Microscopy

Overview

Most of us would have used a microscope for observing various specimen through it. However, when it comes to teaching microscopy to students,we also recall the problems that arise. E.g. in adjusting parts of the microscope, preparing specimen slide, focusing, etc. Students also ask questions like, how is the image formed under the microscope,? ”Why should the specimen be thin?”, etc. The terminology used for microscopy may appear difficult for students to understand. This unit has been designed in a way that students can learn these techniques and terminologies, while working with simple samples we use in our day-to-day life.

Minimum Time required: Two and a half hour or 2 sessions of 80 minutes

Type of Learning unit: Laboratory-based

Learning Objectives

In this learning unit, students will learn

(i) handling and taking care for a microscope,
(ii) the parts of a microscope and their functions
(iii) calculating the total magnification of a microscope.

Then, through first-hand use of a microscope, students will
(iv) learn that the image formed under the microscope is inverted, and
(v) gain the experience of observing minute details while drawing of image observed under the microscope.

Links to Curriculum

Topics relevant to the use and understanding of a microscope are across the NCERT science curriculum from class 7 to 10. The reference chapters are listed below;

Class 7	Class 8	Class 9	Class 10
Chapter 1: Nutrition in Plants **Chapter 12:** Reproduction in plants **Chapter 15:** Light	**Chapter 2**: Microorganisms: Friends & Foe **Chapter 8:** Cell: Structure and Function **Chapter 16:** Light	**Chapter 7:** Diversity in Living organisms **Chapter 9:** Tissue	**Chapter 6**: Life Processes **Chapter 8**: How do organisms reproduce

Introduction

We can see several things in our surroundings through the eyes. But there are limitations to our vision. For example, we can’t see things that are too far and too close to our eyes. Also, we are not able to distinguish between two points which are too close to each other and tiny living forms like microorganisms. For observing such small things, people used different types of lenses. Magnifying glasses is one such lens that helps to enlarge the image of things we observe under it.

Materials Required

- For Task 1: For each goup: 2 Glass slides, 2 pieces of paper (approximately 2cm x 2cm),

ball-point pen, pencil, adhesive tape etc.

- For Task 2: For each goup: Slides (two slides per group), a newspaper cuttings with letter ‘e’ and letter ‘s’, adhesive tape. The cuttings should have the letters in a smaller font. Do not take cuttings of headlines.
- For Task 3: Slides, coverslips, salt, *Hibiscus* flower (*Gurhal* in Hindi, Jaswand in Marathi), Baker’s *Yeast*, leaves of spring onion, safranin stain (optional).

Let us try this..

Hold two magnifying glasses one above the other in such a way that you can read the following words.

Now remove one magnifying glass and observe.

Does this change appear the same when we zoom in a image in mobile phones? When we zoom in an image, we see the finer details of the image.

Assembling two glasses (curved) one above the other forms the basis of a microscope. In this unit, we will learn about the microscope.

Parts of Microscope

The topmost part of the microscope is a lens through which we see. Since this lens is closest to the eye, it’s known as an eyepiece. Below it is a long hollow tube called body-tube.

