

HISTOLOGY LABORATORY

Microscope Orientation and Blood Smear Lab

For practicing “how to use the microscope” DO NOT use the blood smear slide (it is too boring for the lower mags). Use a slide from the white box, e.g., W 48a, it is fairly colorful.

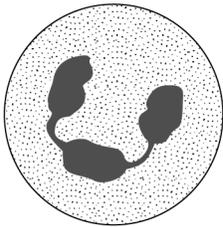
In our slides red (or orange-red or pink) structures are “acidophilic”; blue or purple structures are “basophilic.”



RED BLOOD CELLS (erythrocytes) are the most numerous: about 5,000,000/mm³. If your staining is decent, they are orange-red, about 7.5 microns in diameter (useful comparison for measurement), and may show a lighter-staining area in the middle. With this stain, you will not see reticulocytes. Red blood cells are not real cells since they lack nuclei, they are highly specialized end product of a cell line.

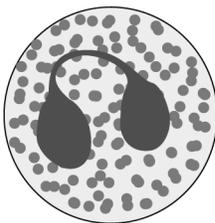
red blood cell

When searching for white blood cells, make sure that you are in an area of the smear where the red blood cells touch each other, but are not on top of each other. (In too thin an area it takes forever to find white blood cells; in too thick an area the white cells are also so crowded that they “curl up” and identification is difficult).



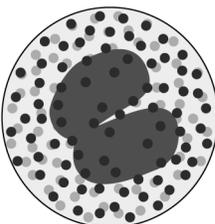
NEUTROPHILS, 55–60% of all blood leukocytes, 10–12 microns in diameter. Dark-staining (dark blue to purple) nucleus is lobulated; young (about 3%) neutrophils have a stab or horseshoe shaped nucleus. There are plenty of grey and purple colored granules in the cytoplasm, but you can see through the cytoplasm (unlike in eosinophils). Neutrophils, often called PMNs (polymorphonuclears, not quite correctly since eosinophils and basophils are also PMNs), are our first line of defense against foreign invaders. Lymphocytes need some time to get ready.

neutrophil



EOSINOPHILS (12–15 microns in diameter) usually have a bilobed nucleus. The cytoplasm is FILLED with orange-red granules, so that you cannot “see through.” Usually 4% of the leukocytes are eosinophils, unless you are allergic to something in which case there are more. Eosinophils may have been created to destroy parasites, but in Western world they do not have that much to do, so they may have turned against us.

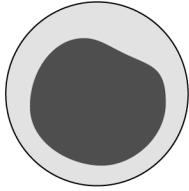
eosinophil



BASOPHILS are so few (less than 1% of leukocytes), that most likely you do not find one in your own blood smear. THEREFORE, go see an example in the demo microscopes in the interlab. Note that the dark blue or purple granules are large, numerous, and often mask the nucleus. Basophils and their connective tissue equivalents MAST cells also play an important role in defense against foreign invaders. HOWEVER, they can kill us by releasing too much histamine and other vasoactive and bronchoconstrictive substances they store or produce upon activation.

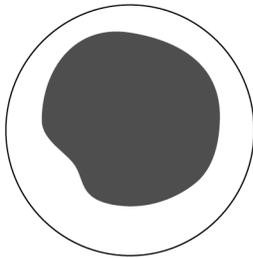
basophil

PLATELETS or thrombocytes can be seen clumped together. They are much smaller than red blood cells (about 2–3 microns) and have a mixture of blue and orange-red staining. They have no nuclei, since they are really pieces of a megakaryocyte cytoplasm.



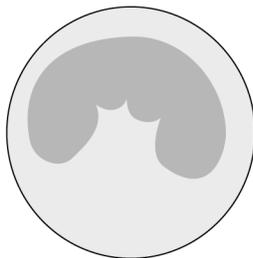
lymphocyte

LYMPHOCYTES (about 30% of all leukocytes) all look alike in ordinary blood smear. They have a very darkly staining, dense, round nucleus. Most lymphocytes are not much larger than the red blood cells, though their diameter may vary as much as 8 μm to 12 μm . The narrow cytoplasmic rim (in a well-stained smear) appears clear, sky-blue. Lymphocytes perform most of our immunological defense, and you will learn more about them later. (For future viewing them in sections: remember that here you are looking at the whole cell, in sections only a slice of lymphocyte is seen and nucleus will not look compact and dense.)



