Introduction to Microscopy Answer Guide



Exercise 1: Getting to Know Your Compound Microscope

Photo 1: Microscope Components

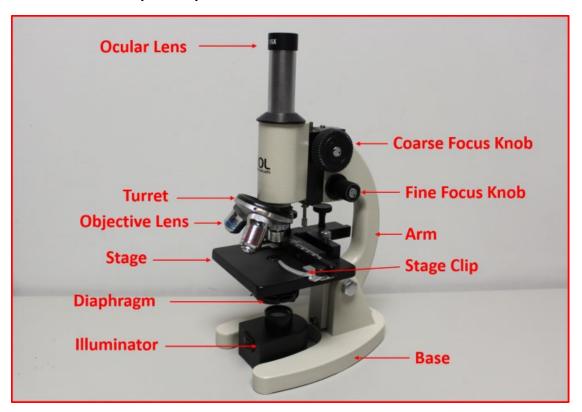


Table 1: Microscope Component Functions

Component Name	Component Function
Arm	Connects the base to the head of the microscope
Base	Flat support for the microscope
Coarse Focus Knob	Quickly moves the stage up or down
Diaphragm	Controls the amount of light on the specimen
Fine Focus Knob	Slowly moves the stage up or down
Illuminator	Light source that shines on specimen
Objective Lens	Lens closest to the specimen used to magnify the image
Ocular Lens	Lens at top of microscope through which the specimen is viewed and additionally magnified.
Stage	Platform that supports the slide to be viewed.
Stage Clip	Secures the slide in place.

Component Name	Component Function		
Turret	Rotates the objective lenses, allowing for magnification to be changed.		

Table 2: Total Magnification and Field of View

Ocular Magnification	Objective Magnification	Total Magnification	Field of View (mm)	Field of view (µm)
15x	4x	60x	2.25 (0.99 V-	2250 (990 V-
			Scope)	Scope)
15x	10x	150x	1.25 (0.38 V-	1250 (380 V-
			Scope)	Scope)
15x	40x	600x	0.225 (0.099 V-	225 (99 V-
			Scope)	Scope)

^{*} Data is for student microscope purchased with kit or V-Scope, Other microscopes may vary

Photo 2: Letter e - 4x Objective

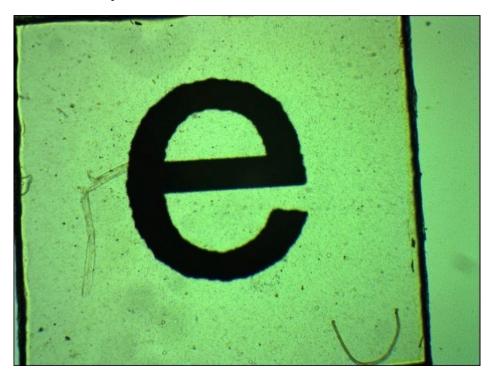


Photo 3: Letter e – 10x Objective

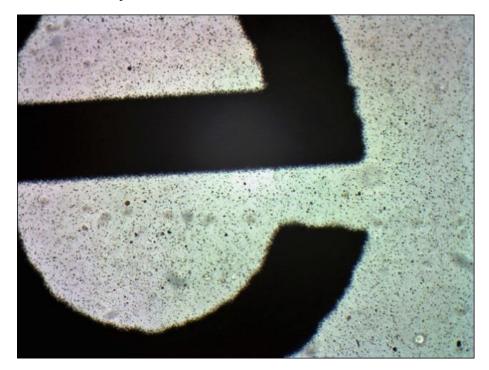


Photo 4: Letter e - 40x Objective

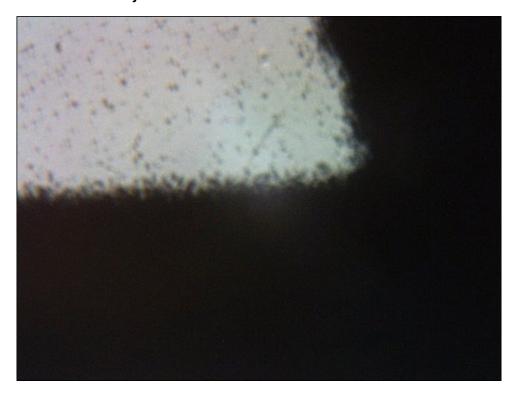


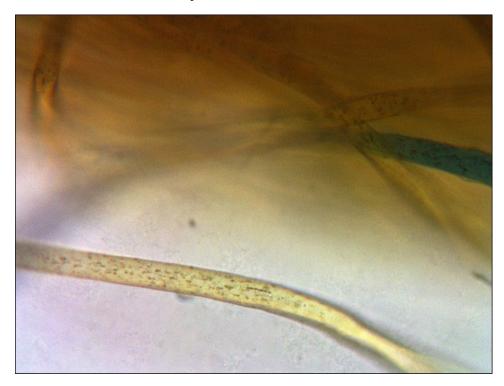
Photo 5: Colored Threads - 4x Objective



Photo 6: Colored Threads - 10x Objective



Photo 7: Colored Threads - 40x Objective



Question 1

How does the compound microscope used in this exercise differ from a stereo microscope? Reference the specific components of your microscope in your answer.

