LIGHT MICROSCOPE

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Biology

First Semester

Week 3

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Outline

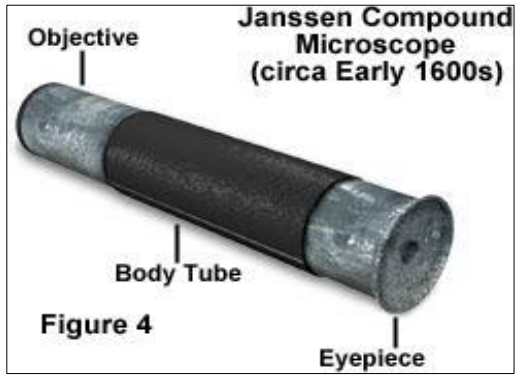
- History of microscope
- Light microscope
- Parts of light microscope
- Microscope safety cautions:
- Steps in using light microscope

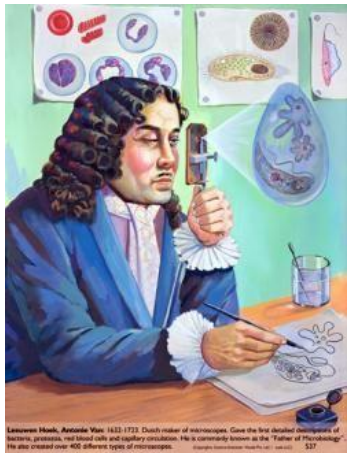
Objectives

- Familiarize students with parts of microscope
- Familiarize the students with steps in using microscope
- Introducing the cautions during the use of microscope
- Introducing the tips in cleaning and preserving light microscope

The Microscope

- Since an unaided eye cannot detect anything smaller than 0.1 mm in diameter, cells, tissues, and many small organisms are beyond our visual capability, so we need equipment to magnified objects which is too small to be seen with unaided eye.
- **Microscopes** are fundamental biological tools. Most of our current knowledge of cell structure has been gained with the assistance of microscopes





- There are several types of microscopes but the only one used in this laboratory is the **compound light microscope.**
- **The compound microscope** (sometimes called the student microscope or light microscope); these microscopes are known as compound microscope because there are two magnifying lenses in the microscope.
- One magnifying lens is in the ocular or eyepiece, which further magnifies the image formed by the objective lens, and one, is in the objective. Each contributes to the magnification of the object on the stage.

Light Microscope

Magnification: the degree to which something is or can be magnified.

Calculating total magnification

Eyepiece lens

Objective lens

Total magnification = eyepiece lens x objective lens

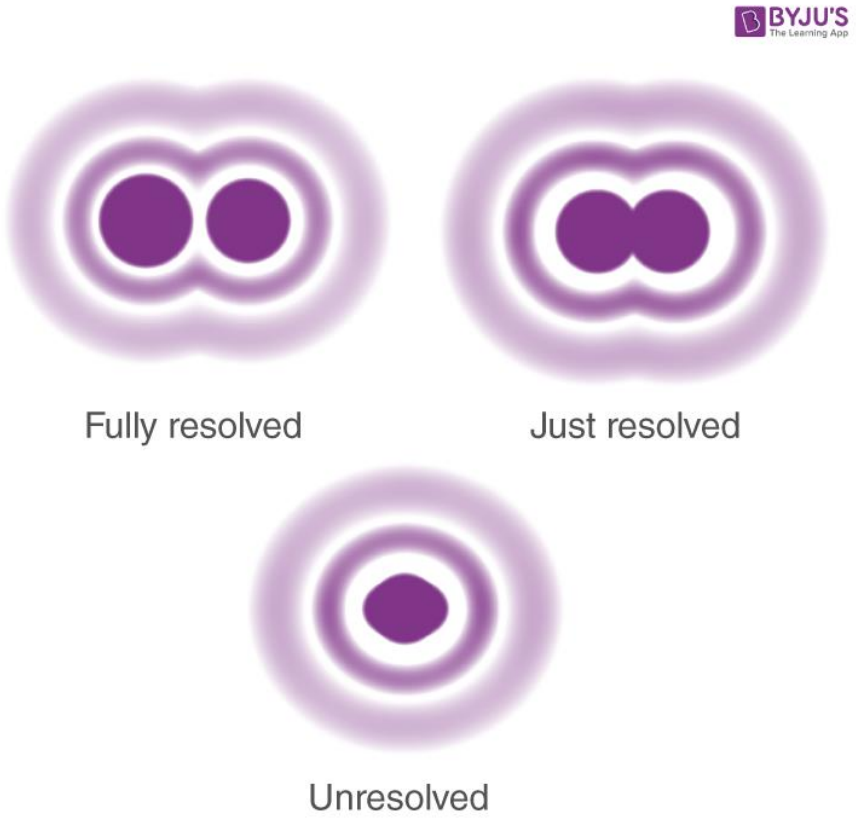
For example, for an *eyepiece of ×10* and an *objective of ×10*, the total magnification of the object is:

10 × 10 = 100

The nose piece rotates the magnification of the microscope. Generally compound microscope magnifies from 40 x to 1000 x.