**Natural Killer
(NK) Cell**

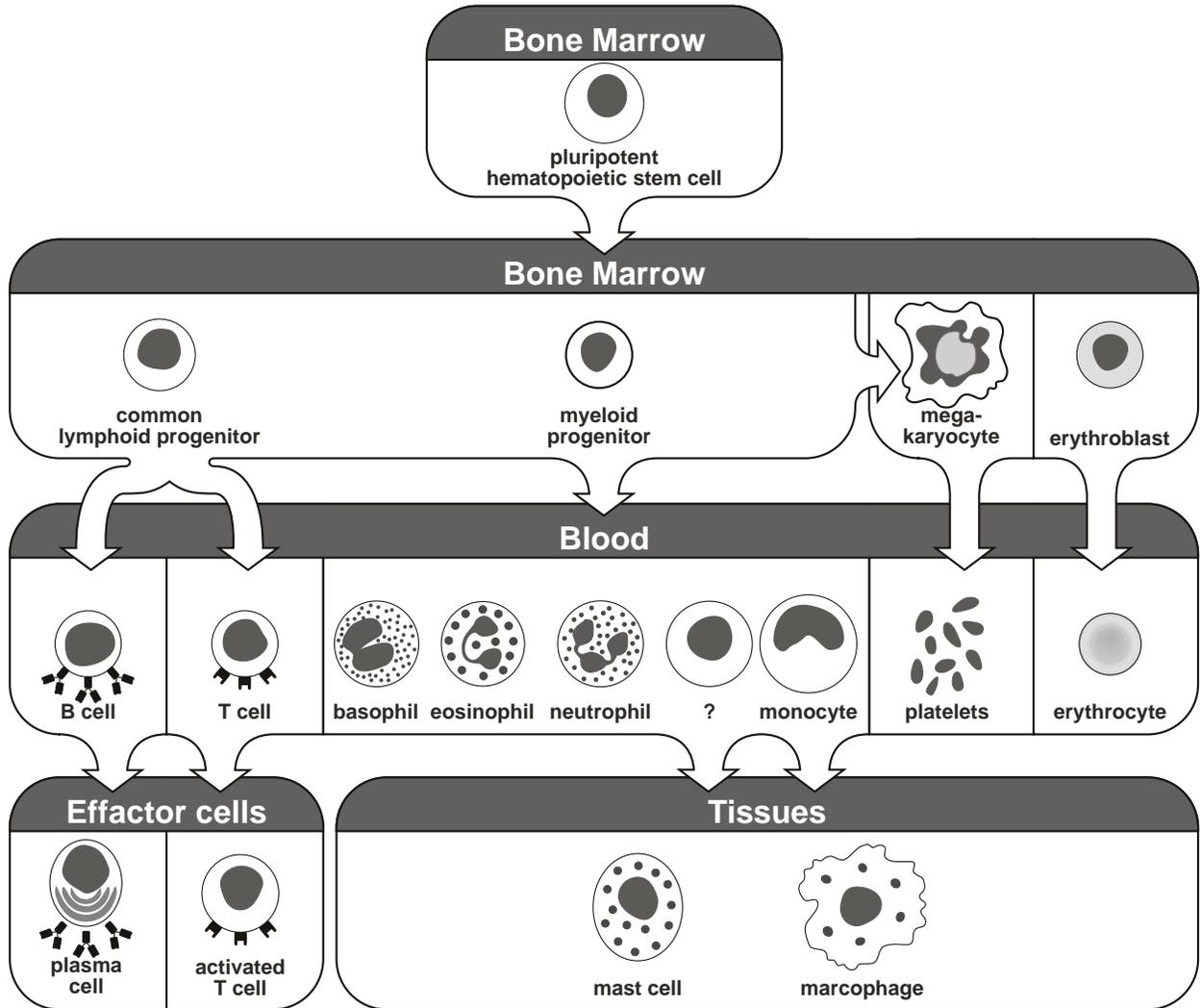
NATURAL KILLER (NK) CELL or large granular lymphocyte (LGL) (about 5% of all leukocytes) looks a lot like other lymphocytes. It is fairly large and the cytoplasm has some purple granules in it, and often does not stain sky-blue. It may be difficult to find these in your smear, HOWEVER, their existence is important to remember. They are different from other lymphocytes in that they can attack foreign invaders without previous sensitization. They also kill cancer cells.



