insect's wing, and in just about any other living tissue you choose to look at. We, and

the world around us, are cathedrals made of cells. We just need some microscopy to appreciate it.

Microscopes and lenses

Although cells vary in size, they're generally quite small. For instance, the diameter of a typical human red blood cell is about eight micrometres (0.008 millimetres). To give you some context, the head of a pin is about one millimetre in diameter, so about 125 red blood cells could be lined up in a row across the head of a pin. With a few exceptions, individual cells cannot be seen with the naked eye, so scientists must instead use microscopes (*micro-* = "small"; *-scope* = "to look at") to study them. A **microscope** is an instrument that magnifies objects otherwise too small to be seen, producing an image in which the object appears larger. Most photographs of cells are taken using a microscope, and these pictures can also be called **micrographs**. From the definition above, it might sound like a microscope is just a kind of magnifying glass. In fact, magnifying glasses do qualify as microscopes; since they have just one lens, they are called **simple microscopes**. The fancier instruments that we typically think of as microscopes are **compound microscopes**, meaning that they have multiple lenses. Because of the way these lenses are arranged, they can bend light to produce a much more magnified image than that of a magnifying glass.

In a compound microscope with two lenses, the arrangement of the lenses has an interesting consequence: the orientation of the image you see is flipped in relation to the actual object you're examining. For example, if you were looking at a piece of newsprint with the letter "e" on it, the image you saw through the microscope would be "ə." ¹

More complex compound microscopes may not produce an inverted image because they include an additional lens that "re-inverts" the image back to its normal state.

What separates a basic microscope from a powerful machine used in a research lab? Two parameters are especially important in microscopy: magnification and resolution.

- **Magnification** is a measure of how much larger a microscope (or set of lenses within a microscope) causes an object to appear. For instance, the light microscopes typically used in high schools and colleges magnify up to about 400 times actual size. So, something that was 1 mm wide in real life would be 400 mm wide in the microscope image.
- The **resolution** of a microscope or lens is the smallest distance by which two points can be separated and still be distinguished as separate objects. The smaller this value, the higher the **resolving power** of the microscope and the better the clarity and detail of the image. If two bacterial cells were very close together on a slide, they might look like a single, blurry dot on a microscope with low resolving power, but could be told apart as separate on a microscope with high resolving power.

High-quality microscopes tend to have higher resolving power than cheap ones simply because they are more carefully made and work better.

However, resolving power is ultimately limited not by microscope machining quality, but by the physical properties of light. If two structures are separated by a distance less than half the wavelength of the light used for imaging, they cannot be distinguished from each other by conventional light microscopy². This phenomenon is called the diffraction barrier.

Electron microscopy (discussed below) gets around this problem by using beams of electrons, which have much shorter wavelengths than light. Also, some recently developed super-resolution microscopy techniques, have allowed the collection (or, more

typically, reconstruction) of light microscopy images whose resolution is beyond the diffraction barrier^{2,3}.

Both magnification and resolution are important if you want a clear picture of something very tiny. For example, if a microscope has high magnification but low resolution, all you'll get is a bigger version of a blurry image. Different types of microscopes differ in their magnification and resolution.

Light microscopes

Most student microscopes are classified as **light microscopes**. In a light microscope, visible light passes through the specimen (the biological sample you are looking at) and is bent through the lens system, allowing the user to see a magnified image. A benefit of light microscopy is that it can often be performed on living cells, so it's possible to watch cells carrying out their normal behaviours (e.g., migrating or dividing) under the microscope.



A light microscope, of the sort commonly found in high school and undergraduate biology labs.

Image credit: OpenStax Biology. Modification of work by "GcG"/Wikimedia Commons.

Student lab microscopes tend to be **bright-field** microscopes, meaning that visible light is passed through the sample and used to form an image directly, without any modifications. Slightly more sophisticated forms of light microscopy use optical tricks to enhance contrast, making details of cells and tissues easier to see.

