8.4
Looking through a microscope

8.4. Looking through a microscope

Overview

Most science teachers would have used a microscope to observe a variety of biological specimens. However, while teaching students to use a microscope, we become aware of many difficulties that students face, for example, while adjusting the light, preparing the slide, focusing, etc. This unit is designed to address some of these difficulties and to help students learn to use a microscope better, using simple samples from their surroundings.

Unit-specific objectives

In addition to the broader objectives discussed in the General Introduction, this Learning Unit is designed to enable students to learn/do the following:

- To handle a microscope and to take care of it
- To understand the parts of a microscope and their functions
- To calculate the total magnification of a microscope

Also, by using the microscope first-hand, students are expected

- To note that the image formed under the microscope is inverted,
- To observe the minute details of specimens while drawing images observed under the microscope.

Minimum time required

Two and half hours, or two sessions of 80 minutes each

Type of Learning Unit

Laboratory

Links to curriculum

NCERT science textbooks from Class 7 to 10 include topics relevant to the use and understanding of microscopes. The reference chapters are listed below.

Class 7	Class 8	Class 9	Class 10
Chapter 1: Nutrition in plants	**Chapter 2:** Microorganisms: Friends & foe	**Chapter 7:** Diversity in living organisms	**Chapter 6:** Life processes
Chapter 12: Reproduction in plants	**Chapter 8:** Cell: Structure and function	**Chapter 9:** Tissue	**Chapter 8:** How do organisms reproduce
Chapter 15: Light	**Chapter 16:** Light		

Table T1

Introduction

Our eyes enable us to see the things in our surroundings. But, there are limitations to our vision. For example, we cannot see the things that are too far and too near. Also, we are unable to see things which are too small or too close to each other such as microorganisms. To see such small things, people use a lens or a combination of lenses. A magnifying glass (a hand lens) is a single convex lens that enlarges the image of an object. A microscope is an assembly or an arrangement of two or more lenses that enlarges the image even more.

Form groups of two or three with your classmates, and do the following tasks.

Before introducing the microscope to the students, use a magnifying glass to demonstrate how it shows an enlarged image of a small object.

Task 1: Let us try this...

You may have used a magnifying lens to view small objects. A magnifying lens helps to make small objects look bigger.

Materials

Two magnifying lenses per group

Take a magnifying lens and observe the following text.

This is how a single lens magnifies.

Let's see what happens when we use two lenses.

Take another magnifying lens. Keep the first lens above the following text at the same height from which you observed before. Hold a second magnifying lens above the first and move the second lens in such a way that you can read the following words.

Now remove one magnifying glass and observe.

An assembly of two magnifying lenses forms the basis of what is known as the microscope. In this unit, we will learn about different parts of a microscope and how to make the best use of these.

Task 2: Parts of a microscope

With the help of figure 1, identify the different parts of your microscope.

The eyepiece typically magnifies the image of an object upto 10 times its original size. This is known as the magnification of this lens, and is indicated by the number '10X' written on its rim or the cylindrical part. Each lens of a microscope has its specific magnification.

Materials

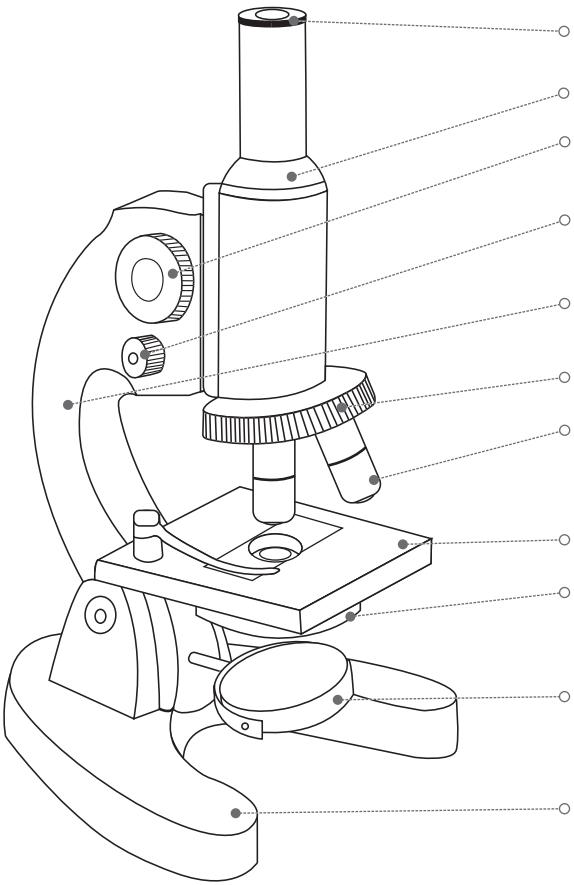
Compound microscope

Q1. What is the magnification of each objective lens of your microscope?

