Activity 1: Microscopy Basics

In this first activity, you will learn the core principles of microscopy – most importantly, how an image is formed within a microscope. In the spirit of the title of this resourse, there will be many illustrations and videos, which will hopefully make the concepts more intuitive.

How does a microscope work?

Lenses

Fundamentally, microscopes are just a set of lenses, arranged in a specific way. The properties of these lenses are what determines the magnification and performance of the microscope. Therefore, the first step necessary to understand microscopes is to understand lenses. For the topics covered in this resource, it is enough to know the basics of ray optics, such as how the light is refracted within lenses, what is the **focal point** of a lens, and how an image is **transformed** by a lens. A great refresher on these topics can be found here.

The lens system of a compound microscope

The size of and the distance to the objects that one wishes to study dictate the design of the optical system. Cells, the objects that life scientists are mostly interested in, are generally about 10-20 µm in size for eukaryotes, while prokaryotic cells tend to be about 1 µm. The naked human eye can discern objects about 0.1 mm in size, which means that a magnification of at least 10 times is required to just about see eukaryotic cells. At a magnification of about 40×, eukaryotic cells can be seen quite clearly, and larger bacteria are just visible. To distinguish details within cells, a magnification of 600× or 1000× is typically used for eukaryotes or prokaryotes, respectively.

It is possible to have a single lens with magnifying power of 100 times; however, using such a lens as a magnifying glass would be highly impractical, and achieving much higher magnification would probably be impossible. However, combining lenses into a system with a shared main optical axis (so-called "optical train") makes this task much easier. This is the working principle of the **compound microscope**. Watch this wonderful explanation of how this system of lenses works, and answer the questions below.

Question 1 Why would it be impractical to use a single lens to magnify small objects >100 times?[1]

[1]This question is not easy! Consider the near point of human eye, and the focal distance and the thickness of such a lens.

Task 1 Draw the optical train of the simplest compound microscope. Make sure you understand how the image is formed. Play around with the positions of the lenses and the object and convince yourself that this design is indeed the best way to obtain magnified images of small objects.

The anatomy of a microscope

A real-world microscope is of course a little bit more than a pair of lenses. This video shows the main parts of a simple upright microscope. Please note that microscope designs vary a lot (for example, some microscopes have the objective below rather than above the stage, and are thus called inverted), therefore this tutorial is only a basic guide.

Task 2 Print[2] or draw a schematic of a microscope and label the parts mentioned in the video and their function.

Illumination and contrast

Contrast

Let us now consider the first of the two defining features of an image – contrast. Contrast is the **difference in brightness that makes an object distinguishable from background** (Figure 1). More formally, it can be defined[3] as $\frac{|Brightness\ of\ object - Brightness\ of\ background|}{Brightness\ of\ background}$. The human eye can usually detect an object if the contrast is only a few percent.

Figure 1: Example of a high- (left) and low-contrast images (right). Original image by Calsidyrose (Flickr).

[2] This is quite a nice one.

[3] This is not the only formal definition of contrast, there are other (generally more complex) variations.

Condenser lens

Most light microscopes use a bright light source and a so-called condenser lens that focusses the light onto the sample, as illustrated in Figure 2. Have a good look at the schematic and answer the following questions.

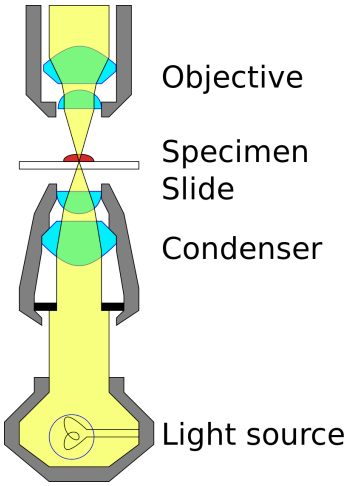


Figure 2: Schematic of the light originating from the light course passing through the microscope's optical path. Image by Egmason (Wikimedia Commons).