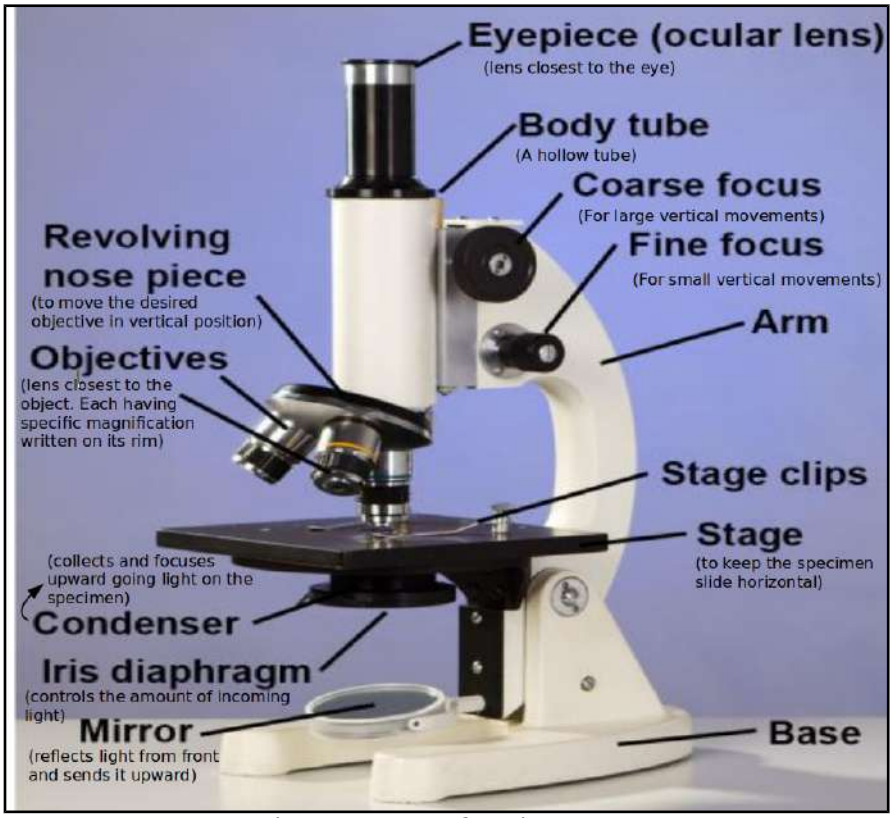


Figure 1: Parts of a microscope

Bottom of this tube has a circular disc, which has three lenses (set in cylindrical cavities) attached to it. These lenses are called the objective lenses (objectives).

Each lens of a microscope has a specific magnification (i.e., how many times the lens can enlarge the image of an object), written on its rim/ on the cylindrical part. Eyepiece typically magnifies an image by 10 times, which is written as ‘10X’ on its rim.

1. Can you find out the magnification of each objective lens on your microscope?

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

> On one of the objective lens, magnification is written as 10X. In addition, there is another number written as 0.25 n.a. - numerical aperture. This is a number that expresses the ability of a lens to resolve fine details in an object than can be observed in its image. A lens with a larger numerical aperture will be able to resolve finer details than a lens with a smaller numerical aperture.

2. When we are using two lenses i.e. eyepiece (10X) and an objective lens (10X), each of them enlarge the image by 10 times. Think how many times the image will be enlarged?

> **# What is magnification?**
> Magnification is a measure of the ability of a lens or other optical instruments to make things look larger than their actual size. It is the ratio of Image size to the object size.
> -*Total magnification* is the product of the eyepiece lens magnification and the objective lens magnification
>
> This number gives an idea about by how many times the specimen under observation will be enlarged. For example- if eyepiece and an objective lens, both have a 10X magnification, then it will enlarge the specimen by 100 times.
>
> -Total magnification of a microscope can be calculated using the following formula;
>
> Total magnification of (M) = Objective magnification (m_o) x eyepiece magnification (m_e)
> Where,
>
> $$m_o = \frac{Least\ distance\ of\ distinct\ vision (25\,cm)}{Focal\ lenght\ of\ objective\ lens} = \frac{D}{Fo}$$
>
> **(Also given on the cylindrical part of the objective lens. Written in a number followed by letter X.)*
>
> $$m_e = \frac{Length\ of\ the\ microsope\ tube}{Focal\ lenght\ of\ eyepiece} = \frac{L}{Fe}$$
>
> **(Given on the rim of an eyepiece)*

The body tube has two knobs – which move the tube (and thus objective lens) in an upward and downward direction. The large knob, known as coarse focus produces large vertical movement. The small knob, known as fine focus, moves objective lens by slight vertical distance. Coarse focus knob is used to quickly bring the specimen to be close to in focus. Once the specimen is close to in focus, the fine focus knob can be used to sharpen the image (or focus on different layers in the specimen).

