School

Literature, Russian Literature

1 Biology 2070H - Lab #1 MICROSCOPY AND CHARACTERIZATION OF CELLS Introduction: Cells can be very difficult entities to study. They are usually very small and transparent, yet extremely complex. Fortunately, there are many tools available to the cell biologist that aid in their study. The light microscope is to this day perhaps the single most important instrument used in Cell Biology. It is used under bright field conditions to study the organization of cells in fixed and stained sections of tissues. With phase contrast optics it is possible to monitor the movements of living cells and to observe changes in their subcellular organization. The light microscope may also be used to monitor certain operations such as cell fractionation and biochemical characterization of cellular components. See the following pages in ECB3 for more useful information: Microscopy: p. 6 -11 Characteristics of cell types: much of Chapter 1, see especially Panel 1-2 Cell Cultures: p. 157 and 161 Since cells are so difficult to examine without the aid of a microscope, they are not things that we are used to seeing everyday. In this exercise we will 1) review how to use a compound microscope 2) examine and compare different types of cells (eg. plant, animal, fungus, eukaryotic, prokaryotic) 3) gain an appreciation of the relative sizes of different types of cells 4) review how to estimate field size and the size of a cell under the microscope. 5) learn about different types of light microscopes and how they are used It is helpful to work with a partner, so that you can discuss what you’re observing. I. COMPOUND MICROSCOPES Review the section on how to use a compound microscope at the beginning of this manual. You should go through this section on your own and consult with the course TAs if you have any questions. If you have not previously learned how to use a compound

microscope, please consult with your TA. II. TYPES OF CELLS AND THEIR RELATIVE SIZES The objective of this part of the lab is to examine a variety of types of cells in order to discover some of their general characteristics. Examine the cells that are available to you (these may deviate somewhat from the following list, depending on availability). Then answer the questions that are at the end of the lab. 2 USE STERILE TECHNIQUE AS DEMONSTRATED BY YOUR T. A. WHEN HANDLING ALL FRESH MATERIALS Material that will be available for you to examine will include the following: A. B. C. D. E. F. G. 0. 85% NaCl suspension washed red blood cells Suspension of yeast Suspension of bacteria Suspension of algae You will prepare a smear of your own cheek cells. Cultured animal cells (cells growing in vitro) There will be several prepared and stained slides for you to examine, to help you in understanding your observations for A to E. Procedure: A to D Suspensions: 1. For A to D above, place a loopful of one of the suspensions on a glass slide. Cover with a cover-slip avoiding excessive air bubbles. If excess fluid escapes from the cover-slip, it may be removed by blotting with the end of a paper towel strip which is then discarded in a disinfectant jar. Examine each wet mount successively under low (10), high dry (40), and oil immersion (100) objectives. You will be given a demonstration of how to use oil immersion. Please follow the instructions very carefully, in order not to damage the microscope objectives. In your lab notebook, describe or illustrate your observations at the most informative magnification. Be creative in your observations. Note the colour of each cell type, its movements, any internal features, anything that makes it distinct from the other cells. Also note any similarities. How do eukaryotic cells differ from

prokaryotic cells? How do plant cells differ from animal cells? For A to D, estimate the size of each type of cell, using the method described at the beginning of the lab manual. If the bacterial cells are too difficult to observe, try preparing a second wet mount of the cells and staining them by mixing equal parts with the stain provided (e. g., 1% aqueous crystal violet). Examine as before. You can also try examining unstained cells using phase contrast microscopy. This is only available on a selection of microscopes and instructions will be given in the lab. Note that the field of view might be a different size on different microscopes. 2. 3. 4. 5. 3 E. Cheek cells 6. Take a toothpick and scrape the inside of your cheek in order to remove a few of the epithelial cells. Smear the scrapings on a clean slide. On the underside of the slide, mark a ring with a wax pencil around the area of the smear so that it can be located under the microscope. Let the smear dry completely and examine under low or medium power. Take the same slide and stain it with safranine for 1 minute. Rinse off the stain with water and blot dry. (It is also possible to do a wet mount by adding a drop of stain and covering the smear with a cover slip.) Examine the slide. You may be able to observe bacteria along with the epithelial cells. Estimate the size of the epithelial cells. F. Cultured cells 7. Cultured cells typically grow in a defined medium in a dish or flask. The presence of the flask walls or the lid of the culture dish prevent the objectives in a regular microscope from getting close enough to the cells to have a clear view. In order to see cultured cells, an Inverted Microscope is used. The objectives are below the sample, and the light source and condenser are above. Examine the demonstration of cultured cells using an Inverted Microscope. How do these cells differ from the other cells you've

