How Far Down The Rabbit Hole Can We Go?

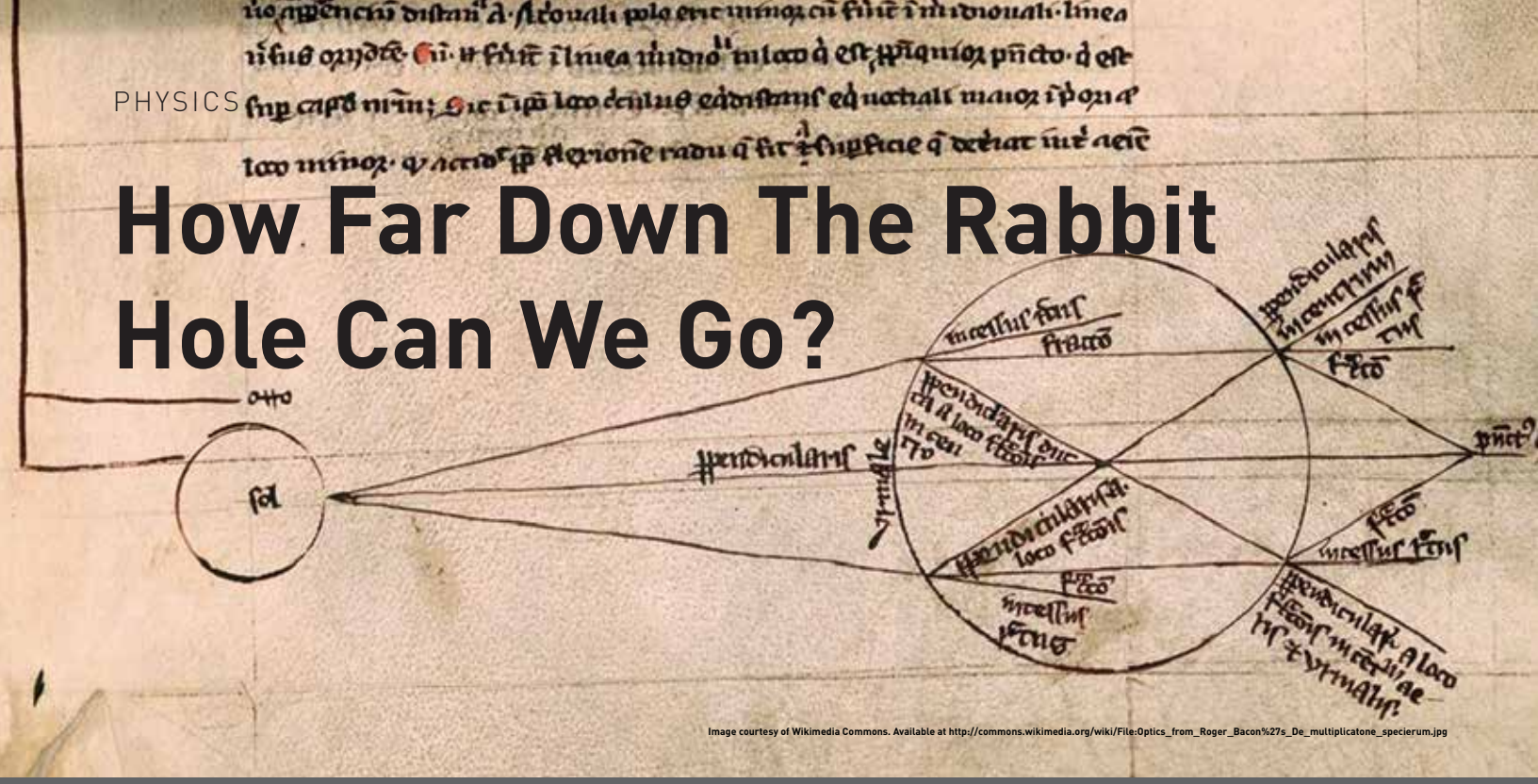

Image courtesy of Wikimedia Commons. Available at http://commons.wikimedia.org/wiki/File:Optics_from_Roger_Bacon%27s_De_multiplicatone_specierum.jpg

BY JAMES HOWE

Introduction

In 1665, Robert Hooke published *Micrographia*, one of the most important biological texts in history. In it, he made a description that provided the foundation for microbiology: "I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular…these pores, or cells… were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this" (1).

These were the first cells ever seen, only visible due to Hooke's invention of the compound microscope, which allowed him to the push the boundaries of the size of structures that could be visualized. Visual advances like this are necessary for the observation of many new phenomena. However, each medium is inherently limited by simple physics, and eventually new methods must be developed once current boundaries have been reached.