monocyte

MONOCYTE, (3 to 8% of all blood leukocytes), precursor of **MACROPHAGES** is a **LARGE** cell (12–19 μm), usually much larger than the lymphocytes. Cytoplasm is especially abundant and lacks the sky-blue color; instead, it is often muddy grey looking. The nucleus is much less dense than in lymphocytes, it looks spongy, you can almost see through it. Often the nucleus is horseshoe shaped, or bent over itself (and resembles brain).

Remember that the red blood cells and platelets are not true cells (no nuclei), however, they are the ones who perform their functions within the blood vessels, whereas the leukocytes perform in the other tissues.



Lab Exercise, Unit 1

I. HOW TO USE THE LIGHT MICROSCOPE

This is meant for those students who have never used a microscope before, but it will do no harm for those with previous experience to follow through the procedure to refresh it in their mind. (Especially since you do not have to rent or buy the microscope you are using.)

Instructions refer mainly to the AO 50. If you use another scope, there will be minor variations to the theme which you should always become familiar with.

1. Remove the microscope from the cabinet by grasping the arm of the instrument and supporting the base with the other hand (Fig. 1). Always carry the scope in this way. Place the scope on your desk.
2. Adjust your chair height so that you will sit comfortably in a near erect posture while viewing through the eyepieces (oculars).
3. Familiarize yourself with the parts of the microscope (Fig. 2).
4. Plug the cord from the microscope to your transformer and the transformer cord into the wall socket. Turn on the illuminator, and switch the transformer to the "Med" setting. (Some microscopes have a built-in transformer.)
5. Since your microscope is equipped with an in-base illuminator, you do not have to fiddle with a mirror. In general, you can leave the light source unaltered for most purposes.
6. Raise the nosepiece using coarse adjustment knob. Rotate the nosepiece so that 10x objective (green ring) is in the operating position (you can feel definite stopping points and hear the click when it is in the right place).
7. Set the condenser iris diaphragm to approximately the half-way point.
8. Place a specimen slide, e.g., No. W31, on the stage and position the specimen above the center of the condenser.
9. Raise the condenser by means of the condenser knob until the top of the condenser lens is almost but not quite touching the slide.
10. Adjust the fine focus to approximately midway in its range.
11. Lower the nosepiece with the coarse adjustment knob until a stop is reached.
12. View through the right ocular and bring the specimen into focus with the fine adjustment knob. If you wear eyeglasses, you may want to remove them and focus the microscope accordingly; however, you may feel more comfortable keeping them on. Note: if you have an astigmatism, leave glasses on to avoid headaches; microscope does not correct for astigmatism.

13. Adjust the left ocular for your eye with the knurled ring (a in Fig. 3), in other words, turn the knob until specimen is in focus for your left eye. **DO NOT** touch the fine adjustment knob while adjusting the left ocular.
14. Adjust the interpupillary distance of the binocular body with the thumb wheel (b in Fig. 3). In some scopes this is done by simply pushing or pulling the binocular body. You should now be able to see comfortably with both your eyes. Try not to lean too closely to the oculars; keep a distance of about 3 cms.
15. Remove one ocular to view the condenser iris diaphragm. Close the diaphragm and then reopen until the leaves (edges of iris diaphragm) just disappear from the view. This step has to be repeated for each objective to obtain the full resolving power of the microscope.
16. This microscope is adjusted (provided all the objectives are well screwed in position) so that you can rotate the nosepiece, after the initial focusing with 10x objective, to the other objectives without having to coarse-adjust the focus. Only very little fine focusing is needed. You may need to increase or decrease the light intensity, though. When going from a lower magnification to a higher one, always center “the thing” that you are interested in the middle of the field in order not to “lose it.”
17. When using the oil immersion objective, the above-mentioned steps apply; however, certain additional steps are needed. After having focused the specimen under 40x, raise the nosepiece using the coarse adjustment knob. Place a small drop of immersion oil on the specimen in the center of the light, and rotate the nosepiece to the oil immersion objective, (100x, red ring). Bring down the nosepiece with the coarse adjustment knob until it stops. **NOTE!** This advice applies **ONLY** to the AO 50 spring-loaded nosepiece with the positive stop. (If you use other microscopes, bring the objective down by looking from the side until the objective just touches the drop of oil.) Bring the specimen into focus by using the fine adjustment knob.