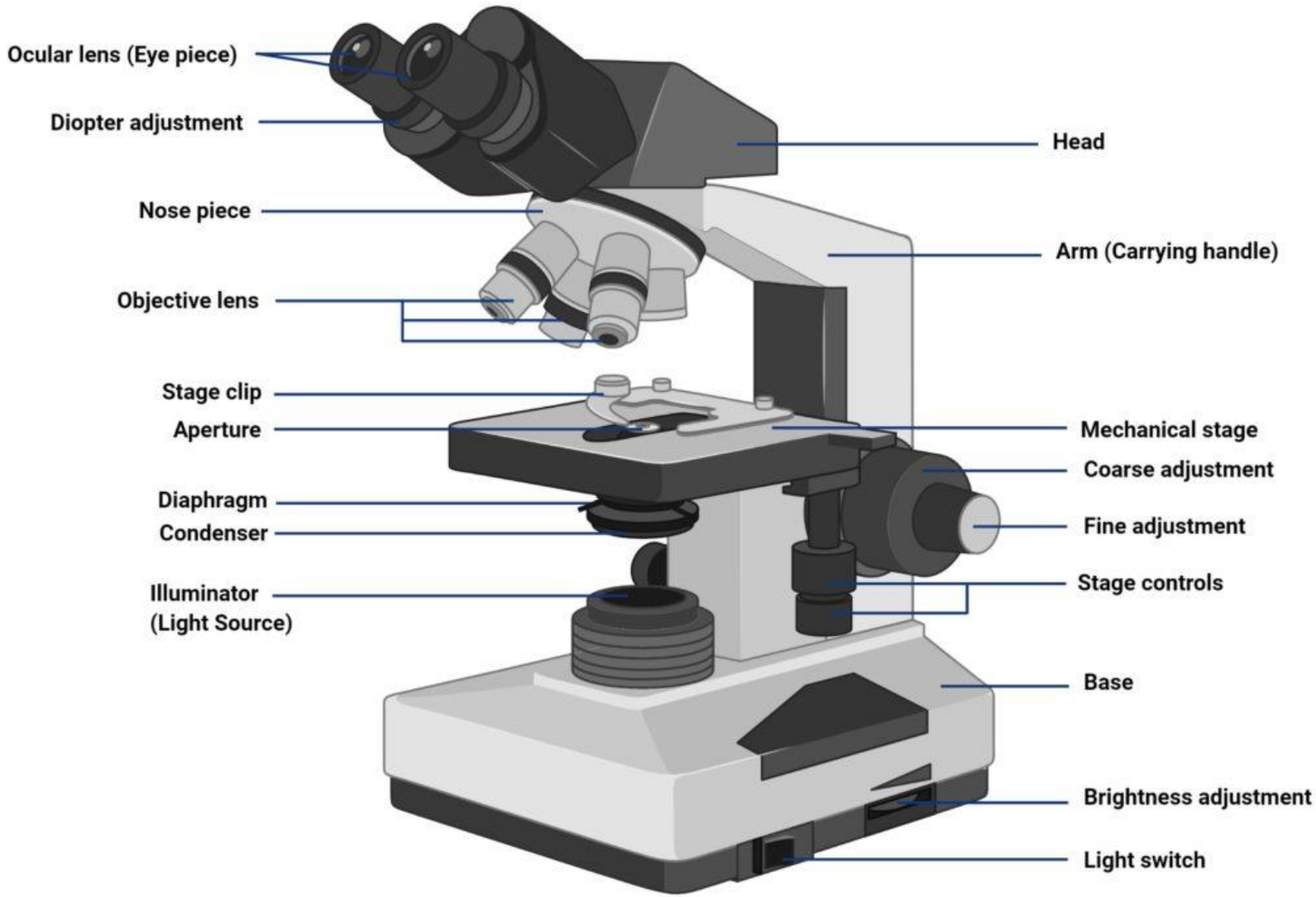
The most important feature of any lens system is its resolving power

- **The resolving power of a lens system is the smallest distance separating two objects that can be distinguished by the lens system and that allows them to be seen as two distinct** objects rather than as a single entity