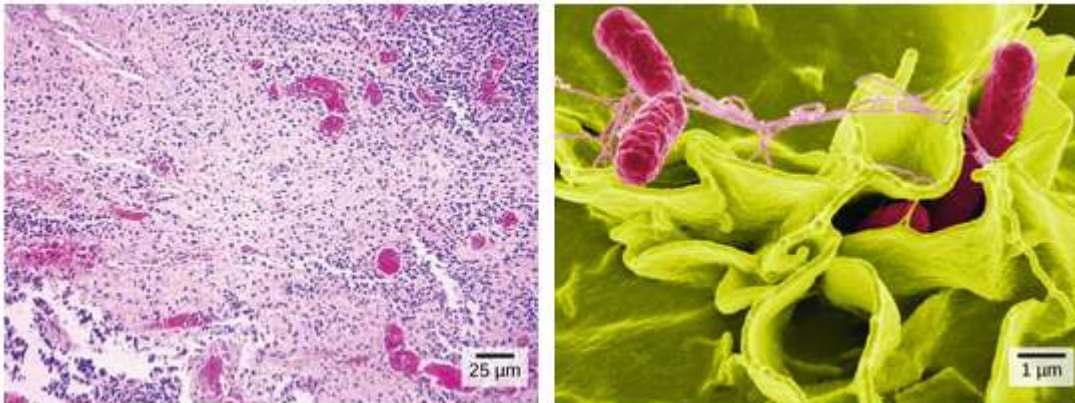
Another type of light microscopy is **fluorescence microscopy**, which is used to image samples that fluoresce (absorb one wavelength of light and emit another). Light of one wavelength is used to excite the fluorescent molecules, and the light of a different wavelength that they emit is collected and used to form a picture. In most cases, the part of a cell or tissue that we want to look at isn't naturally fluorescent, and instead must be labelled with a fluorescent dye or tag before it goes on the microscope.

The leaf picture at the start of the article was taken using a specialized kind of fluorescence microscopy called **confocal microscopy**. A confocal microscope uses a laser to excite a thin layer of the sample and collects only the emitted light coming from the target layer, producing a sharp image without interference from fluorescent molecules in the surrounding layers⁴.

Electron microscopes

Some cutting-edge types of light microscopy (beyond the techniques we discussed above) can produce very high-resolution images. However, if you want to see something very tiny at very high resolution, you may want to use a different, tried-and-true technique: **electron microscopy**. Electron microscopes differ from light microscopes in that they produce an image of a specimen by using a beam of electrons rather than a beam of light. Electrons have much a shorter wavelength than visible light, and this allows electron microscopes to produce higher-resolution images than standard light microscopes. Electron microscopes can be used to examine not just whole cells, but also the subcellular structures and compartments within them.

One limitation, however, is that electron microscopy samples must be placed under vacuum in electron microscopy (and typically are prepared via an extensive fixation process). This means that live cells cannot be imaged.



Images of *Salmonella* bacteria taken via light microscopy and scanning electron microscopy. Much more detail can be seen in the scanning electron micrograph.

Image credit: OpenStax Biology. Credit a: modification of work by CDC/Armed Forces Institute of Pathology, Charles N. Farmer, Rocky Mountain Laboratories; credit b: modification of work by NIAID, NIH; scale-bar data from Matt Russell.

In the image above, you can compare how *Salmonella* bacteria look in a light micrograph (left) versus an image taken with an electron microscope (right). The bacteria show up as tiny purple dots in the light microscope image, whereas in the electron micrograph, you can clearly see their shape and surface texture, as well as details of the human cells they're trying to invade.



Image of an electron microscope. It is very large, roughly the size of an industrial stove.
Image credit: OpenStax Biology. Modification of work by Evan Bench.

There are two major types of electron microscopy. In **scanning electron microscopy (SEM)**, a beam of electrons moves back and forth across the surface of a cell or tissue, creating a detailed image of the 3D surface. This type of microscopy was used to take the image of the *Salmonella* bacteria shown at right, above.

In **transmission electron microscopy (TEM)**, in contrast, the sample is cut into extremely thin slices (for instance, using a diamond cutting edge) before imaging, and the electron beam passes through the slice rather than skimming over its surface⁵. TEM is often used to obtain detailed images of the internal structures of cells.

Electron microscopes, like the one above, are significantly bulkier and more expensive than standard light microscopes, perhaps not surprisingly given the subatomic particles they have to handle!