THROUGH THE LOOKING GLASS

HARINI BARATH

The microscope is the mainstay of cutting edge research in many fields of biology today. When was it invented? What did the initial versions look like? What are some of the latest versions, and what can we use them for? This article provides glimpses into the history of microscopes before recounting some of its more recent and exciting developments.

"Where the telescope ends, the microscope begins. Which of the two has the grander view?"
—Victor Hugo, Book 3 Chap. 3 of Les Miserables.

Few of us can forget the first time we managed to make brick-like rows of cells, peppered with dots of cytoplasm, appear from a flimsy piece of stained onion peel after twiddling the wheels of a school microscope. An indispensable tool in many labs, the microscope is an instrument that helps us examine objects that are too small to be seen by our naked eye. This powerful invention has opened up the previously invisible world of cells and microorganisms to us. Even today, microscopes form the spine of many major areas of life science research, like cell biology.

Figure 1. Skin of a tomato as seen through a microscope. Source: Umberto Salvagnin. License: CC-BY. URL: https://www.flickr.com/photos/kaibara/7781208904/.

A brief history

The first microscopes date back to the early 1600s. While it is not clear who the original inventor was, it

is believed that the term 'microscope' was coined in 1625 by a friend of Galileo Galilei, a German doctor and botanist named Giovanni Faber[1]. In the years that followed, the microscope was increasingly used to examine and record biological structures. The most memorable contributions to the field of microscopy came about 50 years later, by Antonie van Leeuwenhoek, celebrated today as 'the father of microbiology'.

van Leeuwenhoek was originally a trader of drapes and linens. He became fascinated with lenses, which were commonly used as magnifying glasses to count threads, and soon mastered the art of lens-making. He made several hundred lenses and many different types of simple microscopes - small lenses mounted on brass plates - whose magnification far exceeded that of even the compound microscopes his contemporaries could craft. These state-of-the-art microscopes allowed van Leeuwenhoek to make ground-breaking observations. He was among the first people to observe cells; discover bacteria and protozoa; and study animal and plant tissues, as well as mineral crystals[3].

Simple and compound microscopes

A simple microscope typically uses a single lens to magnify an object, much like a hand-held magnifying glass used for reading. van Leeuwenhoek's microscopes were among the best simple microscopes ever made, achieving magnifications of more than 250X. This meant that the image was more than 250 times the size of the sample being viewed. It was more than a century before compound microscopes were able to better this.

Suggested exercise

Give students a magnifying glass and allow them some time to find out, by themselves, how to use it most effectively. They can use a page of their textbooks to experiment. Tell them to look through it first with both eyes open, and then with one eye closed. They can also try holding the magnifying glass at various distances from their eyes. Many will find that closing the non-viewing eye and holding the magnifying glass about half a foot away from the open eye gives the clearest image. Stress that everybody uses it differently and each student should find out what works best for them.

Stick a large leaf on a sheet of white paper and mount it on a board. Ask students to start by holding the magnifying glass very close to the sheet, and slowly moving it further away, stopping at every step, and observing how the image changes. Ask them to draw what they see at 3 or 5 different distances (e.g. 6 inches, 1 foot, 2 feet, 5 feet) from the board in their notebooks. Students can use a measuring tape to measure this distance from the board. They should try to reproduce the detail and size of the image in the magnifying glass as closely as they can.

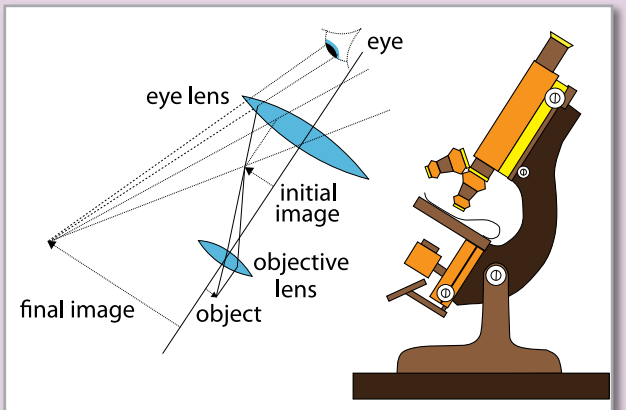

Figure 2. Lens/ray diagram that shows how a compound microscope produces an enlarged, but inverted, image of the object. Source: school physics.co.uk. URL: http://www.schoolphysics.co.uk/age16-19/Optics/Optical%20instruments/text/Microscope_/index.html.

A compound microscope has more than one lens, connected by a tube. The lens closest to the sample is called the objective. The image made by the objective is further enlarged by the eyepiece - the final lens through which the viewer sees the magnified image. Modern microscopes are all compound microscopes. They boast significantly higher (about 1000X or more) magnifications than a magnifying glass.