_ _

When we shift from a 10X objective lens to a lens of higher magnification, we are able to observe finer details of the specimen.

Another number – 0.25 n.a. (numerical aperture) – is also written on the rim of the objective lens. A lens with a larger numerical aperture allows for finer details to be resolved than a lens with a smaller numerical aperture. This discussion is beyond the scope of Class 8 students.



Eyepiece

Body tube

Coarse focus. Move this large knob to quickly move the object/specimen closer to the objective, and bring it into focus

Fine focus. Move this smaller knob to slowly move the objective lens by a small vertical distance.

Arm. Hold and lift the microscope and change the angle of the stage using this.

Nosepiece. Rotate this to select the appropriate objective lens.

Objective lenses or the objectives (lens closest to the object, two to four lenses attached to the nosepiece). Select a suitable objective lens to achieve the required magnification.

Stage. Place the slide here and hold it with the stage clips.

Condenser lens. Use this to focus the light from the mirror below on to the object. Open or close its **diaphragm** to control the amount of light reaching the condenser.

Mirror. Use this to reflect light from front/surroundings towards the object above. Orient it towards the light sources present around so that the mirror captures maximum light.

Base. Support this with one hand while lifting the microscope's arm with the other hand to carry it around safely.

Figure 1 *Parts of a microscope*

Q2. When we use two lenses i.e. an eyepiece (10X) and an objective lens (10X), each lens enlarges the image by 10 times. Can you find out how large the final image looks, if the object is 0.1 mm long?

_ _

Q3. Rotate the mirror and examine the two mirror surfaces. What difference do you see between the two mirror surfaces?

_ _

What is magnification?

Magnification is a measure of the capacity of a lens or other optical instruments to make things appear larger than their actual size. It is the ratio of image size to object size.

Total magnification is the product of the magnifying power of the eyepiece lens and the magnifying power of the objective lens.

This number gives an idea about the total enlargement of the image of an object.

Total magnification of a microscope (M) can be calculated using the following formula:

Total magnification (M) = Objective magnification × eyepiece magnification

For example: The assembly of the eyepiece and the objective lens, each having a magnification of 10, will make the specimen look 100 times bigger than it is.

The microscope mirror is a set of two mirrors fixed back to back in a frame. One of them is a plane mirror and the other is a slightly curved (concave) one. The plane mirror works better for a distant source of light, such as sunlight. The concave mirror works better for a nearby light source, such as a tube-light in the room.

Rotate the circular disc (nosepiece) till the 10X objective lens is vertically below the body tube. When it is set in this position, you hear a 'click' sound.

Open the diaphragm completely with the help of the lever attached to it.

Orient the microscope towards the light source such that the mirror captures maximum light. Now, look through the eyepiece and rotate the mirror such that you achieve maximum illumination.

Best practices while handling a microscope

i. Before observing the specimen, wipe the lenses, the mirror, and the stage of the microscope clean. For the stage and the mirror, use a tissue or a cloth. However, for lenses, use only a dry, soft paintbrush/muslin or silk cloth/lint-free paper tissue. Move the cloth or tissue in a gentle, circular swiping motion, rather than rubbing.
ii. Align the objective by holding the nosepiece and rotating it. The nosepiece should not be rotated by holding the objectives.
iii. While rotating the nosepiece, keep some distance between the stage and the objective. The objectives should not touch the stage.
iv. A microscope should always be kept covered when not in use.

Task 3: Did you ever wonder how things will appear under a microscope?

We have seen the lines drawn on paper using a pen or a pencil. How do these look? Smooth, coloured, and sometimes shiny? Let us imagine for a moment that we are as small as ants, and can walk over these lines. How will they appear to us then?

We cannot become as small as ants, but we can see the lines at that scale.

The teacher can prompt a discussion about whether a pen or pencil line can be observed through the microscope. Ask the students to predict how the lines will appear under the microscope.