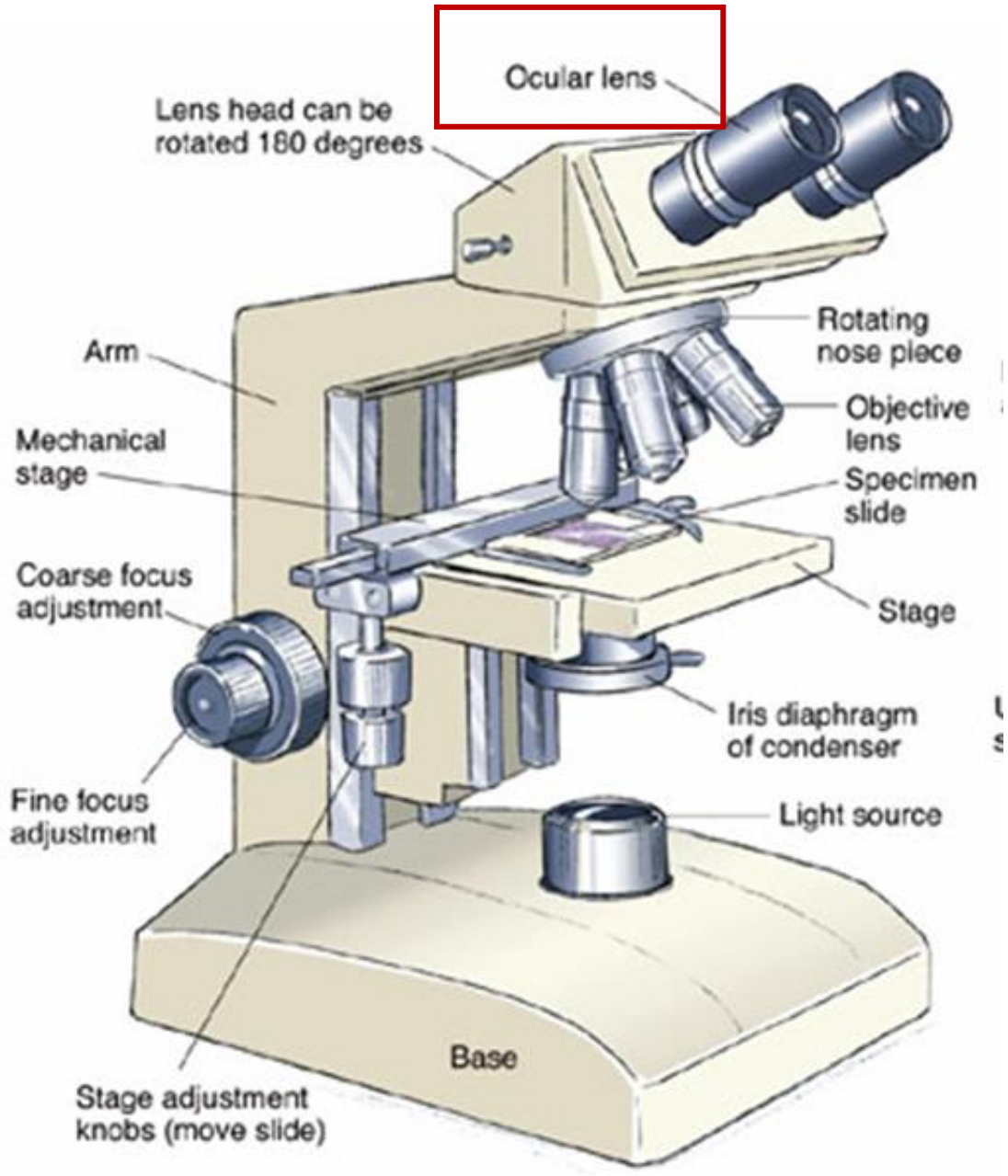
- For example, most humans see two fine parallel lines as two distinct markings if they are separated by 0.1 mm. If they are closer together we see them as a single line.
- Thus the resolving power of the human eye **is 0.1 mm**.
- The light microscope has a resolving power of about **0.0002** mm so it gives useful views of cells and can reveal features of some of the sub-cellular contents of eukaryotic cells.

Microscope Parts



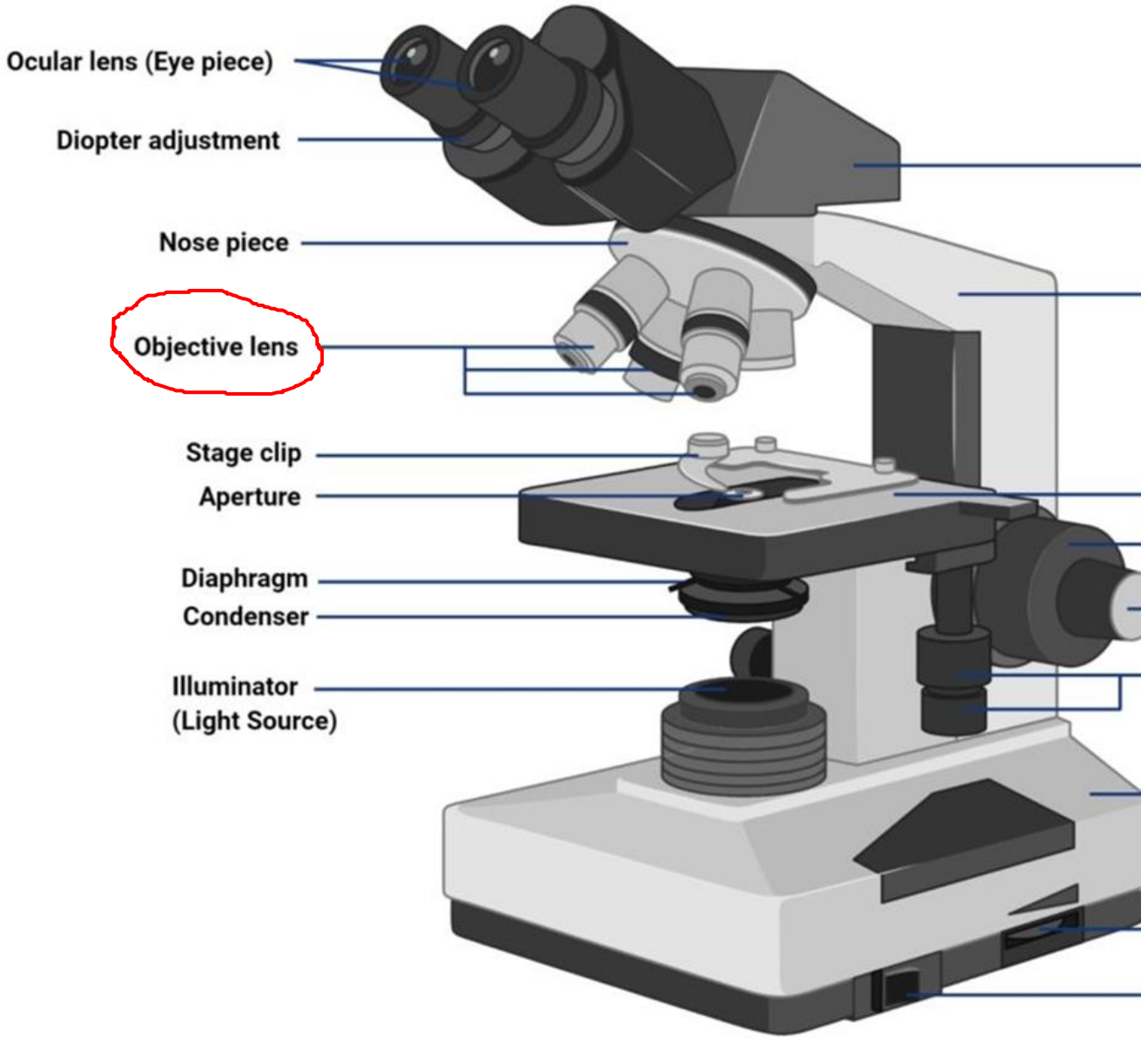
1. Ocular of eyepiece lens.