Below the objective lenses, is the stage, on which we can place our slide and fix it with stage clips. Some stages also have two knobs to move the slide in left-right, and forward-backward directions.

Below the stage, a cylindrical part contains a condenser lens for collecting the light reflected by the mirror and focusing it on the specimen. The cylindrical part also has a diaphragm that

can be opened and closed with a lever to control the amount of light reaching the specimen. You can see the mirror attached to the base. It reflects light upward through the diaphragm.

Open the diaphragm completely with the help of a lever.

3. Rotate the mirror and write types of mirrors you observe.

> The microscope mirror is a set of two mirrors fixed back to back in a frame. One of them is plane miror and the other is slightly curved (concave). The plane mirror is better for a distant source of lighted daylight, i.e. sunlight. The concave mirror is better for near light source, such as tube light in the room.

Rotate the circular disc till the 10x objective lens is vertically below the body tube and it sets in that position with a sound of “click”.

Look through the eyepiece and rotate a mirror in such a way that you can see the maximum brightness.

Task 1: Ever Wondered How it Will Appear?

Before we learn how to observe through the microscope, we should know the correct way of handling and care of the microscope.

1. **Firstly, hold the arm of the microscope with one hand and the base with the other hand. Now we can carry our microscope from one place to another.**
2. Before we observe the specimen, we should wipe the lenses, mirror and stage of the microscope. For stage and mirror, a tissue or a cloth can be used. However for lenses, we should use only dry, soft paintbrush/ muslin cloth/ silk cloth/ lint free paper tissues to clean.

Procedure

i. On a piece of paper, draw two lines: one with a pencil and other with a ball-point pen.
ii. Fix the paper on a slide with the adhesive tape or you can keep it between the two slides. Fix the slide on a microscope stage keeping the pencil line below the objective lens (use the stage clips if available on the microscope).
iii. Bring the objective lens very close to the slide with the help of coarse focus. objective lens should not touch the slide!
iv. If there is too little light, then shine some light on the top part of the paper using a torch.
v. Look through the eyepiece and move the objective lens in the upward direction using the coarse focus until you can see an image of the line. Move the slide on the stage stage if you cannot see the image of a pencil line.
vi. Slowly rotate the fine focus to get the fine details through the eyepiece.
vii. Similarly, observe the ball-point pen line, by moving the slide and bringing below the lens.

> **What does it mean by to focus?**
> It means of moving the objective lens closer to or further away from the specimen to form a sharp image. On some microscopes, the stage can move and on the others, the body-tube can moves.

1. How does the pencil-line and pen-line appear under the microscope? Describe observations in your words. You can make small sketches if you feel the need.

If students get confused about how to describe their observations, then the teacher can help them by asking the similarities and differences between the pen line and the pencil line and why the lines appear differently.

The spread of ink of the pen line depends on the texture of the paper and as the ink is liquid, the paper will be uniformaly coloured and dark at cross fibers of paper. While the pencil lead is made of graphite and clay binders. The pencil line will appear as a scattered dots on a paper. If we provide light from the upper side, graphite will reflect the light and give a shiny appearance.

When you was able to observe fine details of object, have you noticed the distance between slide and objective lens?

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

2. Using those methods, estimate the distance between the objective and the slide.

	Pencil line	Ball-pen line
Distance between the slide and the tip of an objective lens	_____cm/ _____mm	_____cm/ _____mm

The students can come up with techniques using thread/ paper to measure the distance between slide and objective lens.

Task 2: Looking at Letters ‘s’ and ‘e’ !

 # How does light travel inside a microscope?

At this stage (Class 8), teacher need not explain this ray diagram to students. If some students are curious about why inverted image is formed or how the magnification of two lenses is multiplied, then teacher may draw this diagram for students and explain them.

This task helps students understand the concept of magnification as well as inversion of image.

- The reflected light rays of the sun (or a bright lamp) from the mirror at the bottom of the microscope will fall on the specimen (AB). Theses refracted rays will then pass through the specimen carrying information about it in the form of light.