Suggested exercise

Dip a toothpick into curd/yogurt and use this to smear a very small drop of this liquid onto a glass slide. Gently place a coverslip on top of this smear. View under a compound microscope, slowly increasing its magnification. You will be able to see small bacteria, singly or in clusters, and in different shapes - rods or spheres. Encourage students to reproduce what they see through the microscopes in their notebooks[2].

Collaborating with artists

Modern microscopes record their images on computers, and previously on film, so researchers can do without the good drawing skills that are often considered necessary for recording and note-taking in school biology. But what did researchers in the 17th and 18th centuries do? Images were published as engravings. To do this, a drawing was traced onto and carved or etched into a copper plate, which was then printed.

Robert Hooke, a contemporary of van Leeuwenhoek, and author of **Micrographia** - the first scientific bestseller, made his own illustrations. His book contains the first microscopic images of plants and insects. van Leeuwenhoek, however, was not as skilled, and therefore worked with artists who would make the images for him. An engraver would engrave and print the plates[4].

Figure 3. Life magnified: A human liver cell. Source: National Institute of General Medical Sciences. URL: https://www.nigms.nih.gov/education/life-magnified/Pages/1b3_human-hepatocyte.aspx.

Microscopy continues to bring scientists and artists together, even today. Many artists and photographers are inspired by the compelling images captured by powerful modern microscopes. Their interactions with scientists often result in a collection of stunning images, some of which make their way to art exhibits, and others to public spaces like airports, where they serve as an eye-catching way of popularising science[5].

Today's researchers are trying to zoom much further into the cell. They seek to probe and photograph the wheels and cogs of all living systems – the biomolecules. One invaluable technique that has made this possible is fluorescence microscopy. Biochemists have discovered a whole host of fluorescing molecules - proteins that spontaneously emit light of one colour after being excited by light of another colour. By chemically coupling these labels or markers to other proteins of interest that do not emit light of their own, researchers are able to 'see' proteins as they move and interact with each other.

Optical microscopes are limited by what is known as the 'diffraction limit', which only allows them to discern details larger than half the wavelength of light, almost of the order of microns. So, two spots, which are less than a micron apart, will appear as one spot. However, a full understanding of many key bimolecular functions, processes and diseases requires a nano-scale picture. Researchers have developed ingenious ways to overcome this constraint and capture the nano-world. These 'super-resolution fluorescence microscopy' methods work by controlling the fluorescent labels in different ways. In fact, the 2014 Nobel Prize in Chemistry was awarded to Eric Betzig, W.E. Moerner and Stefan Hell for the development of these techniques.

Electron microscopes

All the microscopes discussed in this article are optical microscopes - they use light to generate an image. There are also other types of microscopes, including scanning probe microscopes, ultra microscopes and electron microscopes.

Ernst Ruska and Max Knoll are credited with creating the first electron microscope in 1932. As its name suggests, electron microscopes use electrons instead of light to form an image. Glass lenses are replaced by electromagnets, but the working principle remains the same as an optical microscope. Electrons have a much shorter wavelength than light. This means that the electron microscope can resolve, or reveal details on a much smaller scale than an optical microscope. In fact, the best electron microscopes can tell two atoms apart! Electron microscopes can also achieve significantly higher magnifications – almost a thousand times better than a compound microscope.

Studying biological samples in an electron microscope, however, has one major problem. Samples are studied in a vacuum, and need to be prepared or 'fixed' by one of many methods. This means that live cells cannot be viewed or photographed.

Making better microscopes - an ongoing quest

Even after three centuries of pioneering work that have made microscopes tremendously powerful and sophisticated, there is room for improvement. "As a physicist, I think there is a huge amount of physics left to be explored and exploited in microscopy," says G. V.

Pavan Kumar, Assistant Professor in the Department of Physics at IISER, Pune. His work is a continuation of the legacy of the many physicists before him whose efforts, through the ages, have helped overcome the limitations of microscopes.

Biological samples are largely transparent. Staining these samples with contrast agents is one way to make them visible under the microscope. But this means that the specimens need to be killed and fixed before staining. Is there a way we can look at living cells instead? Transparent samples, that don't affect the amplitude of light rays, diffract it. But they also modify another parameter imperceptible to our eye, and called its phase. The Dutch mathematician and physicist, Frits Zernike, discovered a method to convert these phase changes into contrasts in intensity. Using a special disk and a phase plate, he separated and increased the phase difference between direct light and the light diffracted by a specimen. The subsequent interference of the separated light waves resulted in an amplitude contrast that is visible to the human eye.

Until recently, phase-contrast was a qualitative method to see cells and tissues non-invasively. Efforts are now focused on extracting quantitative information from the phase change, with scientists trying out several experimental approaches to achieve this. Pavan Kumar and his colleagues are currently dabbling with one such state-of-the-art technique, which could benefit both material science and biology. They have technology that can tailor a phase pattern into the light shone on the sample. The diffracted light, whose phase is modified by the sample, is compared with a reference beam by interference. The phase difference information between the two beams of light is then extracted. A very accurate image of the sample can be constructed using this information, with details of cellular structures and motions on a nanometer scale. "Among other things, this is a method of label-free imaging, which has great advantages in biology," says Kumar.