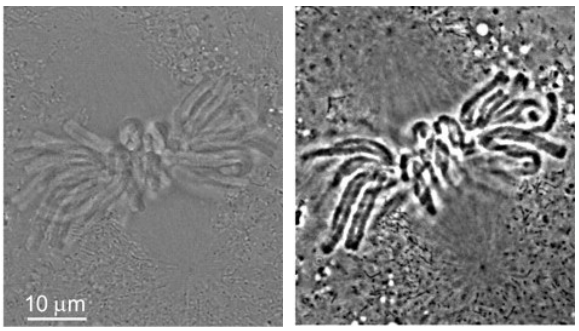
Question 2 Upon increasing the magnification, how does the intensity of light change?

Question 3 What is the function of the condenser lens in a microscope?

Modes of illumination

In the context of microscopy, contrast is generated by the sample interacting with the illuminating light. For white light, these interactions include **absorption** and **scattering**. The simplest mode of illumination and contrast generation involves collecting all the light passing through the sample (Figure 2). The most intensely absorbing elements of the sample then appear dark on the light backround of uniform illumination (Figure 3a). This modality is called **bright-field**, and it is used very widely. However, the contrast in this case is usually low, because most biological specimens do not absorb much light.

Contrast-enhancing techniques enable scientists to obtain micrographs with better contrast and therefore see cells in greater detail. There is a large variety of these techniques; in this Activity, we will have a brief look at **dark-field** and **phase contrast** microscopy (Figure 3b). Both of them rely on the sample scattering the light rather than absorbing it. In dark-field microscopy, the central part of the light cone illuminating the sample is blocked, such that no directly passing light reaches the objective. However, the angle of the light scattered by the sample changes, and some of it is collected by the objective. Phase-contrast microscopy makes use of



(a) (b)

Figure 3: The mitotic spindle imaged using bright-field (left) and phase contrast (right) microscopy. Images from Rieder and Khodjakov (2003).

the phase shift that is introduced upon light scattering (the wave nature of light will be described in the next section). Watch this video that shows in more detail how these techniques work. The Wikipedia page for Phase contrast also has a very good explanation of its working principle.

Magnification and resolution

We can hardly distinguish the texture or even the shape of an object placed far away from us. When we come closer to it, its image occupies a larger space on our retina. As a result, we perceive it enlarged, and this allows us to examine it in more detail. Therefore, naturally we expect that upon magnifying something further and further, we will be able to see it in increasingly finer detail. However, this is not always the case. In this section we will look at the concept of resolution and the fundamental laws of physics that is limited by.

Resolution

Resolution is the **ability to discern closely-situated objects**; in other words, the smaller the distance at which two objects are distinguishable from each other, the higher the resolution of an image. Take a look at this blog post featuring Jackson, the honorary dog of science, for a graphical illustration!

The resolution limit of light microcopy

In the case of digital images, such as the pictures of Jackson, the resolution is determined by pixel size. However, why does a resolution limit exist for real, non-digital objects? The answer lies in the nature of light itself. On our everyday level, light behaves as a stream of particles, and it can be described by the straightforward rules of geometrical optics. However, already in the 17th century a Dutch physicist Christiaan Huygens proposed that **light** must be a **wave**, and was right.

Two superb introductions into **wave optics** are given in the "Diffraction, Interference and Doppler Effect" section of the AK Lectures resource, or this playlist on

the FloatHeadPhysics YouTube channel. In this video I explain how these concepts apply to microscopy and why the **resolution limit** d equals to

$$d = 1.22\frac{\lambda}{2 * NA} = 1.22\frac{\lambda}{2nsin\theta}\,, \tag{1}$$

where λ is the wavelength of light, NA is numerical aperture, n is the refractive index of the medium between the sample and the objective, and θ is the angular radius of the objective.

Task 3 Calculate the highest resolution achievable with red light of 640 nm, using an objective which has an angular radius of 80° and air interface with the specimen.

Question 4 How could you increase the resolution of a microscope at a given wavelength?[4]

References

Rieder, Conly L., and Alexey Khodjakov. 2003. "Mitosis Through the Microscope: Advances in Seeing Inside Live Dividing Cells". *Science* 300 (5616): 91–96. doi:10.1126/science.1082177. eprint: https://science.sciencemag.org/content/300/5616/91.full.pdf.

[4]Tough question again! Look at the equation carefully and see which physical variables can be changed to make d smaller.