BIO 211 Human Anatomy and Physiology I

The Light (Compound) Microscope

Edited for NVCC students.
A special Thanks to the author of this presentation,
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How to carry microscope properly

One hand holding **arm** of microscope

One hand supporting **base** of microscope

2

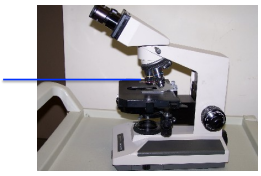
Please bring one microscope to your work station.
Carry it correctly!
Each person should have his/her own microscope today.

3

OCULAR LENSES	SUBSTAGE LIGHT
binocular (2 oculars); never swivel	sends light up toward image
Eyepieces magnifiy 10 X	controlled by light switch on bottom/side of 'scope

4

OBJECTIVE LENSES	ROTATING NOSEPIECE
4X = scanning 10 X = low power	supports objective lenses
40 X = high power or high dry 100 X = oil immersion	changing objective lenses changes magnification



5

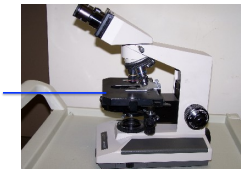
Important!!!

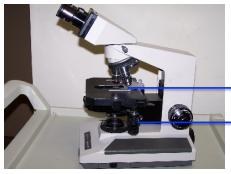
NEVER use Kimwipes or paper towels to clean any lens on your microscope. They will scratch and ruin the lenses.

Use Lens Paper only.

6

STAGE	MECHANICAL STAGE
supports the slide	grips the slide
moves up and down during focusing	moves the slide using control knobs





7

COARSE ADJUSTMENT KNOB	FINE ADJUSTMENT KNOB
stage visibly moves up and down	stage moves up and down; not visible
used for coarse focusing	used for fine focusing

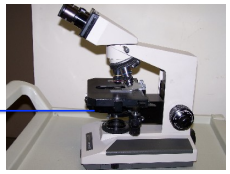
8

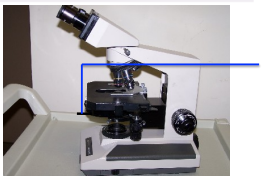
Now plug your microscope in & turn the light on.

Never let the microscope cord hang over the side of the desk.
Students could trip and injure themselves.
The microscope could also be damaged.

9

CONDENSER	IRIS DIAPHRAGM LEVER
raises & lowers (keep closest to stage)	makes light seen dimmer or brighter
focuses light on the specimen (on the slide)	controls the amount of light going through condenser.





10

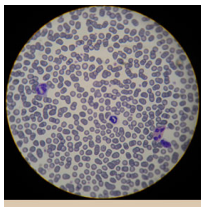
TOTAL MAGNIFICATION

magnification of oculars (10)

Times (X)

magnification of objective lens used

cat blood in field of view of microscope



11

QUESTION

You are observing a slide using the high power objective. What is the total magnification of the image?

under low power?

under oil immersion?

under the scanning objective?

12

Working Distance

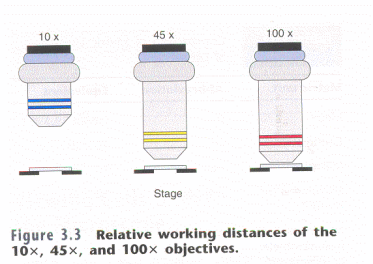


Figure 3.3 Relative working distances of the 10×, 45×, and 100× objectives.

As magnification increases, working distance decreases. What is the significance of this?

13

QUESTION

Are working distance and total magnification directly or inversely proportional to each other?

14

Resolution

Ability to distinguish between 2 closely spaced points.

Resolution of human eye: 100 µm
(100 µm = 0.0039 inches)

If > 100 µm

Will see 2 objects

If < 100 µm

Will see only 1 object

15

Resolution continued …

Resolution of compound microscope: 0.2 µm
(0.2 µm = 0.0000078 inches)

If > 0.2 µm

Will see 2 objects

If < 0.2 µm

Will see only 1 object

16

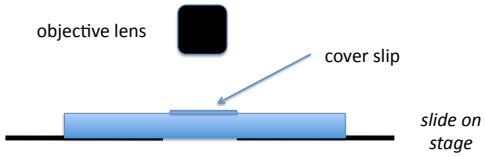
Limit of Resolution continued …

can reach a point where you can increase volume, but the sound won't be clear because you have reached the limit of resolution of the radio

Why can't you just keep putting more powerful objective lenses on a microscope to keep increasing magnification?

17

Oil Immersion: Theory



After passing through the slide, light must pass through the coverslip (glass) and air before reaching the objective.

18

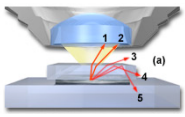
But air and glass have different refractive indexes.

This means that they bend light differently (i.e., to different degrees)

Air and water also have different refractive indexes, so the spoon appears bent at the air/ water interface.

19

Oil Immersion: Theory continued …



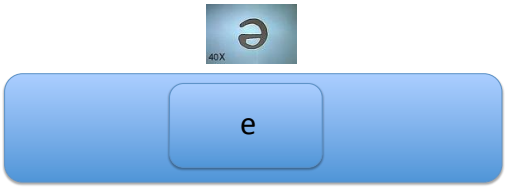
With no oil:
Not all light goes into objective;
Enough light for 4X, 10X & 40X objectives;
Not enough light for 100X objective.