examined? G. Prepared slides 8. The slides we have prepared so far are not permanent. In order to preserve the samples so that slides may be observed on future occasions, samples need to be fixed. The samples may be left whole or they may be cut into very thin sections. Examine the prepared slides that have been set out for you and note how features differ from the slides you made yourself. 4 ASSIGNMENT Worth 15% of the final mark. It will be graded out of 45 marks. You don't need to write a formal lab report, just answer the questions. However, be sure to include citations and the list of references you used for your information, where appropriate. 1. (4 marks) What is the field size at each magnification for the microscope you're using? Show your calculations and give your answer in millimetres. 2. (16 marks) Briefly summarize the observations you made during the lab, using a comparative approach. You may use a table. Using these observations to formulate a specific question regarding some aspect of cells that you would like to explore. State your question and the method of how you would go about answering it. Iinclude one primary reference that uses the technique you are proposing to use and very briefly describe what they were investigating and the primary result. 3. (5 marks) Rank the cells you have observed in order of size, stating the specific size you have calculated for each. Show how you carried out the calculation. Include the cell suspensions and the cheek cell samples (A to E). 4. (8 marks) Choose one cell type that you found interesting and submit a drawing of it. Refer to the section on how to do a scientific drawing. Title each drawing, label the cells properly and include a scale bar. Choose the magnification and conditions (unstained, stained) that you feel are best to illustrate the cells, and in a separate

paragraph (i. e. NOT on your drawing) give your reasons for choosing this particular cell and conditions. 5. (8 marks) Choose one primary reference that uses cultured cells as an experimental model. Describe briefly the type of cell used, why these particular cells were useful for the study and how they were used. List two ways in which the cells used in the study would resemble cells that are growing within a body, and two ways in which they would differ. 6. (4 marks) Using your textbook or other sources, briefly describe two types of microscopes other than light microscopes, and explain for what purposes they would be used. 5 REVIEW OF PROCEDURE FOR USE OF COMPOUND MICROSCOPE (from first year Biology) General considerations: 1. CARRYING - always carry in a vertical (straight up and down) position as parts can fall off and break (e. g. eyepieces, glass filters, etc.). Carry the microscope by its arm, supporting the base from underneath with your other hand. NUMBERING - your microscope has a number. This number corresponds to a numbered space on the shelf. Your microscope should go back in this space. This is done to control inventory. PLUGGING IN - some microscopes plug directly into the electrical outlet, and some plug into a transformer that then plugs into the electrical outlet. You can usually tell by the type of plug the microscope has. CHECK THIS FIRST TO AVOID A SHOCK. 2. 3. Figure 1: Compound Microscope 4. WORKING THE LIGHT - if the microscope can only plug into the transformer one way, it will have a rheostat knob, which when turned to the right, turns on the light and controls the intensity. The further to the right the knob is turned, the brighter the light. Microscopes that plug directly into an outlet have this feature on their base. PRECAUTIONS- keep the intensity low to start (or else it will be too