Materials

For each group: 2 glass slides, 2 pieces of paper (approximately 2 cm × 2 cm), ball-point pen, pencil, transparent adhesive tape etc.

Procedure

i. On a piece of paper, draw two lines, one with a pencil, and another with a ball-point pen.
ii. Fix the paper on a slide with an adhesive tape or hold it between two slides. Put the slide/s on the microscope stage,

keeping the pencil line below the objective lens (use the stage clips, if available).

iii. Bring the objective lens (10X) very close to the slide with the help of the coarse focus knob. The objective lens should not touch the slide.

iv. Bring your head at the level of the stage and check if the pencil line to be seen is vertically below the tip of the lens. If not, then bring it below the lens by moving the slide. Now do the same by looking horizontally along the other perpendicular direction (See Figure 2).

v. We will observe the lines in the reflected light, hence close the diaphragm below the stage. Look through the eyepiece and move the objective lens in the upward direction using the coarse focus knob until you can see an image of the line. If the light is not sufficient, shine some light on the upper surface of the paper, using a torch.

vi. Once the pencil line is visible and close to focus, rotate the fine focus knob to sharpen the image.

vii. Use the same procedure to observe the ball-point pen line.

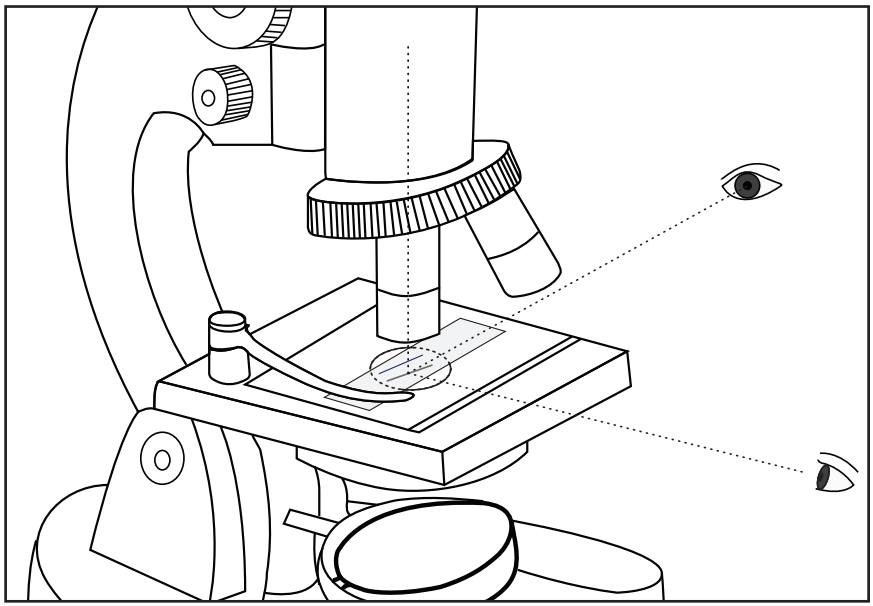


Figure 2 *Positioning the specimen under the lens*

What does it mean 'to focus'?

Focusing means moving the objective lens closer to or further away from the specimen to form a sharp image.

Q1. How do the pencil-line and the pen-line appear under the microscope? Describe your observations in your own words. Would you also like to sketch it?

_ _

_ _

_ _

If the students appear confused about how to describe their observations, ask for similarities and differences between the pen-line and the pencil-line, and the probable reasons for the differences.

The manner in which the ink spreads when a line is drawn with a pen depends on the texture of the paper. The ink being liquid, the paper is uniformly coloured and darker at the cross-fibres of the paper. The pencil lead is made of graphite and clay binders, and it appears as scattered marks on the paper. If you shine the torch light from above, the graphite reflects the light and appears shiny.

Q2. For each objective lens, there is an approximate lens-to-object/specimen distance around which it gives the best/ sharpest image. Let us try to estimate this distance while the object (line/s) is in focus.

It may not be possible to measure the distance between the slide and the objective lens using a scale. Think of other ways in which you could estimate this.

The size of objective lens cavities is so designed that the distance between body tube and stage remains approximately same for all the lenses.

However, the distance between object and objective is smaller for the objectives of higher magnification. To ensure this, the students can come up with techniques of using thread or paper to measure the distance between the slide and the objective lens.