When finished studying with the oil immersion objective, wipe off the oil from the objective and the slide with lens paper. MAKE IT A ROUTINE TO LIFT THE NOSEPIECE UP WHEN FINISHED WITH OIL SO THAT YOU DO NOT GET THE OIL INTO THE OTHER OBJECTIVES. If your oculars and/or objectives are dirty, ask help from the instructors or laboratory personnel.

Resolution: The least distance between two points at which they are seen as two objects, rather than a single blurred object.

Magnification: Simply, objective power times the ocular power.

When looking at the histology slides, you will find it particularly helpful to follow the subsequent procedures.

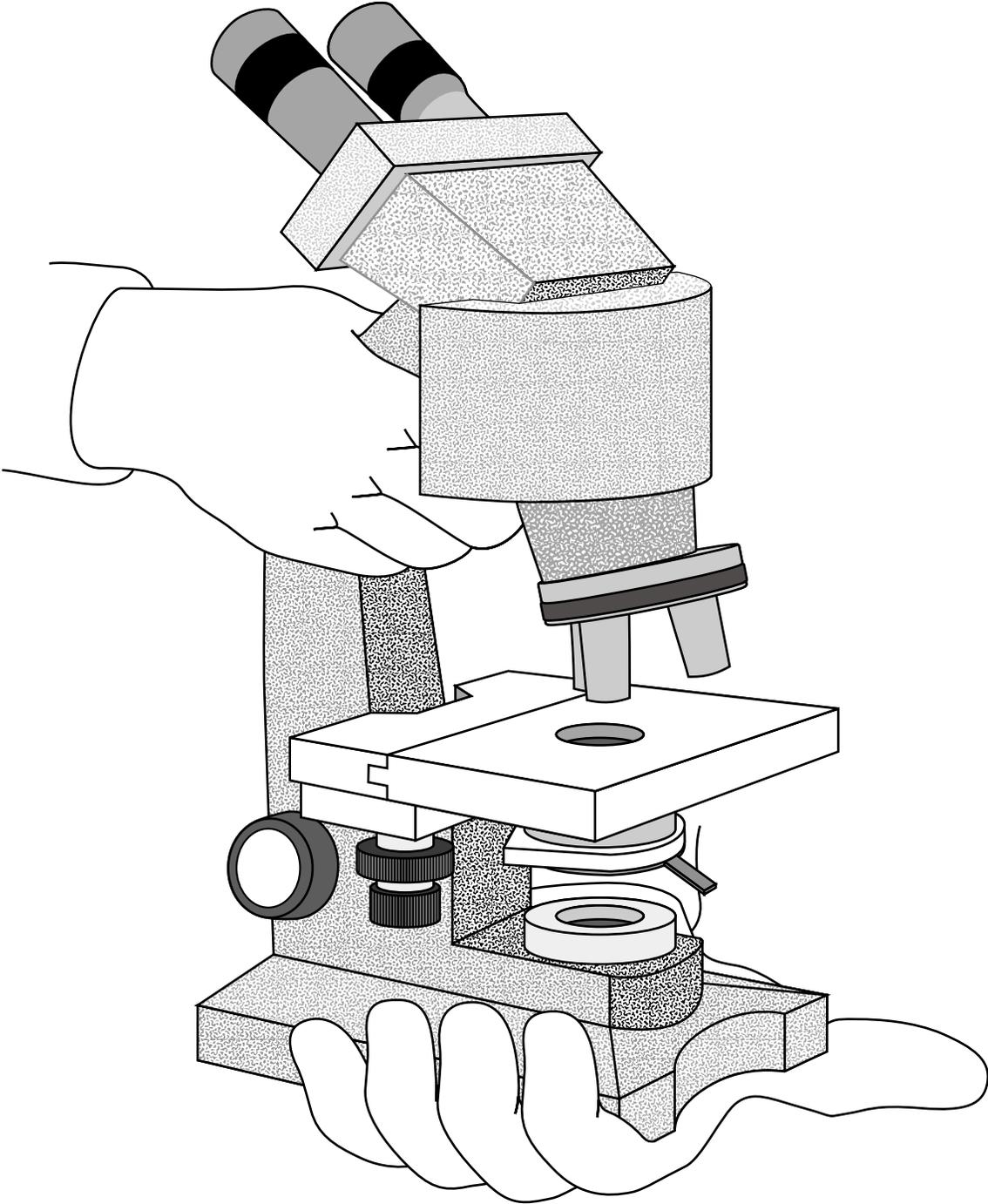


Fig. 1

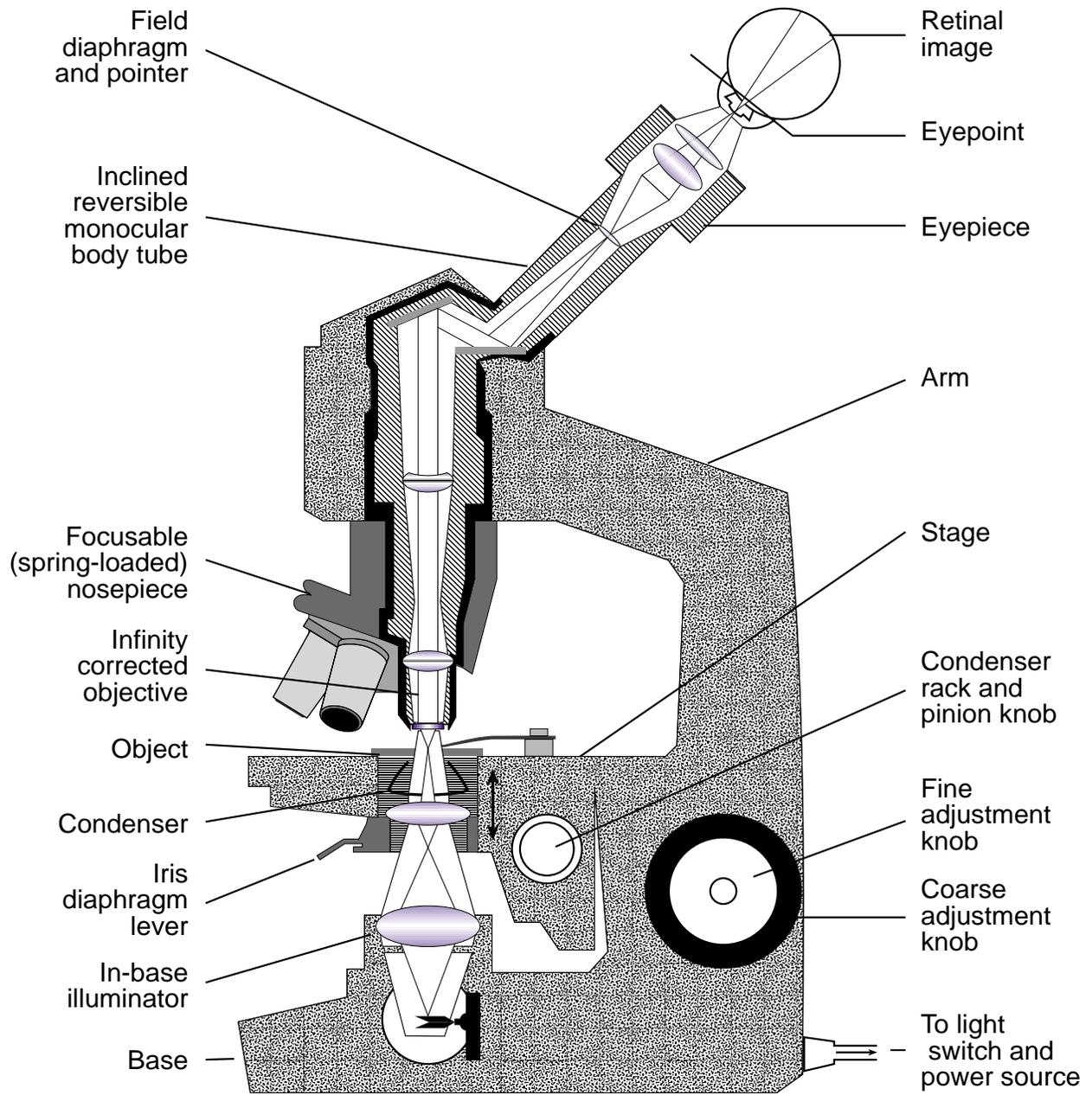


Fig. 2

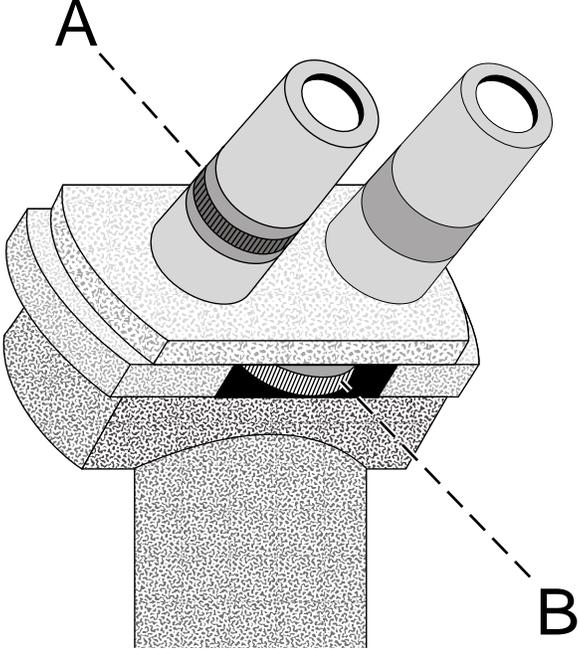


Fig. 3

1. Always look at the tissue on the slide with the naked eye first. Certain tissues have characteristic shapes in section which you should become very familiar with.