The compound microscope used in this exercise contains a turret with three objective lenses, an adjustable stage, an illuminator with a diaphragm and condenser that shines light through a specimen. The images provided by the compound microscope appear in 2D. A stereo microscope lacks a turret with multiple objectives, has lower total magnification, and illuminates specimens from above a fixed platform. The images provided by a stereo microscope appear in 3D.

Question 2

How did the field of view change as magnification increased when viewing the Letter e slide? Reference your calculations in Data Table 2 and Photos 2–4 in your answer.

The field of view decreased as magnification increased when viewing the Letter e slide. As shown in Data Table 2, the field of view at 60x was 2.25 mm (0.99 for V-Scope) but only 0.225 mm at 600x (0.099 mm for V-Scope). When viewed under increasing magnification, less of the letter e

appeared in the field of view. In Photo 2 the entire letter appeared, compared to Photo 4 where only a small portion of the e was displayed.

Question 3

How did the depth of field change with increased magnification when viewing the Colored Threads slide? Reference Photos 5–7 in your answer.

Depth of field decreased as magnification increased when viewing the Colored Threads slide. Photo 1 displays the numerous individual threads appeared in focus at 60x. At 600x only a small portion of one thread appeared in focus.

Exercise 2: Observing the Fine Details

Table 3: Fruit Fly Observations

Fruit Fly Viewing Predictions	Students should state that they may only see a portion of the fly at one time since the specimen will be larger than the field of view. They should also state that they will be able to see details of the surface structure of the fly, but that depth of field will be decreased at higher power magnifications.
Fruit Fly Viewing Observations	Students should note that only a portion of the specimen was visible at one time, and that surface features such as bristles became more visible as magnification increased. Students may also note that only surface features could be clearly focused, and that many areas appeared dark because the light could not effectively penetrate the specimen.

Photo 8: Fruit Fly - 4x Objective

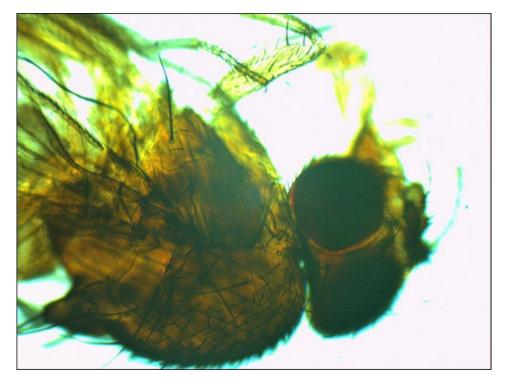


Photo 9: Fruit Fly – 10x Objective

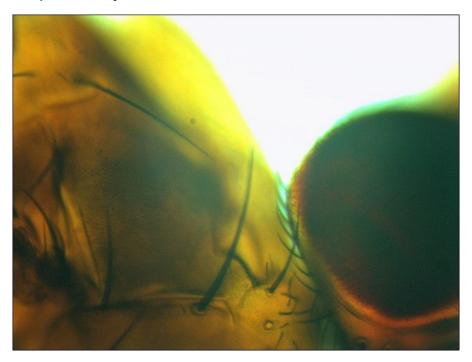


Photo 10: Onion Root Tip – 4x Objective

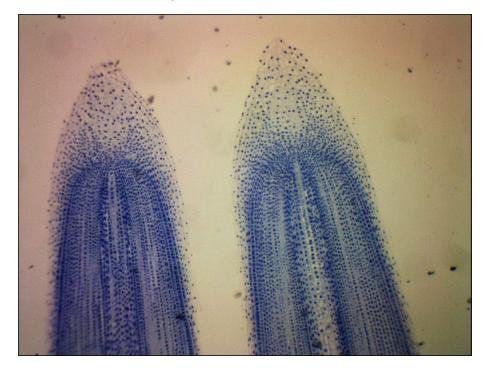


Table 4: Onion Root Tip Observations

Objective Lens	Observations
4x	The entire width of the root is visible. It appears that there are small dots located in some of the small square boxes (cells) in the root tip.
10x	The entire width of the root is no longer visible, and the details of the individual boxes (cells) are now visible. The "dots" in the center of the cells appear to have different shapes from one another.
40x	The details of the "dots" in each of the cells are visible, showing a series of patterns that is unique from cell to cell. The individual "thread" of each design in the cell is now visible.