Using these methods, estimate the distance between the objective and the slide.

	Pencil-line	Pen-line
Distance between the slide and the tip of the objective lens	_____cm/	_____cm/
	_____mm	_____mm

Table 1 *Distance between the slide and the objective lens*

Task 4: Looking at the letters 's' and 'e'!

In this task, we will look at printed letters in a newspaper under the microscope. This activity requires newspaper cuttings. Keep these ready at the beginning of the task.

Cut a small piece of printed newspaper that has the letters 's' and 'e'.

Stick this newspaper piece on a slide as done in the previous task, and observe it under the 10X objective lens.

The back of the newspaper cutting should not have a dark or coloured background because it can interfere with the observations. Make sure that the students observe the letters carefully. When students observe the letter 's' under the microscope, the image formed will be inverted but appear straight due to symmetry in the letter. In case of the letter 'e', they will see an inverted image. The image formed under the microscope is always a virtual and inverted image.

Materials

(For each group) 2 glass slides, a newspaper cutting that has letters 'e' and 's', transparent adhesive tape. The letters need to be in small (regular) font, not from headlines that are printed in large and bold.

Draw the observed images in the following circles.

(Note: The circle is the field of view that you see through the microscope. Compare the size of the image that you saw to the size of the field of view, and try to draw it just as you observed under the microscope.)

Magnification______X

Magnification______X

Q1. Do the letters ‘s’ and ‘e’ appear different in any way from the way they appear without the microscope (besides appearing bigger)?

How does light travel inside a microscope?

This task helps students understand the concepts of magnification as well as inversion of image. However, at this stage (Class 8), the teacher need not explain this ray diagram to students. If some students are curious about why an inverted image is formed or why the magnification of the two lenses is multiplied, then the teacher may use this diagram and explain.

The light at the object/sample (shown as the arrow AB in the diagram) comes either from the mirror at the bottom of the microscope or will fall on the paper from above (background light or torch light). Some of this light is absorbed/ reflected by the object. The transmitted and reflected light from the object reaches the eyepiece and then our eyes, helping us see the image of the object.

The objective lens focuses light from the specimen to produce its larger, real and inverted image (intermediate image A'B'). This is the primary image.

The rays from the image then pass through the eyepiece lens, which further magnifies the primary image, and forms a focused final virtual and inverted image (A"B"), seen by the eye.

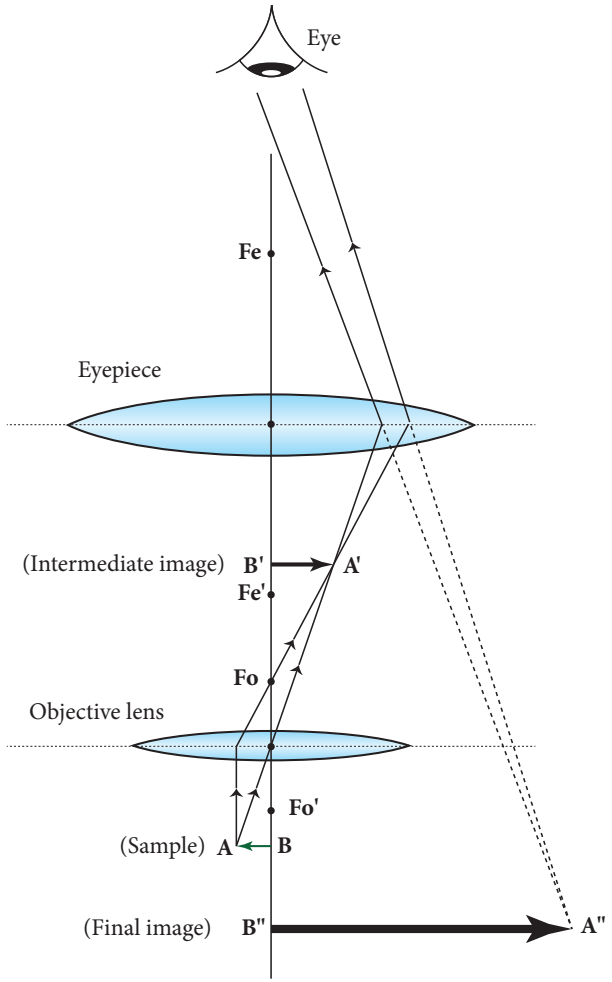


Figure 3 *Microscope ray diagram*

Task 5: Preparing slides for smaller samples

So far, we have learned to observe the surface of paper under reflected light, and to adjust the light of a microscope. When we want to look at the internal structure of small objects/samples, we need to use transmitted light coming from the mirror below. For this, it is important that the sample/object is thin to allow sufficient light to pass through. In this task, let us understand this process by observing a few other (smaller) specimens from our surroundings. Open the diaphragm, and orient the microscope and the mirrors to get sufficient light when observed through the eyepiece.