The Human Eye

The very first visual tools available to humans were the human eyes. Vision tends to be the primary sense humans use to interact with the world, and for good reason. Primate eyes have a relatively high density of cones, or cells in the retina that allow us to see fine details in color (2). Humans are also one of only a few trichromat primate species, possessing three different types of cones. This allows them to see a larger portion of the color spectrum than most other animals, who tend to be dichromats (3). The combination of high cone density and trichromacy allows humans to have one of the best senses of sight among all of M*ammalia*, with comparatively high fine detail (human eyes are able to discern details as small as 0.09 mm from a foot away) and color perception (3). However, as good as human vision is naturally, it still has lower boundaries above the level needed to resolve nature at the microscopic level.

To fully understand how optics work, it is necessary understand how magnification works in the human eye. The mammalian eye has a lens that focuses incoming light onto the back of the retina. The light is pinpointed onto a retinal surface with a very high density of cones, the fovea centralis, which is roughly 20 mm away from the lens (4). The focus is sharpest when the object being viewed is around 25 cm away, a position known as the nearpoint (4). Both of these distances become important in the magnification equation, where the magnification of an object is equal to the distance from the lens to the image divided by the lens to the object, or d_i/d_o (4). Using this equation, where d_i is 20 mm and d_o is 25 cm, the maximum proportion of the size of the image on the retina to the size of the object, or the magnification (m) is .08.

Figure 1: Roger Bacon's original notes on the magnifying glass. The small circle to the left is the object of interest, and the larger circle is the lens, with the rays of light between the two objects being bent by the lens.

Simple Magnification

The first device to increase visual acuity, the magnifying glass, was described by the philosopher Roger Bacon in 1268 (5). While the magnifying glass itself consists of one lens, it actually uses two: the glass itself and the lens in the viewer's eye. Magnifying glasses are fixed lenses, with only one possible level of magnification by the lens itself. The magnification is dependent on its index of refraction of the constituent material (how much it slows the light) and the curvature of the lens, with more convex lenses bending the light to a greater degree and focusing the rays closer to the lens (6).

The external lens magnifies images by focusing all of the light onto the lens of the eye, which is then projected onto the retina. When viewing images, the brain traces the path of the light rays back to their supposed origin (7). The bending of these rays causes the brain to perceive the source of these rays at the nearpoint to be larger than it actually is. The maximum magnification possible with the lens is limited mainly by its diameter, where the power of the lens (maximum ratio of the magnified image to the normal image, 4x is equal to 4 power) is limited to double the diameter of the lens in mm (8).

Compound Microscopy

Because magnification is directly dependent on the size of the lens, there is a practical limit to the magnification possible with only one lens. This led to the invention of the compound microscope in 1597 by Zacharias Janssen (9). In order to further magnify very small objects, he added another small lens, the objective lens, very close to the object. The objective lens is convex, and scatters the light coming from the object of interest. This creates a larger image on the surface just past the objective lens, called the intermediate image plane (7). The image is then magnified by another larger lens near the viewer, called the eyepiece. This lens acts like a magnifying glass on an already-enlarged image. The result is an even larger image at the nearpoint (7).

The compound microscope allows a very high level of magnification, as the maximum resolution is limited by neither the size of any of the lenses nor the angle the lens focuses light at, but by the light itself. At such high levels of magnification, the main limiting factor is the index of refraction of the medium, or the material used to view the specimen (7). As the index of refraction increases, so does the deviation of rays of light from the original path, allowing for less loss of light (10). Air, with its relatively low index of refraction, can be replaced by oil, which has a higher index, increasing the fidelity of the image being projected (10). With oil as the medium, very powerful compound microscopes can resolve structures down to 150 nm away from each other, a limit imposed by the wavelength of light itself (11).

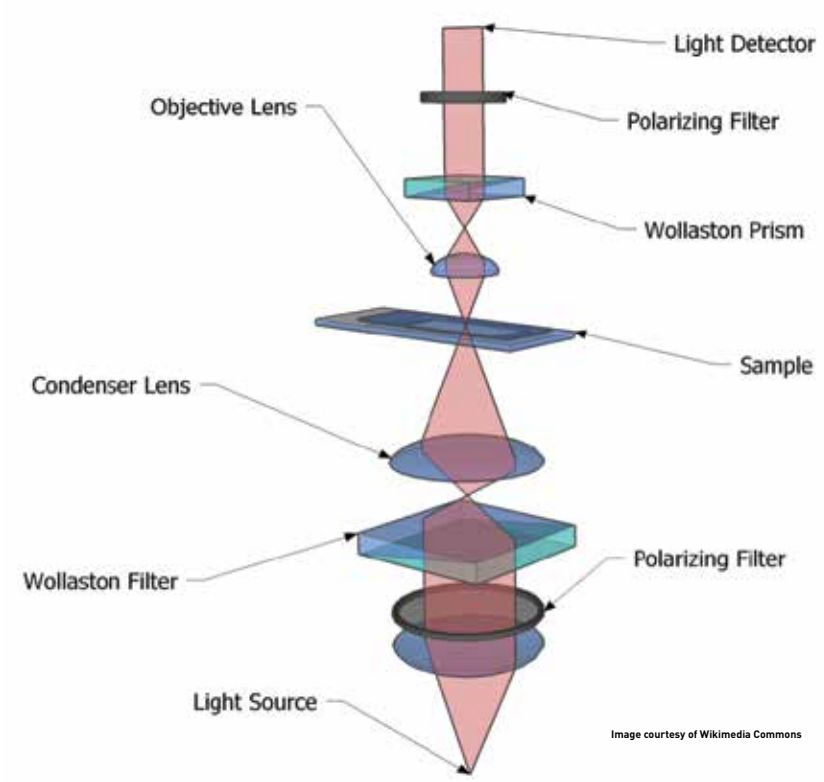

Figure 2: Diagram showing how a compound microscope functions. A number of filters can be used to increase the clarity and/or contrast of the image.

