

LU 8.4 Looking through a microscope

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 students each, and conduct the following tasks.

Materials

Task 1: Two magnifying lenses per group.

Task 3: (For each group) 2 glass slides, 2 pieces of paper (approximately 2cm x 2cm), ball-point pen, pencil, transparent adhesive tape etc.

Task 4: (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.

Task 5: Slides, coverslips, salt, *Hibiscus* flower (Gurhal in Hindi, Jaswand in Marathi), Baker's Yeast, onion, safranin stain (optional).

Task 6: Transparent scale/ruler with a minimum division of 1mm.

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.

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.

1. 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.

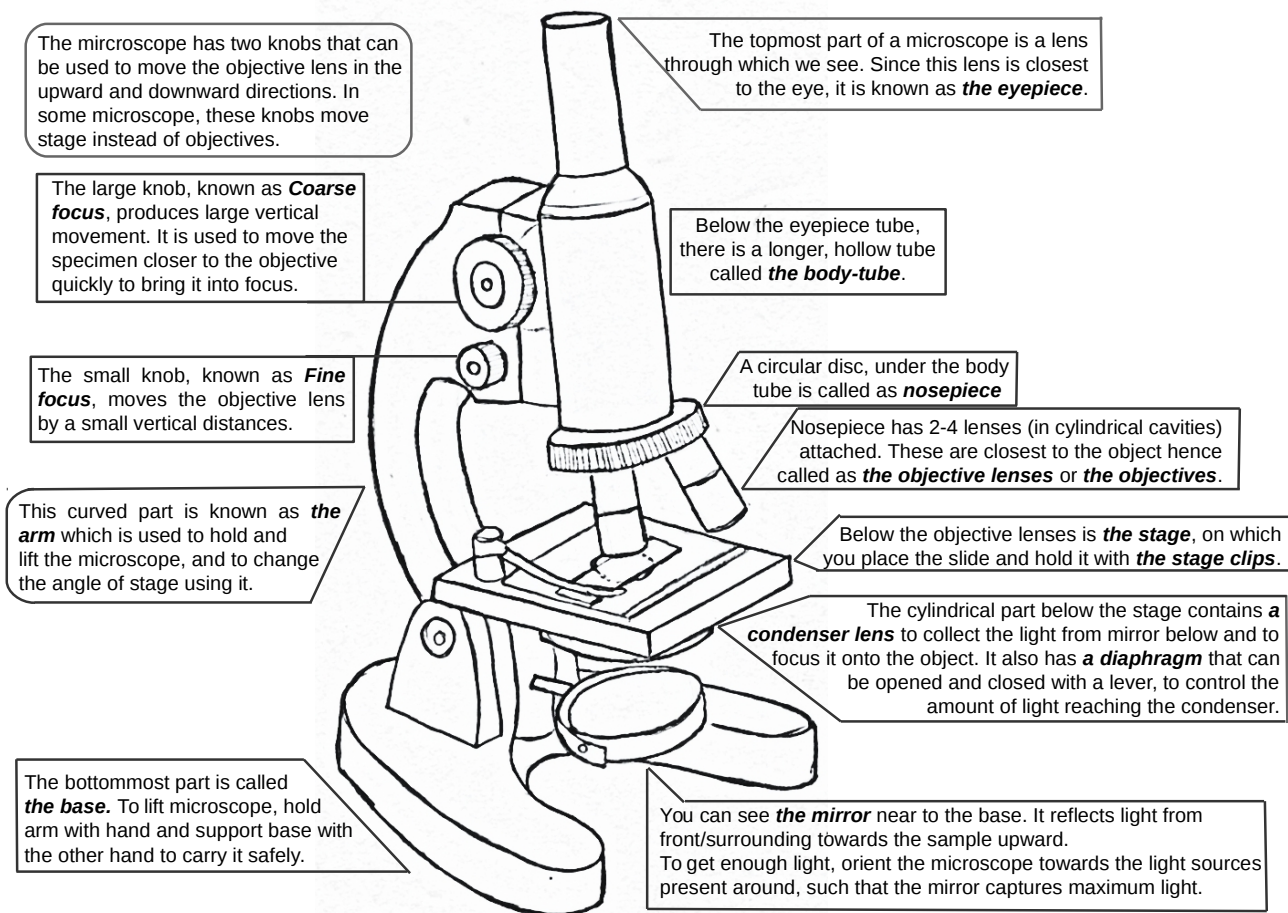


Figure 1: Parts of a microscope

2. 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?