- The ocular lens is the lens you look through, it is inserted at the top of the body tube. If your microscope has one ocular, it is a monocular microscope, if it has two, it is binocular. Its magnification is written on it (Mostly10 x)



2. Objective lenses.

- The objective lenses are a set of three to four lenses mounted on a rotating turret at the bottom of the body tube. The four objective lenses of your microscope and their magnifications are:



Scanning lens	**4X magnification**
Low power lens	10X magnification
High power lens	40-45X magnification
Oil immersion lens	100X magnification

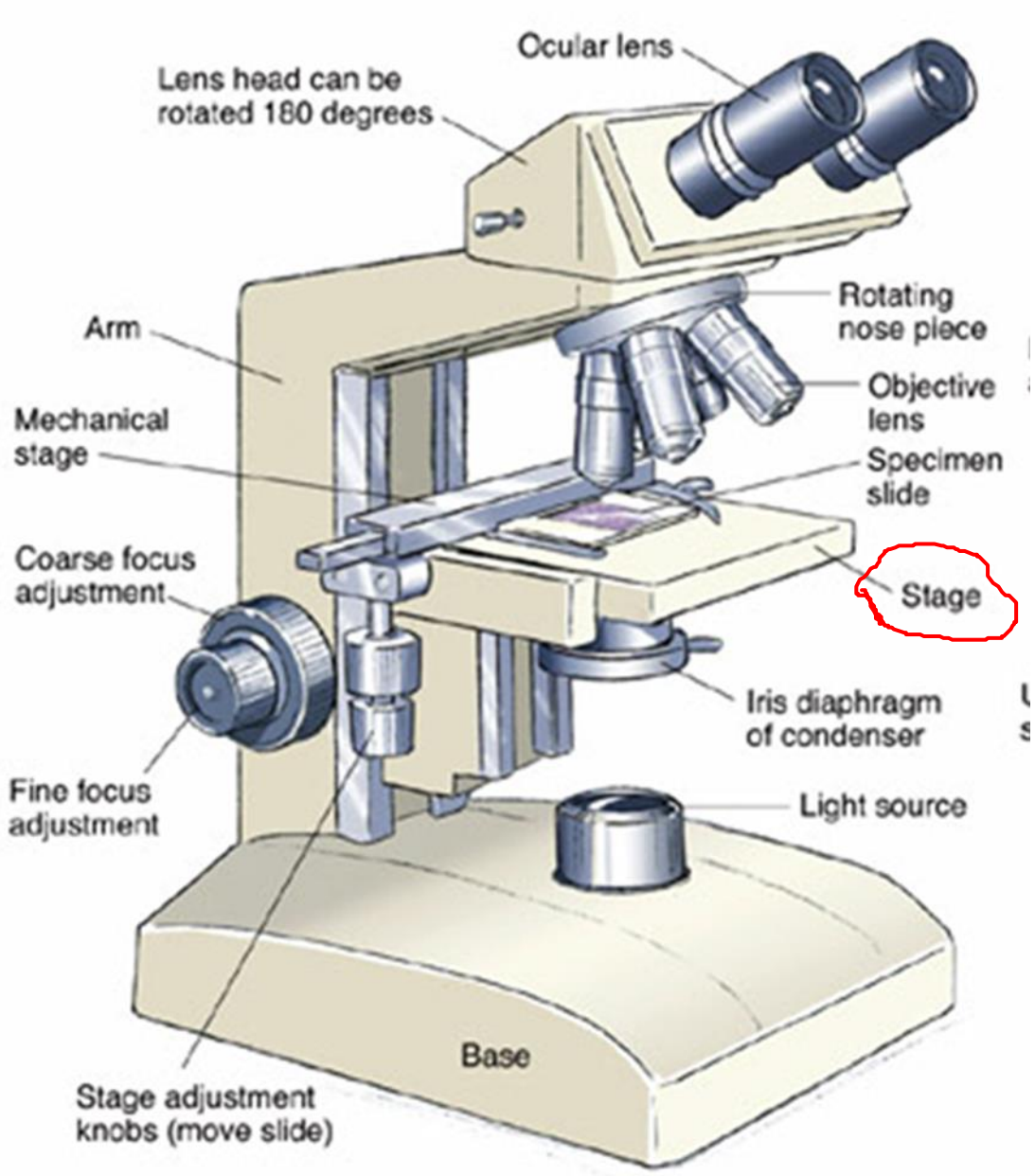
The magnification of the objective lens is written on the lens.