Materials

Slides, coverslips, salt, hibiscus flower (*gurhal* in Hindi, *jaswand* in Marathi), Baker's Yeast, onion, safranin stain (optional)

Procedure

i. Salt: Put a few granules of salt on a slide, and fix it below the objective (10X). Use coarse focus to bring salt particles in rough focus. Now use fine focus to observe different parts of the granules. You will notice that it will be difficult to focus on all the granules at the same time. By slightly varying fine focus, you will be able to focus on one horizontal section of granules at a time. The thickness of object/specimen which can be focussed on at a given time is known as depth of focus (for a given objective lens).

When using salt as a specimen, observe the slide within ten minutes, otherwise the salt will absorb water from the air, and its appearance will change.

ii. Now prepare one or more of following slides, which involve biological specimens. Water is added to these slides to prevent the shrinking of specimens due to drying, and a coverslip is placed on it.

A cover slip or cover glass is a very thin square piece of glass (or plastic) that is placed over the water layer containing a specimen. Because of surface tension, uncovered water drop on a surface forms a curved surface, which can deflect the path of light rays passing through it. With a cover slip in place, the water layer remains flat with even thickness between the coverslip and the slide surface. The cover slip also protects the objective lens from accidental smearing by the water drop. Cover slips also protect the specimens from contamination by airborne particles or other substances.

- Hibiscus pollen: Place a drop of water on a slide, dust some pollen grains from the flower, and place a coverslip over it. Alternately, we can dust some pollen grains on a transparent adhesive tape and stick it on a slide.
- Yeast cells: Add 2-4 beads of Baker's yeast in a small quantity of water and mix well. Take a drop of this water on a slide. Place a coverslip over it.
- Onion peel: Place a drop of water on a slide. Take a piece of the inner transparent skin of an onion leaf or an onion ring, and put it on the slide. Add a drop of dilute Safranin stain (if available) on it, and place a coverslip over the specimen.

Teachers may also dust some hibiscus pollen grains on a watch glass and add a few drops of water to it. From this watch glass, students may take a single drop for their slide.

The teacher can provide dilute Safranin solution (1 drop of stain in 2 mL of water) to students.

Staining technique is used to increase the contrast by changing the colour of some parts of the specimen structure. Using a stain will demonstrate to the students the importance of stains in microscopy.

Sometimes, at very high intensity of transmitted light, the image contrast may decrease. For good contrast, try to maintain optimum light intensity using the diaphragm.

iii. Observe the specimen under the 10X objective lens (as done in task 1), and draw what you observed in the first circle given below.

Next, observe it with the objective lens of 45X.

iv. Move the 10X objective lens slightly in the upward direction with the help of the coarse focus knob.

v. Rotate the circular disc in such a way that the 45X objective lens will set vertically below the body tube with a "click" sound.

vi. Using the coarse focus knob, bring the objective lens close to the slide.

vii. Slowly rotate the fine focus knob until you see the fine details of the object.

(Note: While changing the objective lens, the slide should not move.)

Draw what you observed in the second circle, and note down the magnification of the objective lens.

Specimen 1: ____

Magnification______X

Magnification______X

What happens when you zoom in on an image in a mobile phone camera? When you zoom in, you see the finer details of the image.

Q1. What happened when you changed the objective lens from 10X to 45X? What can you say about the distance between the slide and the tip of the objective lens?

____

____

____

If the object is not visible or in focus,

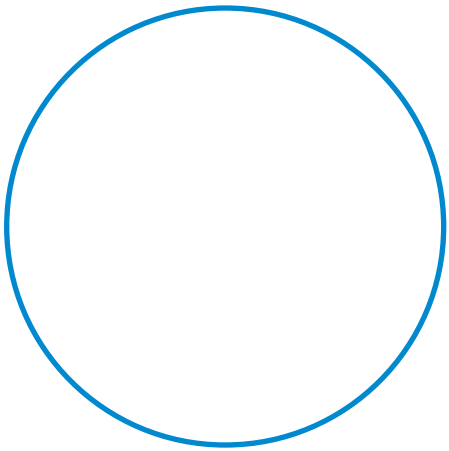
i. try moving the lens a little using the fine focus knob,
ii. if the light is less, adjust the diaphragm,
iii. and if the specimen is not within the field of view, try moving the slide.