- The objective lens gathers and focuses light from the specimen to produce a larger, real and inverted image (intermediate image (A’B’)) of it.

- These image rays will then pass through the eyepiece lens providing further magnification to the primary image and forms a focused Final image (A’’ B’’), which can be seen through the eyes.

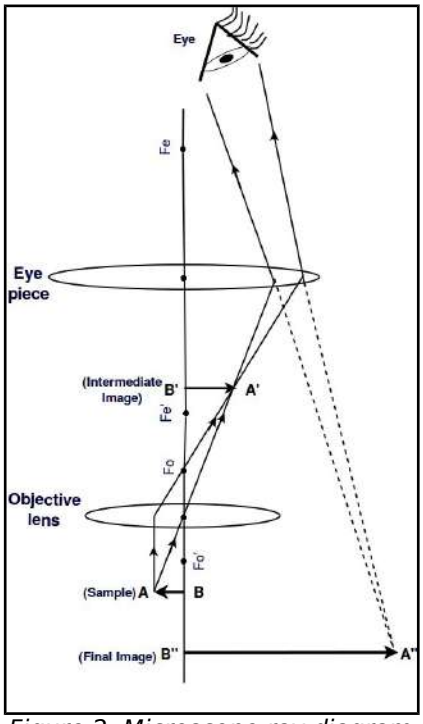


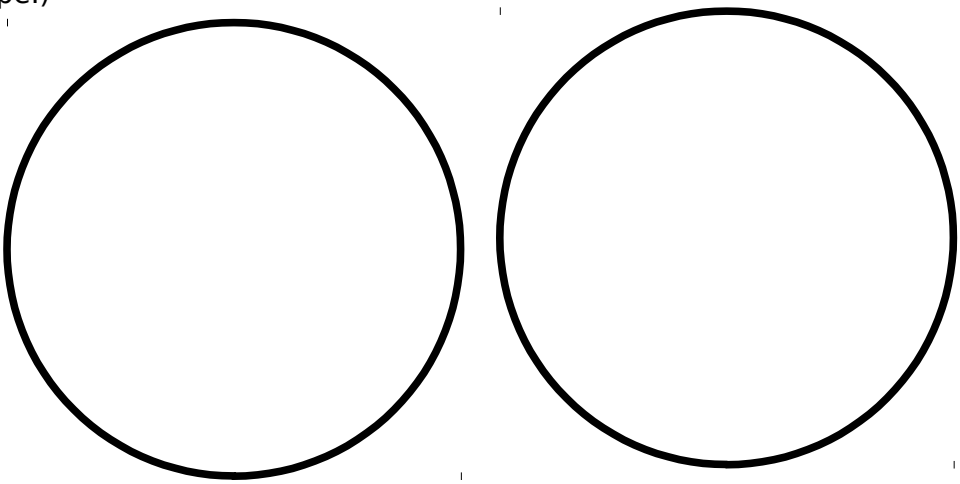
Figure 2: Microscope ray diagram

Here we will look at printed alphabets on a newspaper under the microscope. Cut a small piece of printed newspaper that have the letters-‘s’ and ‘e’.

Prepare a slide as we have done in previous tasks and observe it under the 10X objective lens.

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

Draw your observations in the following circles.
(Note: Compare size of a image to the field of view and try to draw it as observed under the microscope.)



Magnification_____X Magnification_____X

1. Do the letters-'s' and 'e' appear different in any way from how they look without the microscope (besides being bigger)?

_____

_____

_____

_____

> In case if students get confused about how to describe their observations, then the teacher can help them by asking "how did we see the letter on a stage by naked eyes and how is it appearing under the microscope?".

Task 3: Looking at Some Other Specimens

So far, we learned to prepare a slide and adjust the light of a microscope. In this task, we will observe few other specimens in our surroundings.