2. Next examine the section with the 10x eyepiece (ocular upside-down). Note the general organization plan of the tissue (lumen, connective tissue capsule, cross-section, longitudinal section, overall color of the staining, etc.).
3. Put the slide on the specimen stage and proceed from low to high magnification in examining the tissue. Remember that the microscope “reverses,” thus rotating the slide 180° horizontally will give you the same view as the naked eye view.
4. Always look for an optimal cross or longitudinal section. In general, look for a “typical example” of a cell or a tissue. With practice and experience you will learn to recognize atypical examples as well. Ask for help frequently in order not to get overwhelmed. You will need extra help particularly during the first three laboratories.
5. Return to low magnification and review the features learned in that slide thus far.

II. MICROSCOPIC SLIDE COLLECTION

Our slide collection is composed of a box of commercially available microscopic slides (referred to as the Black Box) and a box of SBMS-UC made slides (referred to as the White Box). Letters B and W in front of a slide number in the lab exercises indicate to which slide box we are referring. The commercial slides are paraffin embedded, 10 µm thick, mostly hematoxylin-eosin stained material. The homemade slides are of very high quality, glycol-methacrylate embedded, 1 µm thick (thin), and most of them are stained with methylene blue-azure and basic fuchsin. Whenever possible, use slides from the White Box because they are made from better preserved material, are thinner and, in general, give more information than the commercial slides. However, the new collection is not complete yet, so we still have to use material from the Black Box. During the year, new material will be added to the White Box and when applicable, new, detailed information concerning those slides will be distributed as an addendum to the lab exercises.

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