Foldscope

Microscopes don't come cheap. Or they didn't, until now. A paper microscope called the Foldscope (https://indiabioscience.org/columns/indiabioscience-blog/foldscope-events-in-india-the-delhi-photoblog), developed by Manu Prakash, a biophysicist at Stanford University, is set to change the status quo. This origami-based microscope, which can be printed and assembled from a sheet of paper, costs about a hundred rupees. Yet, it provides a magnification of over 2000X, weighs less than a 1 rupee coin, and doesn't need an external power source to work[6].

What is phase?

Waves are defined by several properties. It is easy to understand some of these properties by generating a wave on a long string of rope tied at one end. The amplitude is simply the height of the wave - wiggling the string harder makes waves of larger amplitude. Wiggling it faster, on the other hand, increases the frequency of the wave. Phase is another property of a wave, but it cannot be perceived easily. Perhaps the easiest way of understanding it this - when the crests and troughs of two waves are lined up, they are said to have the same phase. If they don't line up, the distance ('theta', measured in angles) between the two crests is the phase difference between the two waves. In a sense, the phase of a wave defines its starting point.

When light passes through any object, its phase changes; some objects alter this phase (or delay the light, in a manner of speaking) more than others. Very cleverly taking advantage of this, phase contrast imaging techniques allow us to look more clearly at biological samples that are transparent to light, or very similar to the background.

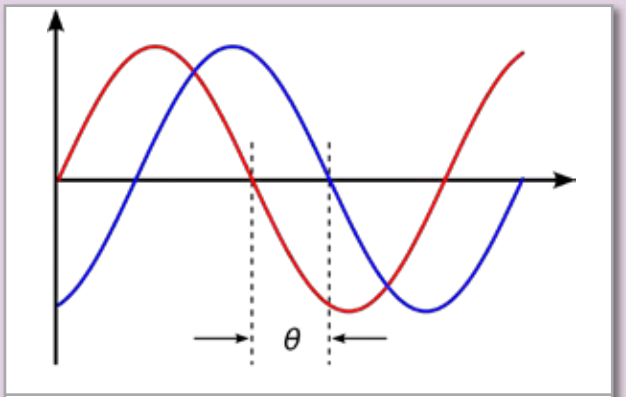

Figure 4. Two sine waves offset from each other by a phase shift. Source: Peppergrower (own work), Wikimedia Commons. URL: https://en.wikipedia.org/wiki/Phase_(waves)#/media/File:Phase_shift.svg. License: CC-BY-SA.

The on-going quest for nano-scale clarity has many takers - maybe not among drapers anymore; but, certainly from chemists, physicists and engineers, among others. Their efforts will go a long way in furthering our understanding of the wonderful mechanisms of biology, and hopefully, give us ways to correct those that go wrong. Biology, however, is not merely a muse for innovators. After all, it has been experimenting with the nano-world eons longer than us and has learnt a trick or two, which we can only hope to mimic.

Additional readings/resources

1. Microscopy Society of America. Web. 10 January 2016. http://www.microscopy.org/.
2. Chapter 10.11: Microscopes. "Fundamentals of Optics", Jenkins & White. McGraw-Hill International Edition.
3. Cells Alive! Web. 10 January 2016. http://www.cellsalive.com/
4. Microscopedia. Web. 10 January 2016. http://www.microscopedia.com/Resource/Application/22?ccgid=4
5. AAAS ScienceNetLinks "Pond 2: Life in a drop of pond water". Web. 10 January 2016. http://sciencenetlinks.com/lessons/pond-2-life-in-a-drop-of-pond-water/

References

1. Wikipedia: Microscopy. Web. 10 January 2016. https://en.wikipedia.org/wiki/Microscope
2. Microscopes for schools. Web. 10 January 2016. http://www2.mrc-lmb.cam.ac.uk/microscopes4schools/yoghurt.php
3. Wikipedia: Antoine van Leeuwenhoek. Web. 10 January 2016. https://en.wikipedia.org/wiki/Antonie_van_Leeuwenhoek
4. Gizmodo "How 17th Century Artists Helped Make the Microscopic World Visible". Web. 10 January 2016. http://gizmodo.com/how-17th-century-artists-helped-make-the-microscopic-wo-1736249872
5. NIH National Institute of General Medical Sciences "Life: Magnified" Online. Web. 10 January 2016. http://irp.nih.gov/catalyst/v22i4/nih-microscopy-lights-up-dulles-airport
6. Foldscope: Microscopy for everyone. Web. 10 January 2016. http://www.foldscope.com/

Harini Barath is a science writer based in Bangalore. She writes and curates content for IndiaBioscience. Her background and research training are in the field of condensed matter physics. She can be reached at harini@indiabioscience.org