When you change the magnification from a lower magnification to a higher magnification objective lens, try not to move the slide. While rotating the nosepiece without changing the focus, you may accidentally move the slide from its place.

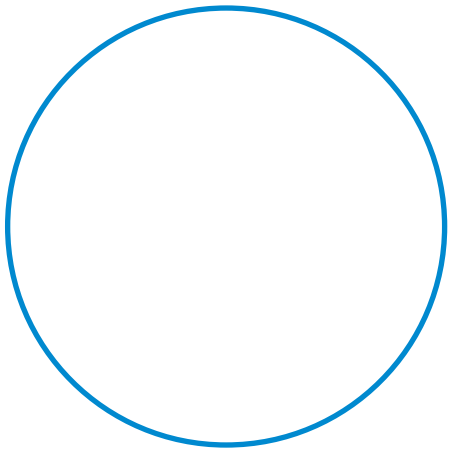
The distance between the objective lens and the slide reduces as the magnification of the objective lens increases. If you change the focus and try to adjust the focus using the coarse adjustment, the slide comes too close to the lens, crushing the specimen or sometimes the slide as well. Therefore, when you shift from lower to higher magnification use the fine adjustment to focus.

Repeat the procedure with the second specimen.

Specimen 2: _____



Magnification_____X



Magnification_____X

State whether True or False

1. Objects viewed under the microscope appear upside down (inverted). _____
2. Eyepiece is attached to the body tube and is closest to the specimen. _____
3. While working with a high magnification objective, we should use the coarse adjustment knob. _____
4. We use the diaphragm to adjust the amount of light entering the microscope. _____

Tick the correct answer

1. What is the correct way to hold the microscope when carrying it?

a) By the eyepiece

b) By the arm

c) By the stage

d) By the slide

2. A microscope is set to 10X eyepiece and 40X objective. What is the total magnification?

a) 140X

b) 410X

c) 400X

d) 100X

3. If we place a letter 'e' under the objective of a compound microscope and moved the slide to the left, in what direction would the 'e' appear to move?

a) To the left

b) To the right

Answers

State whether True or False

1. True
2. False; eyepiece is the topmost part of a microscope, which is closest to our eye.
3. False; while working with high magnification we should never use the coarse adjustment, it can break the slide.
4. True

Tick the correct answer

1. b)
2. c)
3. b)

Task 6: Estimating the size of a specimen

This task may be considered as a possible extension of this Learning Unit.

Materials

Transparent scale/ruler with a minimum division of 1 mm

A microscope is not only useful for observing small specimens but can also be used to estimate their sizes. To do so, we must first get an approximation of the diameter of the bright circle seen through the eyepiece. This bright circle is called the field of view.

The size of the field of view reduces with the higher magnification of objectives.

Procedure

Place the scale/ruler on the stage. Click the 10X objective lens into position. Rotate the coarse focus knob till one of the markings on the ruler is in focus. If you are able to observe at least one division of the scale/ruler then the diameter of field of view will be approximately 1 mm. If you can observe 2 divisions then the diameter will be approximately 2 mm.

1. In your microscope, the diameter of the bright circle (field of view) for 10X objective is _ _ _ _ _ mm. You cannot observe samples/objects bigger than this size completely using this set of lenses.

1 mm = 1000 micrometer

Therefore, diameter of the field of view is _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ micrometers.

Now look at your drawings of the pen/pencil lines observed in Task 3, and of the specimens observed in Task 5, to estimate their size.

Width of pen line _ _ _ _ _ _ _ _ _ _ _ _ Width of pencil line _ _ _ _ _ _ _ _ _ _ _ _

Size of specimen 1 particles _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Size of specimen 2 _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

For example, if you were looking at a specimen that took up half the field of view (for example, a diameter of 1300 micrometers), its length would be approximately 1/2 × 1300 micrometers = 650 micrometers. If a specimen appeared to be 1/5 the width of the field of view, you would estimate its width to be 1/5 × 1300 = 260 micrometers.