Slide Preparation:

- For Salt: Put a few granules of salt on a slide and spread it. Place a coverslip over it.
- For Hibiscus flower: Take a drop of water on a slide, dust some pollen grains from Hibiscus flower and place a coverslip over it. Alternatively, we can dust some pollen grain on adhesive tape and stick it on a slide.

> Teacher may also dust some pollen grains of Hibiscus flower on a watch glass and add a few drops of water in it. From this watch glass, different students may take single drop for their

slide.

- For Yeast- cells: Add 2-4 beads of a Baker's yeast in water and mix it thoroughly. Take a drop of water on a slide. Place a coverslip over it.
- For onion: Take a drop of water on a slide. Take a piece of inner transparent skin of onion leaf or onion ring and put it on the slide. Add a tiny bit of safranin stain (if available) on it and place a coverslip over it.

In this task, the teacher can select at least two specimens to observe. While observing the salt, observe it within 10 minutes. Otherwise, salt can moisten due to humid surroundings.

Sometimes at very high light intensity, image contrast may decrease. So try to maintain optimum light intensity for good contrast.

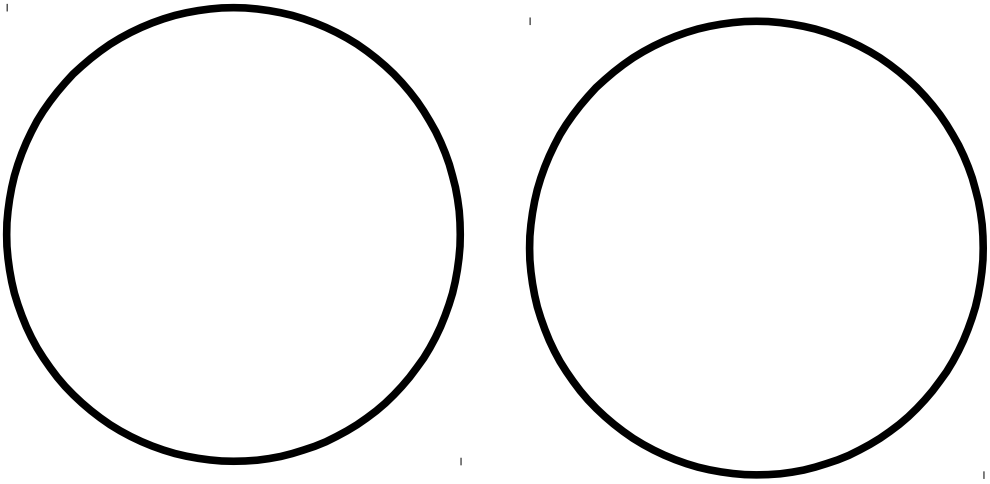
Staining technique is used for increasing contrast through changing the colour of some of the parts of the structure observed. In addition, they will know the importance of stains in microscopy.

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

Observe the specimen under 10X objective lens (as done in task 1) and draw your observations in the circle given below.

Note: Draw your observation in one of the cirlce and follow the instructions.

Specimen 1: _____



Magnification: _____X **Magnification: _____X**

Now we will observe it with the objective lens of 45X.

i. Move the objective lens in upward direction with the help of coarse focus knob.
ii. Rotate the circular disc in such a way that 45X objective lens will set vertically below the body tube with a sound of "Click".
iii. Using a coarse adjustment knob bring the objective lens close to slide.
iv. Slowly rotate the small knob until you get the fine details of an object.

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

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

_____

_____

2. What will happen if we change the objective of 10X to 45X-
   i. without changing the distance between lens and slide?
   ii. by changing the distance between lens and slide?

_____

_____

_____

_____

When we change the magnification of the low power objective lens to a high power objective lens, we mostly try not to move the slide. While rotating the nosepiece (circular disc) without changing the focus, possibly we can move the slide from its place.

On the other hand, if we change the focus and try to bring focus using coarse adjustment, then slide can come too close to the lens that can crush the specimen or sometimes slide as well. Therefore, when we change the magnification to higher magnification we should use the fine adjustment for focus.