Photo 11: Onion Root Tip - 10x Objective

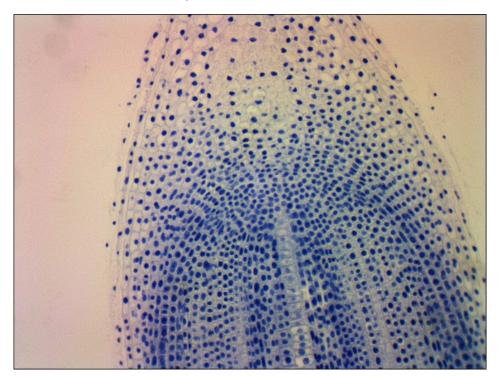
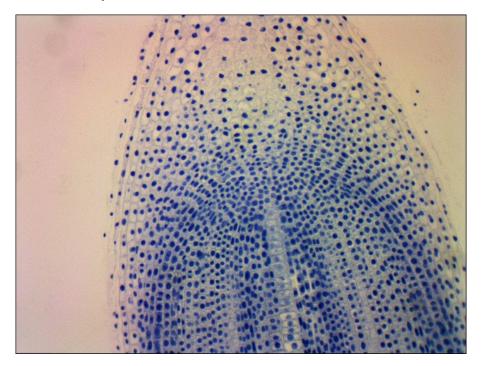


Photo 12: Onion Root Tip - 40x



Question 1

Did your predictions match your observations when viewing the fruit fly? Explain your answer referencing Data Table 3.

Student's answer should match the predictions/observations recorded in Data Table 5. Students should note that the fruit was larger than the field of view of the lowest power magnification such that the entire specimen could not be viewed as shown in Photo 8. The light source did not penetrate many areas of the specimen when with the 10x objective as displayed in Photo 9.

Question 2

What was the best magnification for viewing individual rectangular-shaped cells of the onion root tip? Reference Photos 10–12 in your answer.

Students should state 600x or the total magnification of their microscope when using the 40x objective. Students should also note that Photo 12 provided the best image of individual cells.