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

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 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.

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.

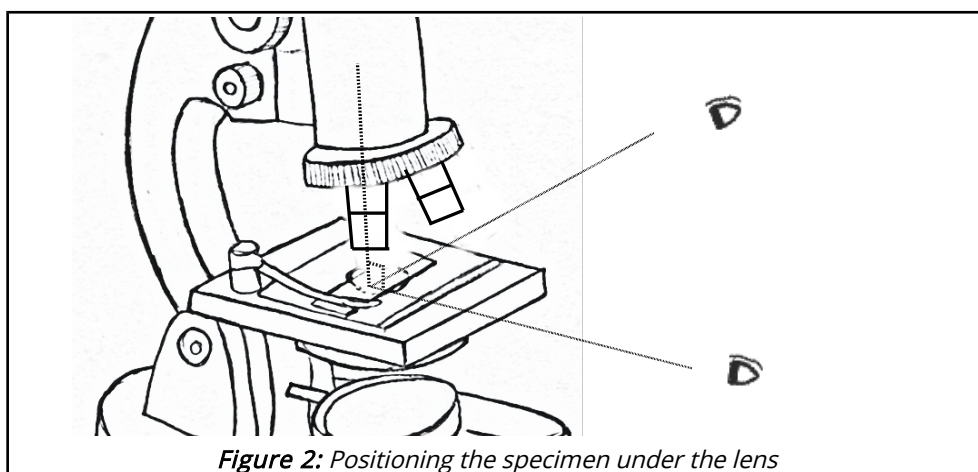


Figure 2: Positioning the specimen under the lens

1. 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?

2. 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.

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

Table 1: Distance between the slide and the objective lens

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

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

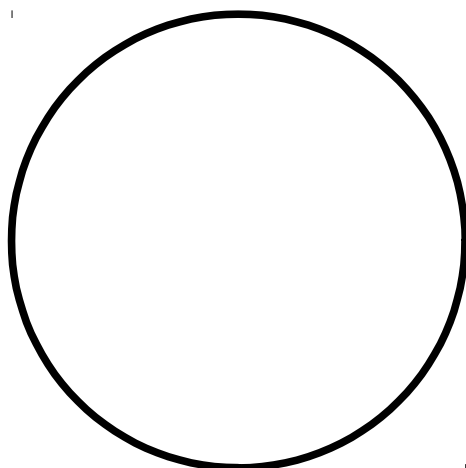
In this task, we will look at printed letters in a newspaper under the microscope. This activity requires newspaper cuttings. Keep these ready at the beginning of the task.

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

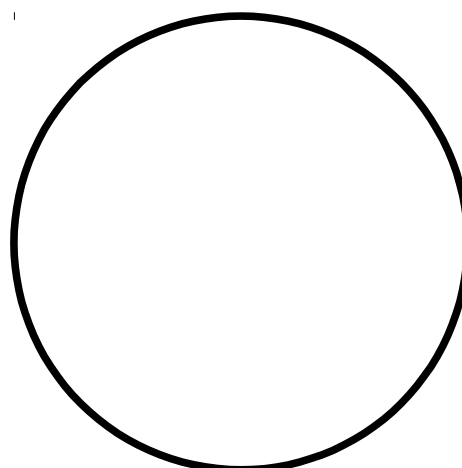
Stick this newspaper piece on a slide as done in the previous task, and observe it under the 10X objective lens.

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

1. Do the letters 's' and 'e' appear different in any way, from the way they appear without the

microscope (besides appearing bigger)?