The distance between an object and a tip of the objective lens will remain the same at a particular magnification. The distance between the objective lens and slide reduce as the magnification of objective lens increase.

Now draw your observations in a above circle and note down the magnification of objective lens.

Repeat the procedure to observe 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 closest to the specimen. _____
3. While working with high power magnification, we should use the coarse adjustment knob. _____
4. We use the diaphragm to adjust the amount of light entering the microscope. _____

5. We can clean microscope lens with any paper or cloth. _____

Tick the correct

1. What is the recommended part of the microscope to hold when transporting it?
   a) Eyepiece
   b) Arm
   c) Stage
   d) 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 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 closer to our eye.

3. False; while working with high magnification we should never use the coarse adjustment, it can break the slide.

4. True

5. False; Microscope lens is a delicate part of it. Using any paper or cloth to clean the microscope lens can leave scratches on the lens.

Tick the correct one

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

Task 4: Estimate the Size of a Specimen

This task will be considered as a possible extension for this learning unit.

To estimate size of the specimen we should get the idea of a diameter of the bright circle observed under microscope. This bright circle is called as field of vision. In this task we will try know the diameter of field of vision.

The size of the field of vision depends upon the magnification, i.e. the higher magnification, size of field of vision is less. It is as similar as we zoom in the image in our mobile phones. The more we zoom in the lesser part of an image we can see.

Requirements: Transparent scale with minimum division of 1mm.

Procedure: Place the scale on the stage. Focus and observe it under a 10X magnifying lens.

1. What is the width of the bright circle? _____

Now _____ is a standard metric unit, but when we use a MICROscope we tend to

use MICROmeters. To convert from millimeters to micrometers, we should multiply it by 1000 (or move the decimal 3 places to the right). Our _____mm estimate becomes _____ micrometers.

Now we can get the ruler out of the way, and estimate the size of things we observed!

For example, if something we were looking at took up half of the field of vision (i.e. 1300 micrometers), its size would be approximately 1/2 x 1300 micrometers = 650 micrometers. If something appeared to be 1/5 of width of the field of vision, we would estimate its size to be 1/5 x 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.
- Figure:Parts of a microscope- https://assist.asta.edu.au/resource/2879/sop-use-and-care-compound-light-microscope