Note: with the exception of the oil immersion lens all the objective lens is used dry.

The magnification of oil immersion lens requires using the lens with special immersion oil for proper resolution.

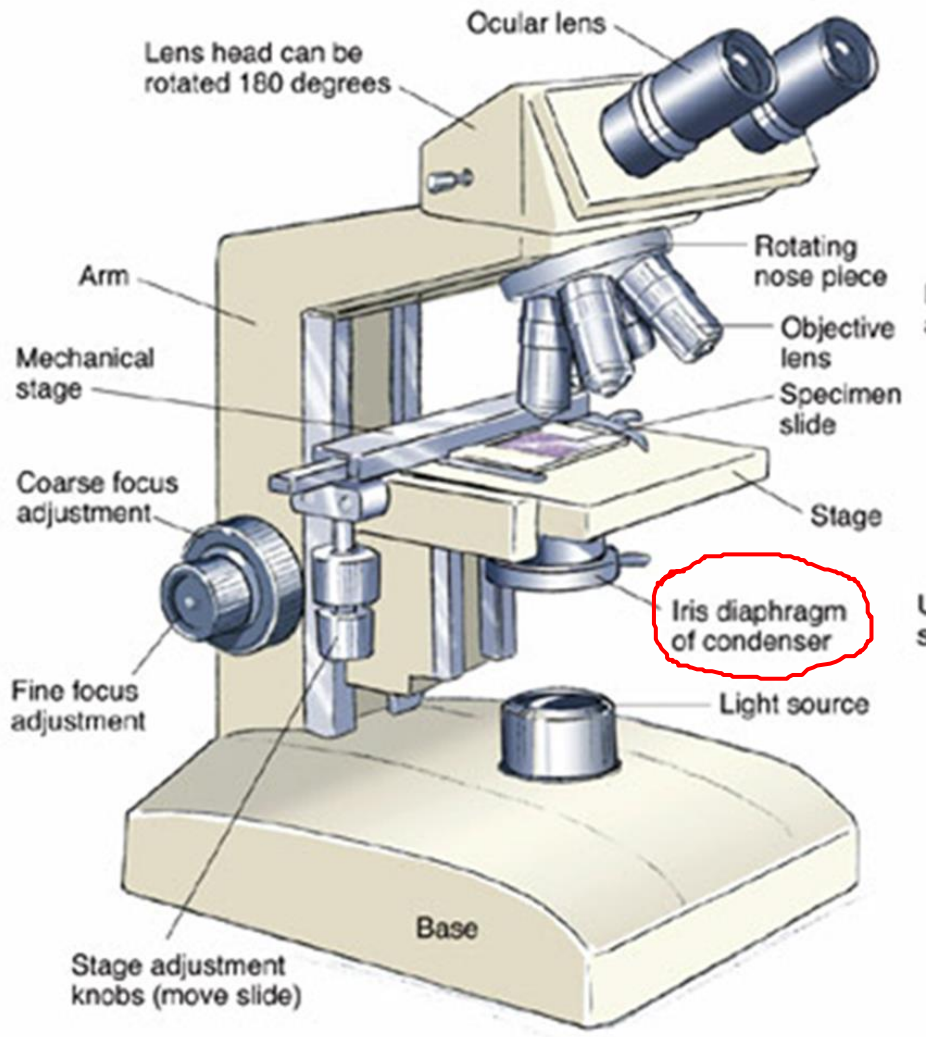
3. Stage

- The horizontal surface on which the slide is placed is called the stage. It may be equipped with simple clips for holding the slide in place or with a mechanical stage, a geared device for precisely moving the slide. Two knobs, either on top of or under the stage, move the mechanical stage



4. Iris diaphragm

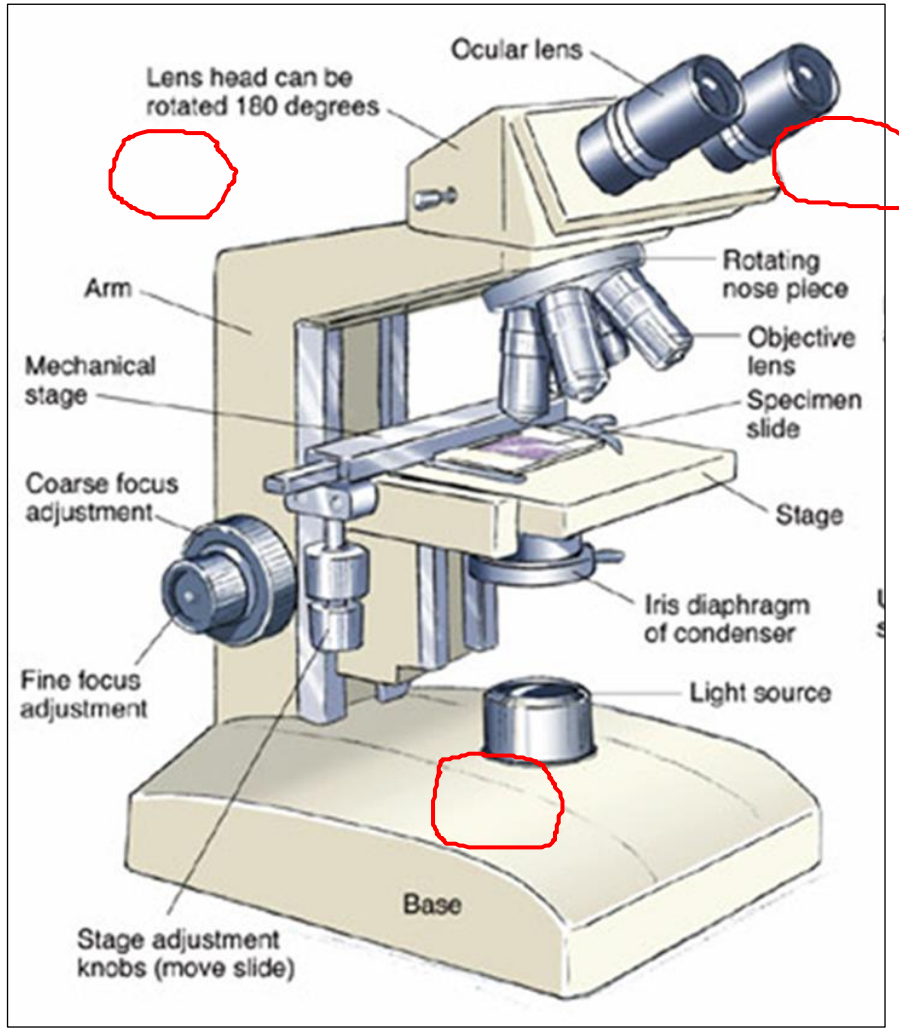
- Iris diaphragm is located below the condenser or immediately below the stage in microscopes without a condenser. It functions in regulating the light intensity passing through to the stage. More light is required at higher magnification