With oil:
Glass & oil have similar refractive indexes;
Almost all of the light goes to 100 X objective

20

Letter “e” Slide and Proper Focusing

Activity 2



21

1. Obtain letter “e” slide from side counter

2. Move stage as far away from objectives as possible.

3. Put the 10x objective in place.

Always use the low power objective to focus your slide – even if you want to view your slide under high power.

22

4. Look at your letter “e” on the slide with your eyes (i.e., NOT through the ‘scope). Note the orientation of the letter “e” in your lab book.

5. Put the letter e slide on the stage of your microscope; be sure it is clipped into place by the mechanical stage. You can test if your slide is in the mechanical stage correctly by trying to move it around with the appropriate dials.

6. Move the slide so that the “e” is centered in the hole the light comes up through.

23

7. Looking to the side of the microscope (NOT through the oculars), move the stage as close as possible to the objective lens without touching the slide to the objective lens.

Why do you have to look to the side of the ‘scope when you are moving the stage closer to the objective lens?

_____

24

8. Adjust the oculars for your eyes.

9. Looking through the oculars, slowly move the course adjustment knob until the image is almost in focus.

When you do step 9, are you moving the slide toward or away from the objective lens?

_____

When moving stage & objective closer together, you must look at the side of 'scope rather than through the oculars. When moving the stage & the objective away from each other, you can look through the oculars.

25

10. Use the fine adjustment knob to finish focusing the slide. *NOTE: if the slide doesn't come into focus, repeat steps 2 – 10.*

11. Once the slide is in focus, note the orientation of the letter "e" in your lab book.

12. Compare the orientation of the letter "e" in steps 4 and 11.

What changes do you see in the orientation of the letter "e" when looking at it without a microscope (step 4) and with a microscope (step 11)?

_____

26

13. Now move the slide to your right while looking through the ocular.

In which direction (left or right) does the letter "e" appear to move?

_____

14. Now move the slide away from you while looking through the ocular.

In which direction (toward you or away from you) does the letter "e" appear to move?

_____

27

QUESTION

What implications do the answers to the questions in steps 13 and 14 have in terms of looking at tissues under the microscope?

28

15. Make sure that the letter "e" is in the center of your field of vision and is in focus.

16. Looking at the side of the microscope, switch from the 10x objective to the 40x objective.

In step 16, why do you need to look at the side of the microscope rather than through the oculars?

_____

29

17. The slide should be in focus because our microscopes are **PARFOCAL** (i.e., if they are in focus using one objective, they should be in focus using all objectives).

Microscopes are not perfectly parfocal, though. If your image is not quite in focus, use the FINE adjustment knob. **DO NOT TOUCH** *the course adjustment knob.*

Can you see the whole "e" ?

_____

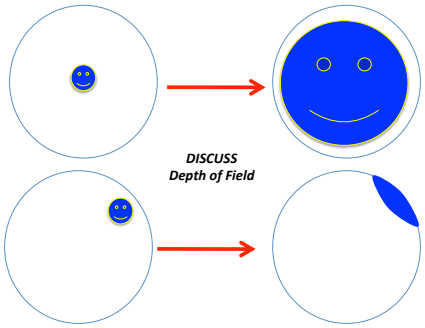
Is there enough light? _____

(If not, move the iris diaphragm lever until there is enough light)

30

Field of View

10x 40x



DISCUSS Depth of Field

31

QUESTION

As magnification increases, do you see more or less of the "e"? What implications does this have if you are looking at a tissue under the microscope?

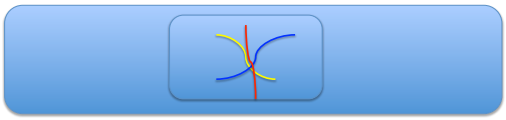
As magnification increases, does the field of view increase or decrease? What implications does this have for looking at cells under the microscope?

As magnification increases, does the amount of light getting through to your eyes increase or decrease? What implication does this have in terms of looking at something under the microscope?

32

Depth of Field

(Activity 4)



3 colored threads are crossed on a slide.

Each thread is 3 dimensional.

If you focus on the point at which the threads cross, the threads should not be in focus at the same focal point.

33

Which thread should come into focus first – top or bottom?

objective lens 

NOTE:
NVCC student slides may contain different colored threads.

3 colored threads on slide

When focusing correctly, are you moving the threads toward or away from the objective while looking through the oculars?

_____

Therefore, should the focal point be above the orange thread or below the green thread? _____

34

objective lens 

3 colored threads on slide

Focal Point

Therefore, which should come into focus (i.e., reach the focal point) first – the top thread or the bottom thread?

_____

35

1. Obtain a crossed colored thread slide from the side counter.
2. Focus correctly using the same steps used to focus the letter “e” slide.

   HINT: once the slide is almost in focus, you have to do this very slowly!
3. Which thread (blue, red or yellow) is on top? _____ on the bottom? _____

36

QUESTION

3 threads are crossed on a slide – a green thread, a blue thread, and a silver thread. When focusing correctly, first the green thread comes into focus, then the blue thread comes into focus, and, lastly, the silver thread comes into focus. Which thread is on the top? Which thread is on the bottom?

37

What were the purposes of looking at the letter "e" slide?

What was the purpose of looking at a crossed colored thread slide?
What implications does this have in terms of looking at tissues through the microscope?

END

38