

Human Anatomy & Physiology I

Lab 4 Microscopy I – Basics of Microscopy

Lab exercises 4.1

There is a compound microscope for each student. Carry out the activities listed below and fill in the blanks as you do so.

1. Pick up your microscope and physically move it to a new location. Bring it close enough that you can look into it comfortably from where you are sitting. Arrange it so that the stage is facing you and the eyepiece is rotated towards you. What part of the microscope did you grab in order to pick it up and move it?

2. Where are the locations of the two stage adjustment knobs on your microscope?

3. Where is the location of the coarse focus knob? _____

4. Where is the location of the fine focus knob? _____

5. Is there a condenser adjustment knob? If so, where is it located? _____

6. Find the diaphragm lever. Looking in the hole in the center of the stage, what happens when you slide the diaphragm to the right?

7. Still looking down at the hole in the center of the stage, what happens when you slide the diaphragm to the left?

Lab exercises 4.2

1. Write down the magnification factor for the eyepiece lenses on the microscope in front of you.

2. Using the microscope in front of you, write out all the words and numbers written on each objective on your microscope. There are probably three objectives, but some microscopes might have four. Start with the smallest objective and move through them in order of increasing size.

Objective one _____

Objective two _____

Objective three _____

Objective four _____

3. In the above list, for each objective, circle just the magnification factor for that objective. Remember, the magnifying factor is a whole number, and differs for each different objective.
4. Write down the total magnification (eyepiece magnifying factor * objective magnifying factor) when using each objective on the microscope in front of you.

Objective one _____

Objective two _____

Objective three _____

Objective four _____

5. If you observed two features on a slide with your naked eye that were 0.5 mm, how far apart would they appear to be if you observed them with the microscope in front of you, using the second objective?

Lab exercises 4.3

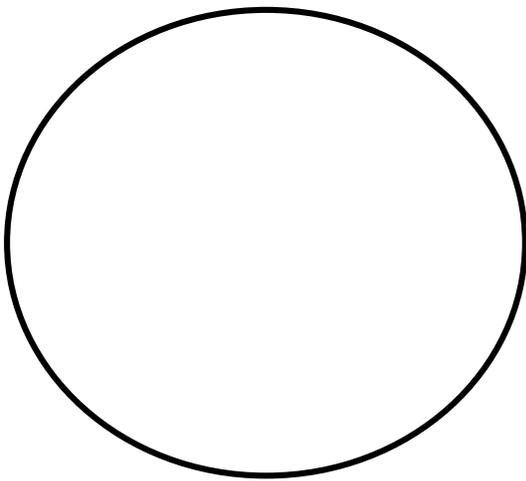
Get a slide with the letter “e” on it. Then following the checklist below, checking off each item as you complete it. For this slide, the letter “e” is the specimen we are interested in.

Checklist for setting up a microscope and beginning with a new slide.

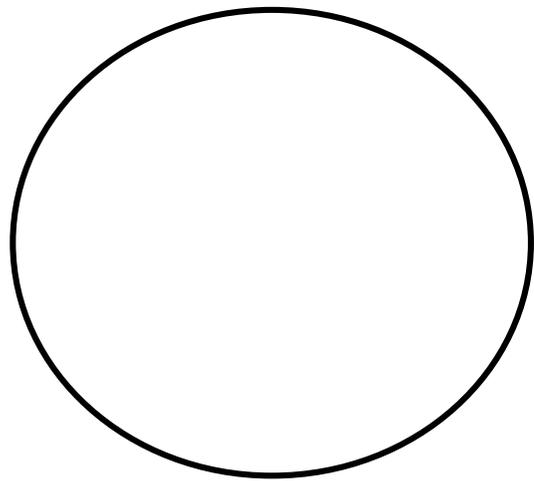
1. Plug in microscope & turn on light source.
2. Pick up microscope by carrying arm, position it so it is accessible to your seat, with open side of the stage facing you.
3. Rotate the objectives so that the lowest power objective (smallest in size) clicks into place.
4. Look at slide with your naked eye and find the location of the specimen.
5. Clip slide into place with stage clips. The cover slip on the slide must be face up. Find the stage controls and make sure that, when they are turned, the slide moves smoothly left & right or up & down, depending on knob.
6. Use stage controls to move slide so that light source is shining directly on to the specimen to be magnified.
7. Find the coarse and fine focus knobs. Watching the stage and objective, use the coarse focus knob to bring the low power objective as close to the slide as it will go.
8. Put your eye to the eyepiece (or eyepieces, if the microscope is binocular) and rotate the coarse focus knob in the lowering direction until some aspect of the specimen comes into focus.
9. Move your hand to the fine focus knob and get the specimen into perfect focus for your eyes. Do NOT touch the coarse focus knob again.
10. Use the stage control knobs to move your specimen to close to the exact center of your field of view.
11. Move to the next highest power objective (do not skip individual objectives) and use only the fine focus to get your image into perfect focus for your eyes.
12. If you need further magnification, move to the next highest power objective and use only the fine focus to get your image into perfect focus for your eyes.
13. Do not use the 100x objective (if you have one) in this course. It must be used with immersion oil and we won't have students doing that.