5. **Base:** Base – also called the supporting stand, rests on the bench.

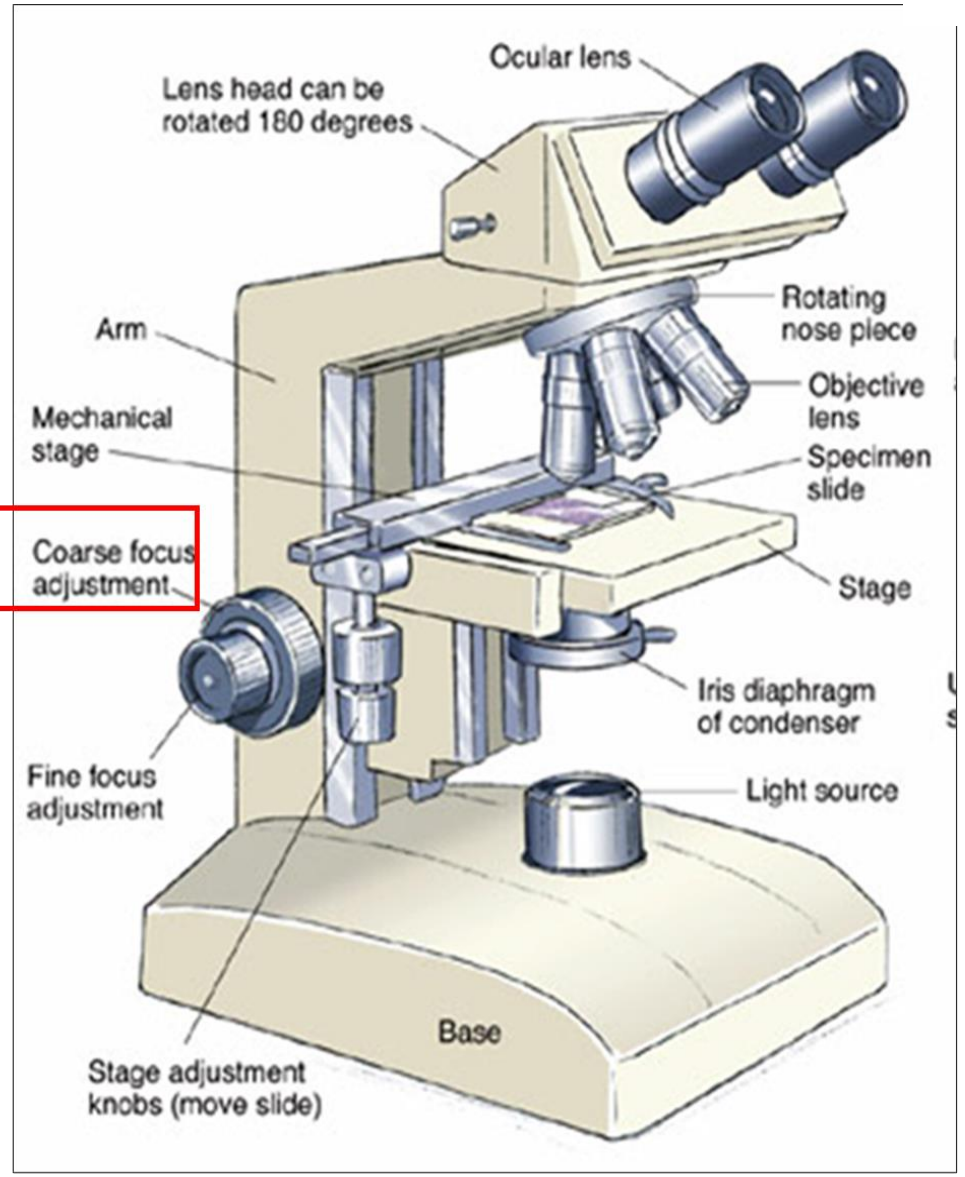
6. **Body Arm:** The body arm is used when carrying the instrument.

7. **Nose piece:** Nosepiece is the mounting for the objective lenses which rotates to bring the desired objective into position.