Electron Microscopy

In order to bypass this physical limit, two German engineers invented the electron microscope in 1931 (12). Instead of using glass to bend light, its lenses use magnets to focus electrons. These electrons are emitted by a heated filament, and their trajectories are bent by the magnets towards the specimen (12). When the electrons bounce off of the specimen at different angles, they come into contact with a sensor that measures the angles to visualize the image (12).

Using electrons to visualize the image allows the detrimental effect of high wavelengths, the ultimate limit of resolution in light microscopes, to be minimized. Unlike light, an electron has mass. Mass and wavelength are inversely proportional, so any particle with mass has a much lower wavelength than massless light waves, and thus much higher maximum resolution (13). Electron microscopes can magnify objects up to two million times larger, allowing them to resolve distances of up to .05 nm (50 pm), which is small enough to distinguish

individual atoms (14). Increasingly for many fields, the limiting factor for resolution is not based on the microscope, but rather on small imperfections in the specimen being imaged, causing slight distortions in the image (15).

The electron microscope has such high resolution that more recent microscopy techniques, such as atomic force microscopy and scanning tunneling microscopy (both of which use probes to detect the forces of individual atoms) are unable to significantly improve on the images produced (16). As of now, electron microscopy remains one of the highest-resolution tools known. Additionally, given the degree of conceptual resolution already available, such as the visualization of individual atoms and covalent bonds, it is becoming increasingly unclear whether further developments in microscopy techniques will be able to resolve higher magnifications.

The Smallest Measurement Ever Made

Early in 2014, a group of researchers at UC Berkeley managed to record a force of 1 x 10^{-24} newtons by adding very small amounts of force to a cloud of ultracold (just above absolute zero) atoms (17). This amount of force is not only incredibly small, but also very close to the theoretical limit of any measurement, known as the quantum limit. At such low levels of force, feedback is limited by quantum effects, specifically the Heisenberg uncertainty principle. The uncertainty principle describes the inherent imprecision in all measurements, which is caused by randomness found in all matter at infinitesimally small scales (17). At the quantum limit, a measurement becomes so small that it cannot be distinguished from the noise produced by the sensor simply existing (17).

If scientists can make measurements of force that approach limits set by the universe itself, then it should be possible to take an even closer look at matter. When resolving images at the atomic scale, we are not yet limited by quantum physics, but by the current scale of engineering. The current level of scientific growth, coupled with the efforts of engineering, suggests that we could soon be taking ever closer views of nature. D

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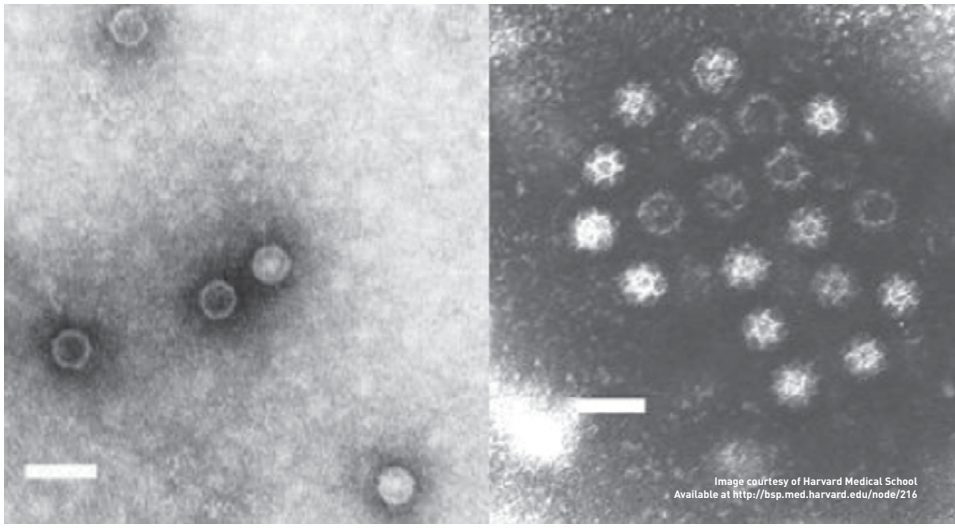

Figure 3: Electron microscopy images of Poliovirus (left) and Calicivirus (right). Many important biological phenomena, like viruses and proteins, are only able to be visualized at a scale requiring an electron microscope. The white bar for scale is 50 nm.

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