Lab exercises 4.4

1. Get an “e” slide. If it is already under your microscope, rotate the lowest-power objective into place, use the coarse focus to lower the stage, and remove the slide.
2. Look at the unmagnified “e” on the slide by eye. Rotate the slide around in your hand so that the “e” is right side up. Now clip the slide onto the microscope stage with the stage clips so that the “e” is facing you right side up when you look at it with your unaided eye.
3. In the right-hand circle below, draw what the “e” looks like when you are looking at it right side up. Assume the circle below is the size of the entire coverslip. Draw the “e” you see unaided in the correct proportion to the coverslip. (The unmagnified “e” will take up a tiny portion of the coverslip area.)



Total magnification: _____



Total magnification: _____

4. Clip the slide into your microscope stage so that the “e” is still facing you right side up. Follow the checklist in Lab Exercise 4.3 to ensure you are setting up the microscope properly for use, but stay on the lowest power objective. Get the “e” into your field of view and in focus.
5. In the left-hand circle above, draw what the “e” looks like when viewing it through the microscope under the lowest-power objective. Under the circle, write the total magnification of the image.
6. When viewed under a microscope, how is a specimen rotate? _____
7. Look at the stage and slide directly (not through the eyepieces.) Move the stage control knob that causes the slide to move away from you on the stage, then move it back to its original position.
8. Now move the stage control knob the exact same way you just did, but view the “e” through the eyepiece. When the stage is moving away from you, what direction does the virtual image appear to be moving?

9. Again, look at the stage and slide directly (not through the eyepiece.) This time, move the stage control knob that causes the slide to move to your right, then move it back to its original position.

10. Now move the stage control knob the exact same way you just did, but view the “e” through the eyepiece. When the stage is moving to your right, what direction does the virtual image appear to be moving?

11. The **field of view** is the entire area you can see when looking through an eyepiece. Use the stage control knobs to move the virtual image of your “e” to one side of the field of view. Keep most of the “e” in the field of view, but move it to one side or the other.

12. Now switch to the next-power objective. (Do not skip.) To get to the next-power objective, and not the highest-power objective, which way did you have to rotate the objectives, clockwise or counter-clockwise?

13. Using only the fine focus knob (you do NOT use the coarse focus knob on any objective other than the lowest objective), get the “e” in focus.

14. When moving to the next objective, which part of the field of view do you zoom in on?

15. Move away from the eyepieces and look at the distance between the slide and the bottom of the objective. Rotate back to the lowest power objective. Now rotate to the next objective (do not rotate to the highest-power objective by accident). Now rotate to the third-highest objective. What happens to the distance between the slide and the bottom of the objective as you rotate to higher power objectives?

16. With the third-highest power objective still in place, how space is there between the slide and the bottom of the objective?

17. Notice there is a danger of smashing the objective lens into the slide if you were to use the coarse focus. Why are you instructed to only use the coarse focus with the lowest-power objective?

Lab exercises 4.5

1. Get a human blood smear slide. Rotate your lowest power objective into place on your microscope.
2. Follow the checklist in Lab Exercise 4.3 until you are viewing the blood smear under you 40x objective.
3. You will see mostly red blood cells. They will probably be pinkish and they will be the circles without nuclei. Occasionally, some will appear to have blank circles in their centers, but these are not nuclei. If you search around your slide using your stage controls, you will find the rare circular cells with nuclei. These are white blood cells. There will be less than one white blood cell for every 100 red blood cells. These white blood cells will probably be light blue or grey and have purple or dark blue nuclei. Their nuclei will not always be round.
4. Find a section of your slide with two or more white blood cells among all the red blood cells.
5. In the circle below, draw four or five representative red blood cells (do not draw all the red blood cells you see) and draw all the white blood cells in your field of view. Pay careful attention to drawing the white blood cell nuclei as accurately as possible.
6. Do not remove or change the position of your slide until the instructor has verified that your white blood cells are drawn accurately.