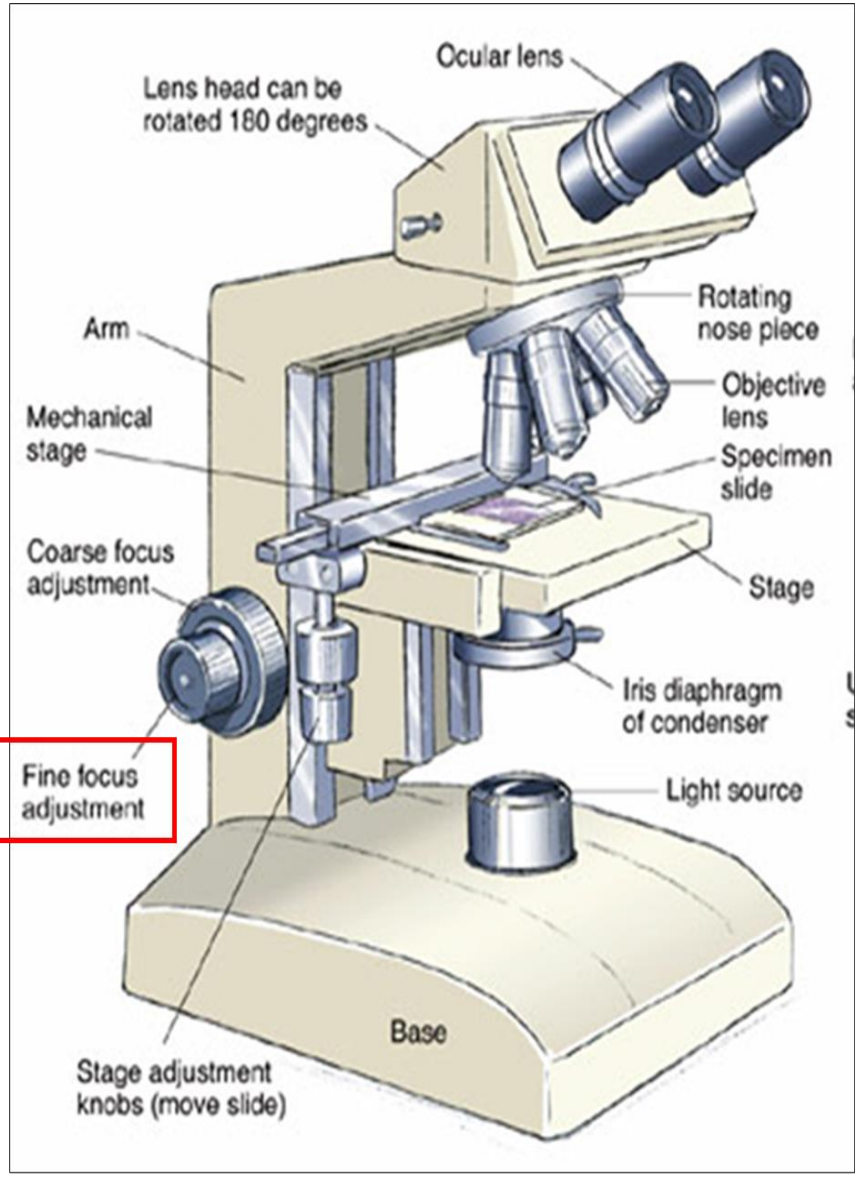
8. Coarse adjustment

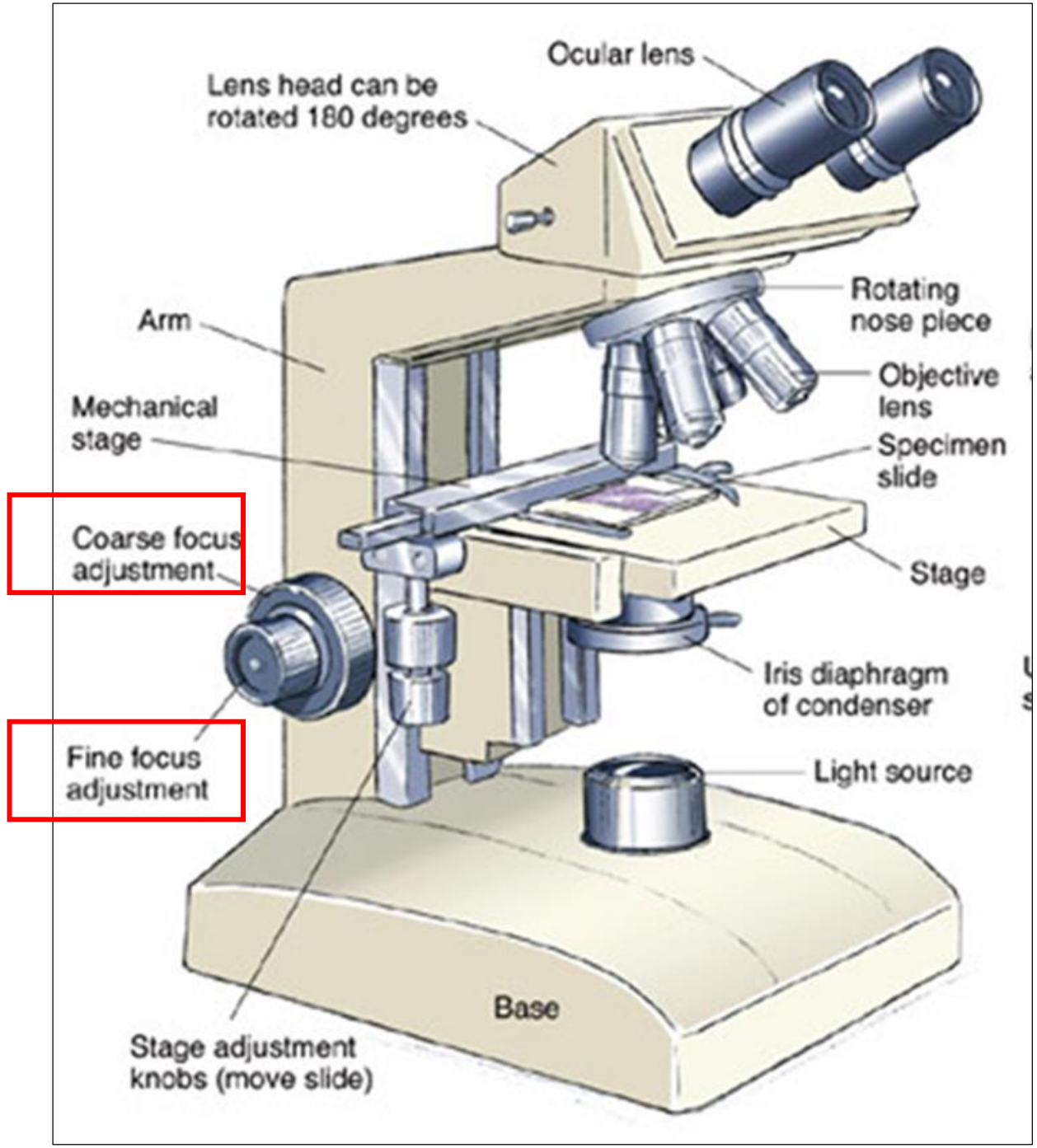
- Coarse adjustment knob is a large knob located at either side of the microscope which functions in controlling the distance between the objectives and the stage. Use the coarse adjustment **only with the scanning (4X) & low- power (10X) objectives. Why**? So coarse adjustment is used for rapid focusing of the specimen until the specimen is roughly in focus & then left alone, in which the fine adjustment knob controls precise focusing of the object.



9. Fine adjustment

- Fine adjustment is a small knob located at either side of the microscope. This is used for the control of the object, precise focusing you should use just the fine adjustment knob with the higher magnification objective lenses; Because using the coarse adjustment knob with the higher objective lenses may damage the lens &/or the slide you are observing.




Ocular lens
Lens head can be rotated 180 degrees
Rotating nose piece
Arm
Objective lens
Mechanical stage
Specimen slide
Coarse focus adjustment
Stage
Iris diaphragm of condenser
Fine focus adjustment
Light source
Base
Stage adjustment knobs (move slide)

Microscope safety cautions:

1. Always carry the microscope in an upright position using both hands.
2. Keep the microscope away from the edge of the table.
3. **Always examine a slide first with the low-or medium power objective, never use the high – power objective to view thick specimens.**
4. **Remove slide only after low-power objective has been rotated into viewing position, never when high – power objective is in positio**n.
5. Keep the stage dry at all times. A wet stage will prevent the slide from being accurately positioned.
6. When returning your microscope to its proper place in the cabinet always:
    a) Remove the slide from mechanical stage.
    b) Clean all lens surface and the stage.
    c) Rotate the nosepiece that the scanning lens is in place

Steps Used in viewing a slide:

1. Obtain a slide.
2. Check that the ocular and all objective lenses as well as the slide clean.
3. Use the coarse adjustment knob to obtain maximum working distance.