bright to focus.) 6 - remember to turn the light down and/or off when you are done. - use of the bulb at high intensity reduces bulb life. There are other ways to control the brightness of the light (associated with the CONDENSER). 5. MOUNTING THE SLIDE - The first two things to do are to: a) Lower the stage away from the objectives to give working room (2-3 cm is good). To do this use the coarse focus knob on the column. b) Rotate the lowest power objective into place. Now you are ready to place the slide on the stage. Pull back the clip on the left, place the slide on the stage so that the lower right hand corner fits snugly into the 90 degree angle of the slide holder, then GENTLY release the clip to hold the slide in place. The slide should be held securely at this point. Find the slide controls on the stage, and move them until the specimen is centred below the objective. 6. FOCUSSING a) Check that the lowest power objective is in place. Now use the coarse focus to raise the stage as high as it will go. b) Look through the eyepieces. You cannot see the specimen at this point, but you will notice the intensity of the light. Adjust this intensity for suitable viewing. c) Adjust the interocular distance by gently pulling the eyepieces apart, or gently pushing them together, until you see a single, circular field of view. d) Rotate the coarse focus knob. It will only move in one direction, ie. lowering the stage, until the specimen comes into focus. Complete focussing using the fine focus knob, which may be located with the coarse focus knob, or separately on the column. NOTE: IF YOU HAVE DIFFERENTLY SIGHTED EYES you should adjust the focus of the individual eyepieces. To do this, you should first close your left eye and adjust the focus with the fine adjustment knob for your right eye. Then close your right eye and open your left eye. Rotate the left eyepiece until the

image is clear. Check that both eyes are now in focus. People with similarly sighted eyes should also check that both eyes are in focus, and adjust if needed. 7. POWERING UP - Higher power magnifications may now be used by moving the next higher power objective into place and adjusting the focus accordingly. NOTES: a) Very little focussing should be necessary (fine focus only), as you have already focussed the instrument on the lowest power. On most microscopes the objectives are PARFOCAL with one another. This means that if you have focussed with a lower power objective, the next higher one will already be " partially" focussed. If you find you need too much focal adjustment, it means that you have lost focus. To avoid breaking the slide and the objective lens (a very costly mistake), YOU MUST START OVER WITH THE LOWEST POWER 7 OBJECTIVE IN PLACE. b) As you move to a higher objective, you are making the centre of your field of view bigger. Therefore move the object you wish to look at more closely into the centre of your field of view with the slide controls. c) The 100X objective is an OIL IMMERSION lens. It should only be used with special immersion oil. The technique for doing this is covered in Section III. 8. USING THE CONDENSER -- As you power up, it becomes more important to use proper lighting. The more you magnify an object, the darker its image, as less light is reaching your eye. The CONDENSER can solve this problem. It is a small unit located under the stage, and its role is to focus the light that is emitted from the source onto the specimen. Its proper use is critical for specialized techniques, such as Koehler illumination, described in Section III. a) There is a knob below the stage on the left of the microscope that raises and lowers the condenser. The higher the position of the condenser, the brighter the

light. For our purposes the condenser should generally be set 1-2 mm below its highest point. b) In front of the condenser is a lever which can be moved to the left or right. This opens and closes an IRIS DIAPHRAGM, which controls the amount of light passing through the condenser. Opening the iris diaphragm makes the image brighter, so if your image looks dark and grainy open the iris diaphragm. However, if your image looks washed out, try closing the iris diaphragm just until the image begins to dim. Closing it even more may increase contrast, but resolution, or in other words detail, will be lost. c) On the right hand side of the condenser is a knob, which will move a lens called the auxiliary lens into place below the slide. This lens should be moved into place when using the higher powers, as it focuses the light on the specimen and makes the image brighter. d) Some condensers have a swing out filter on the bottom that is used to diffuse the light. e) IMPORTANT: all condensers have two centring screws at the front. DO NOT MOVE THESE as all of the condensers have already been centred for your use. 9. CLEANING - Glass surfaces are hard to keep clean. Fingers leave an oily print and dust collects. Slides and cover slips should be handled carefully by their edges. (BE CAREFUL--COVERSLIP EDGES CAN BE QUITE SHARP.) You can tell how clean your slide is by looking through your slide at a light source. Slides can be cleaned by polishing with a paper towel. However, ONLY SPECIAL LENS PAPER must be used to clean the optics on the microscope itself (eg. the surface of the eyepiece lenses, and objective lenses.) You will find lens paper in the drawers under the bench. FINISHING UP - Turn down the light intensity and turn the transformer off. Unplug the microscope or the transformer from the electrical outlet. Lower the stage away from the