Exercise 3: Wet Mount Slides

Photo 13: Prepared Slide - Unstained

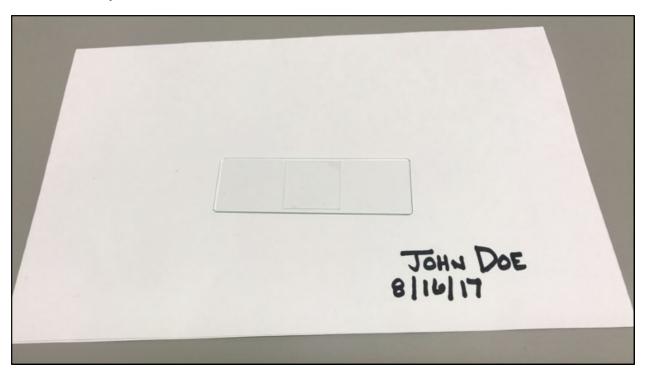


Photo 14: Unstained Cheek Cells - 4x Objective

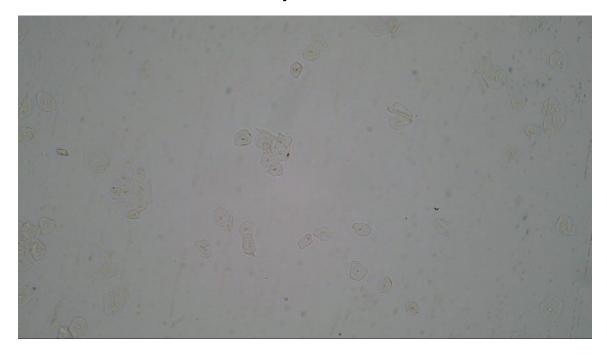


Photo 15: Unstained Cheek Cells - 10x Objective



Photo 16: Unstained Cheek Cells – 40x Objective

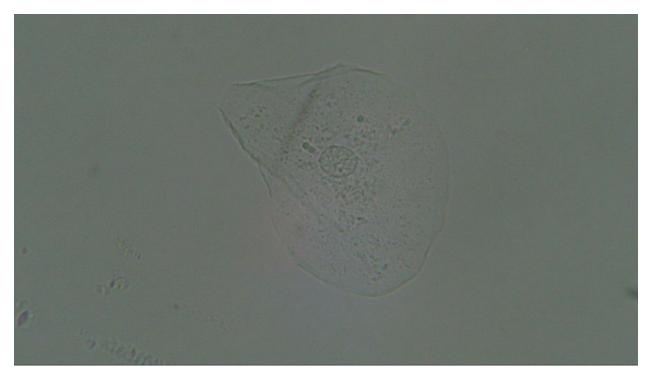


Photo 17: Prepared Slide- Stained



Photo 18: Stained Cheek Cells - 4x Objective

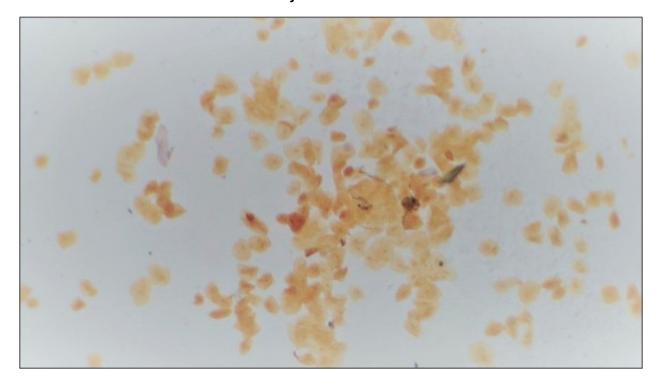


Photo 19: Stained Cheek Cells - 10X Objective

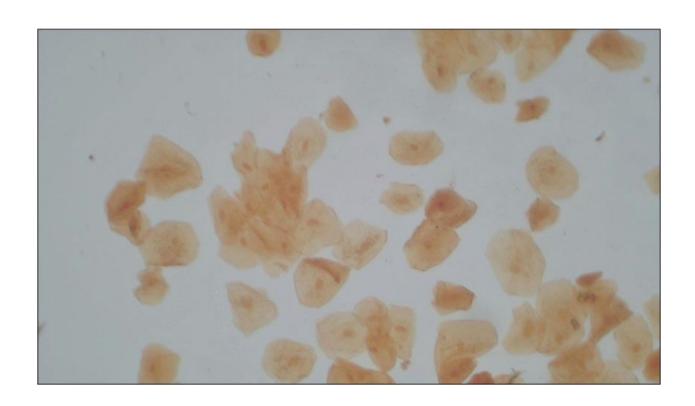
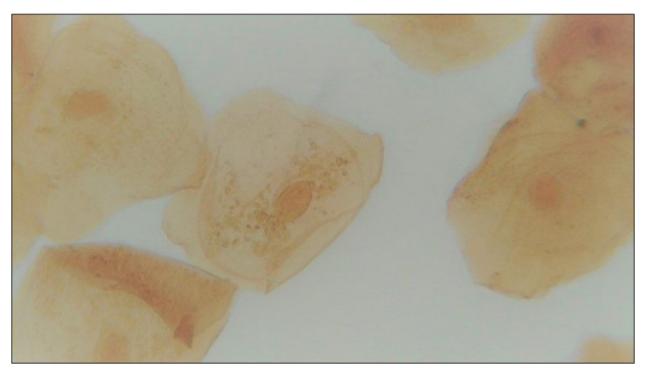


Photo 20: Stained Cheek Cells - 40x Objective



Question 1

Which objective lens was best for viewing individual cheek cells? Reference your photos in your answer.

Students should state that the 40x objective was ideal for viewing individual cells and reference the corresponding Photos 16 and 20 in their answer.

Question 2

How did the appearance of IKI stained cheek cells differ from that of unstained cheek cells? Reference your photos in your answer.

The IKI stained cheek cells appeared an amber color with darker internal structures, such as the nucleus. The unstained cells appears colorless with shadows indicating the cell shape and nucleus. Student will likely state that the stained cells were easier to observe.

Question 3

Why is it important not to trap air under the cover slip when creating a wet mount slide?

Air bubbles trapped under the cover slip prevents the cover slip from flattening the specimen which leads to difficulty viewing the specimen under the microscope and challenges when applying stain to the slide.

Extension Question

Amoeba proteus is a free-living microbe 0.2 - 0.5 mm in size found in ponds, streams, and puddles. Apply your knowledge of microscopy to describe the type of microscope and slide preparation techniques that would be ideal for testing a water sample for the presence of this microbe.

A compound light microscope should be used to view **Amoeba proteus** since the specimens range in size from 0.2-0.5 mm. Either the 4x or 10x objective should be used on the microscope to fit the specimen into the correct field of view dimensions. A wet mount slide would be created of the water sample by adding a drop of the sample to a clean microscope slide and gently lowering a cover slip. Pulling a simple stain such as IKI through the wet mount would result in the **Amoeba proteus** becoming more visible.