4. Place the slide on the stage, the slide should fit into the slide holder. Use the stage adjustment knob to move the slide over the hole in the stage.
5. Rotate the lower objective in place.
6. Look through the ocular. Adjust the light with the iris diaphragm level if necessary. Slowly turn up the coarse adjustment knob until something comes into focus. Use the fine adjustment knob to sharpen the focus.

8. Using the stage adjustment knob move the slide around until you find an area you wish to examine more closely. Move the slide until the object you wish to examine is in the center of the field.
9. Rotate the high-power objective into place. Use the fine adjustment knob to sharpen the focus. **Do not use the coarse adjustment knob**. Adjust the light using the iris diaphragm lever if necessary.
10. Rotate the high-power object halfway to the next position, place a drop of immersion oil on the slide, and then rotate the oil immersion objective into place. The objective should be immersed in the oil on the slide. Use the fine adjustment knob to sharpen the focus. Adjust the light using the iris diaphragm lever if necessary.
11. When finished viewing the slide use the coarse adjustment knob to maximize the working distance and remove the slide from the stage. If you are finished with the microscope clean the microscope and return it to storage.

Procedure for cleaning a microscope:

1. Turn off the light.
2. Using the coarse adjustment knob to obtain maximum working distance and remove the slide from the stage.
3. Using lens paper cleans all the lenses starting with the **cleanest first ocular**, and objectives lens.
4. Clean any oil off of the stage using paper towels.
5. Rotate the scanning objective into place. Use the coarse adjustment knob to obtain minimum working distance.
6. Cover the microscope with its **plastic cover** and Return the microscope to the appropriate storage area .

References

- Urry, L. A., Cain, M. L. 1., Wasserman, S. A., Minorsky, P. V., Reece, J. B., & Campbell, N. A. (2017). Campbell biology. Eleventh edition. New York, NY, Pearson Education, Inc.
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