objective and remove the slide. Clean up any remaining oil at this point. Rotate the lowest 10. 8 power objective into place (NOTE: The microscope must be stored with this objective in place.) Replace the microscope in the correctly numbered spot in the cupboard. SPECIALIZED TECHNIQUES A. Oil Immersion This is essentially a simple technique made tricky by three things: -- the lighting is critical and must be adjusted for optimum viewing. In general, the condenser should be adjusted so that more light is reaching the specimen. -- the objective is almost sitting on the slide at the point so there is little room for focal adjustment. It is very easy to loose focus. -- you must immerse the 100X objective lens in use a special oil, called immersion oil. This oil must be cleaned from the 100X objective, as well as from the microscope slide when done. USE ONLY LENS PAPER TO CLEAN THE OBJECTIVE LENS. If you lose focus, the oil must be cleaned off, and you must start at lower power, focus again, and reapply the oil. The steps for oil immersion are as follows: 1. Focus on the specimen at the 40X objective. Centre the specimen. Move the objective nosepiece halfway between 40X and 100X. Apply a drop of oil at the point of light on the slide. Move the 100X objective into place. Adjust the focus using the fine focus only. Adjust the lighting if necessary. When you are finished, lower the stage and wipe the 100X objective free of oil with lens paper. The slide must also be cleaned free of oil with lens paper. 2. 3. 4. 5. 6. HELPFUL HINT: When examining unstained translucent cells, it may not be possible to see a clear light microscopic image by following the procedure for proper focusing. In this case, contrast may be increased by moving the condenser farther away from the specimen and closing down the iris diaphragm. Try adjusting the

condenser and iris diaphragm until a clear image is seen. C. Estimating specimen size An accurate measurement of a specimen under the microscope may by obtained using an eyepiece micrometer. If one is not available, it is still possible to estimate the size of an object by comparing it with the diameter of the field of view. To do this, it is first necessary to measure the size of the field. Read through the following instructions and apply them to the cells which you will be examining. 9 1. With the 4x or 10x objective in place, move a transparent ruler over the opening in the centre of the stage so that the lines are visible through the microscope. 2. Move the ruler so that a vertical millimetre mark is just visible at the left edge of the circular field of view. 3. Count the number of millimetres from the left side to the right. If the right side of the field does not line up with one of the vertical markings, estimate the fraction of a millimetre. This is the diameter of the low power field of view. Record your measurement in millimetres (mm) and in micrometers (Î¼m). 4. Carefully move the 40X objective into place. Note that the diameter of the field is less than 1 mm. Rather than measuring the field directly, we can calculate the diameter based on the direct measurement of the low field diameter and the following equation: diameter of the high power field low power magnificat ion = diameter of the low power field high power magnificat ion You will notice that the decrease in field size is proportional to the increase in magnification. If the magnification is ten times greater (eg. 1000X vs. 100X), the field size will be ten times smaller (0. 1mm vs. 1. 0mm). For the microscope you are using, what is the diameter of the field for each objective in millimetres? in micrometers? Record the figures in your lab notebook. How do your measurements compare to those

of others in the lab? 10 The following drawing shows a field of view at 40X and 400X magnifications. Note that when the magnification increases the area that the field of view covers decreases. In effect, you see only a fraction of the tissue at the higher magnification, although it is in much greater detail. Finally, in order to determine specimen size, estimate the fraction of the field size that the specimen occupies. Ask yourself how many of the cells could fit across the field of view. If, for example, you estimate that you could fit six cells across a field, the size of the cell will be one-sixth of the field diameter. For example, if the field diameter in A below is 0. 1mm and you estimate 6 cells could fit across it (B), the size of the cell would be 0. 1 ï€© 6 = 0. 017mm or 17Î¼m. HINT: At this point, you may need to refresh your memory regarding units of measurement. Recall that 1 millimetre (mm) = 1 X 103 micrometres (Î¼m) = 1 X 106 nanometres (nm). 11 D. Scale Bars If necessary, the relative size of the specimen should be indicated by a scale bar, often to the bottom left of the drawing. This can be calculated as follows: actual size of specimen length scalebar represents = size of specimen as drawn length of scalebar as drawn Usually the length the scale bar represents is chosen to be an appropriate " round" number, such as 1 Î¼m or 5 Î¼m, (depending on the actual size of the specimen) and the equation is solved for the length of scale bar to draw on your figure.