Appendix For Teachers

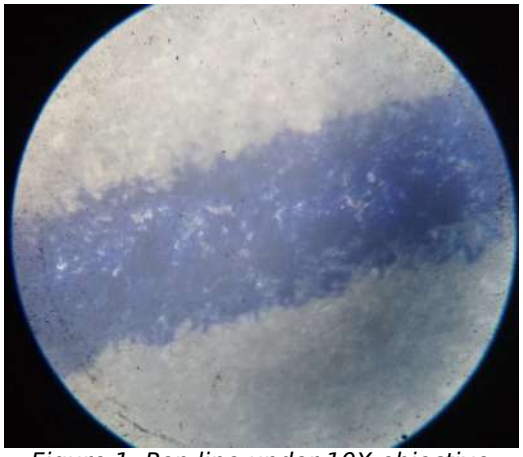


Figure 1: Pen line under 10X objective lens

Figure 2: Pencil line under 10X objective lens

Figure 3: Letter 's' under 10X objective lens

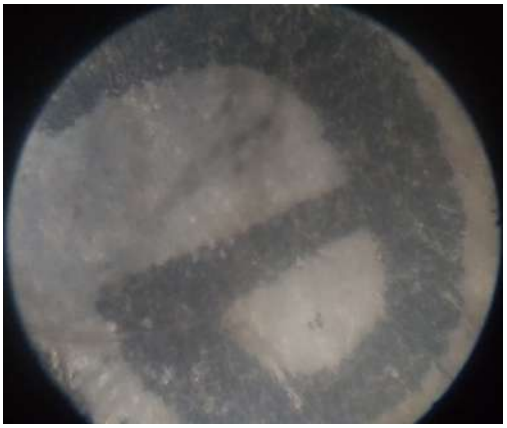


Figure 4: Letter 'e' under 10X objective lens

Figure 5: Salt under 10X objective lens

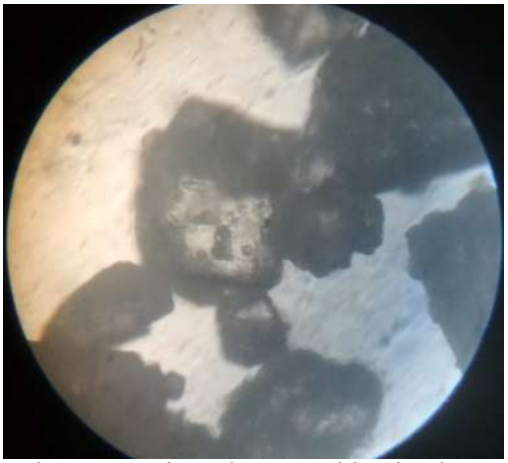


Figure 6: Salt under 10X objective lens

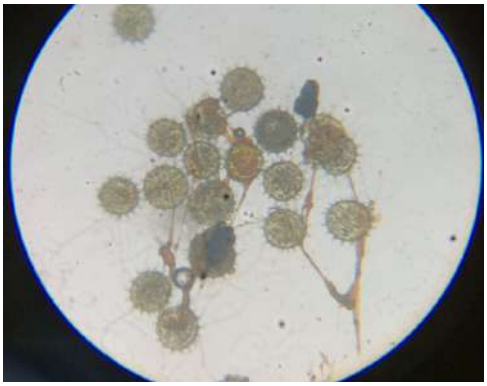


Figure 7: Pollen grains under 10X objective lens

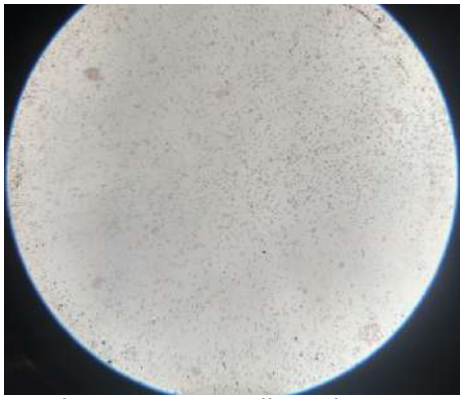


Figure 8: Yeast cells under 10X objective lens

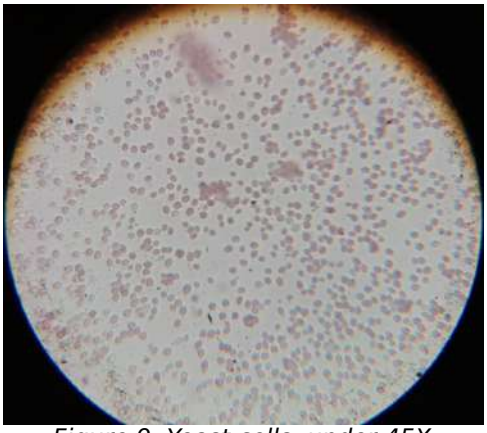


Figure 9: Yeast cells under 45X objective lens

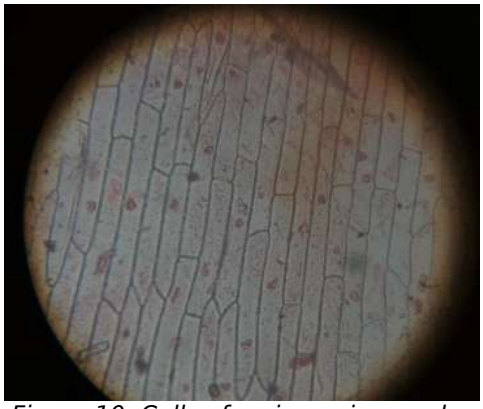


Figure 10: Cells of spring onion under 10X objective lens

Figure 11: Cells of spring onion under 45X objective lens