References

- Brown, M. T., Munn, M., & Tyler, L. (2007). *Amazing cells: A cell biology unit for grades 5 through 7.* Washington MESA and University of Washington, Genome Sciences Education Outreach. Retrieved from: https://gsoutreach.gs.washington.edu/files/amazingcellsbook.pdf
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Appendix for the teachers

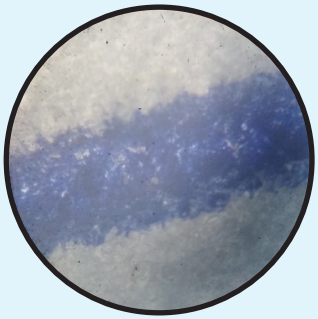

Figure 1 *Pen line under a 10X objective lens*

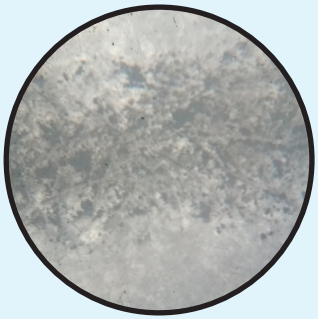

Figure 2 *Pencil line under a 10X objective lens*

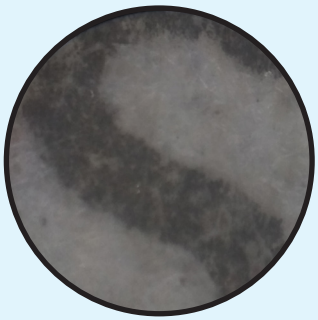

Figure 3 *Letter 's' under a 10X objective lens*

Figure 4 *Letter 'e' under a 10X objective lens*

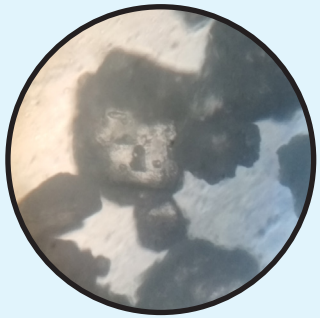

Figure 5 *Salt under a 10X objective lens*

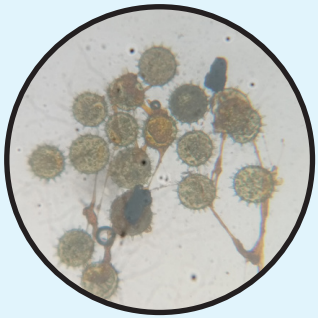

Figure 6 *Pollen grains under a 10X objective lens*

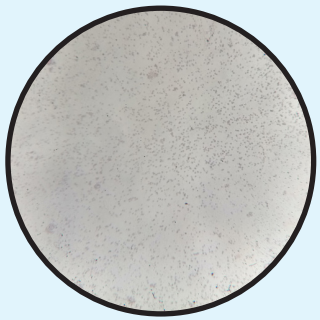

Figure 7 *Yeast cells under a 10X objective lens*

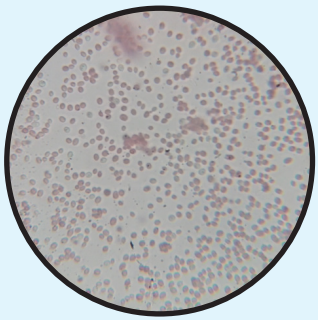

Figure 8 *Yeast cells under a 45X objective lens*

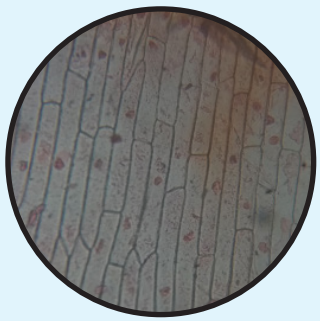

Figure 9 *Cells of spring onion under a 10X objective lens*

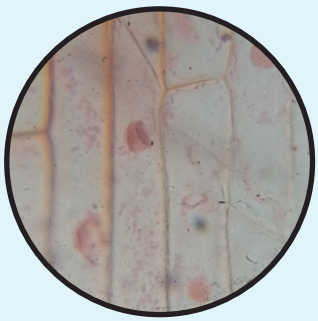

Figure 10 *Cells of spring onion under a 45X objective lens*

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