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.

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).
- 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.
 - **Hibiscus pollens:** 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 water and mix well. Take a drop of 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.
- 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.

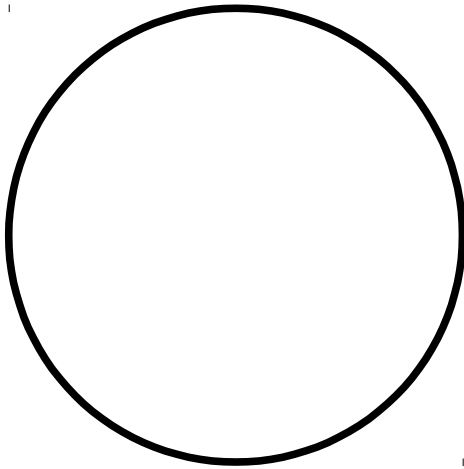
Next, observe it with the objective lens of 45X.

- 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.

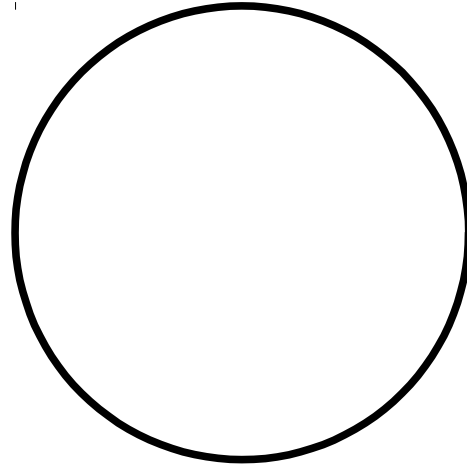
(Note: While changing the objective lens, the slide should not move.)

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

Specimen 1: _____



Magnification: _____X



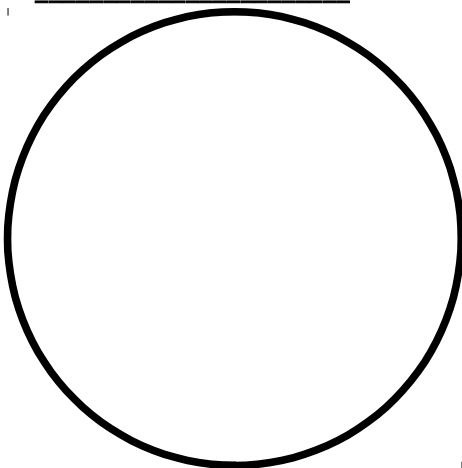
Magnification: _____X

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.

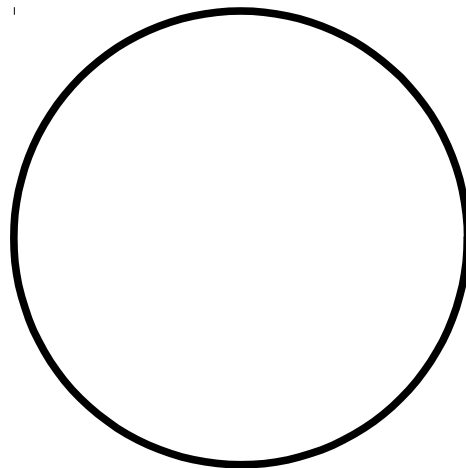
1. What happened when you changed the objective lens from 10X to 45X? What can you say about the distance between the slide and the tip of the objective lens?

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

Task 6: Estimating the size of a specimen

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.

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 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 $1/2 \times 1300$ 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 $1/5 \times 1300 = 260$ micrometers.

References

- Brown M. T. (2007), Amazing cells book: Washington MESA and University of Washington, Genome Sciences education outreach. Retrieved from: <https://gsoutreach.gs.washington.edu/files/amazingcellsbook.pdf>
- Deshmukh N. D., Agarkar S. C. (2010). All About Microscopes History, Use and Care. Homi Bhabha Centre For Science Education.

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