LAB : MITOSIS AND MEIOSIS

Lab format: this lab is delivered through a combination of lab kit (LabPaq) and RWSL

Relationship to theory: In the textbook (Reece et al., 9th ed.), this lab is related to Unit 2 The Cell and Unit 3 Genetics

LEARNING OBJECTIVES

AFTER COMPLETING THIS LABORATORY, YOU SHOULD BE ABLE TO PERFORM THE FOLLOWING:

1. Describe the cell cycle.
2. Identify stages of mitosis from prepared slides.
3. By examining the mitotic region of an onion root tip (Allium cepa), calculate the percentage of time a cell spends in the various stages of the cell cycle.
4. Recognize the process of meiosis in female and male reproductive tissues.

INTRODUCTION

To the Student:
You are part of an exciting opportunity to utilize a remote microscope to add to your educational experience. CCCS is working together with the North American Network of Science Labs Online to develop additional laboratory experiences using Remote Web-Based Science Laboratories (RWSL). This opportunity will give you access to microscopes with higher resolving power and image capture technology. In the future other equipment such as spectrophotometers will be added to the RWSL giving online students the same opportunities to use laboratory equipment that up to this point was limited to students in traditional settings.
We hope that you feel this opportunity adds a new level of excitement and education to your class.

EQUIPMENT

- Unlined paper
- Pencil
- LabPaq microscopy lab and included equipment
- Slides
- Computer (access to remote microscope)
ACTIVITY 1: PREPARING TO USE THE RWSL MICROSCOPE

Before you connect to the RWSL microscope:
Follow the LabPaq instructions to complete the laboratory as it is written.

Connecting to the RWSL microscope
Please use the following instructions to connect to the RWSL microscope:

1. **If you have not already done so**, please ensure that your computer system is capable of interacting with the RWSL microscope. Currently RWSL works only on the Microsoft Windows operating system (XP or later) and a relatively up-to-date browser. Unless you have already done so, confirm that your system meets minimum requirements, visit this website: [http://at.ccconline.org/rwsl/installguide/](http://at.ccconline.org/rwsl/installguide/) (for BCcampus students: [http://rwsl.nic.bc.ca/installguide/](http://rwsl.nic.bc.ca/installguide/)) and follow the steps provided. For more information about certifying your system’s readiness for RWSL, see Appendix A.

2. When you first log in to the RWSL microscope, you will see a view similar to Figure 1 (below). Note the variety of controls and their relationship to controls on other microscopes you have used.
Figure 1: RWSL microscope view

3. The **camera controls** appear on the right side of the screen. These controls enable you to view the sample on the slide, change the viewing according to several special effects, and control white balance. Clicking on ‘picture in picture’ will let you view the position of the objective and the action of focusing. There are pan and zoom controls for the PIP camera that allow you to “look around” at the microscope and its surroundings.

4. You can also capture a high resolution image for saving to your own computer. Click on the “Capture Image” button and it will glow green for about 20 seconds as it downloads the image. After the “Capture Image” button turns dark again, click on “View Captured Image” at the bottom of the screen and the high resolution image will appear in a separate browser window. Right-click on this image and save it to your own computer. Make sure you give the saved image a unique name and notice where you save it so you can find it later.

5. The **stage and magnification controls** appear on the left side of the screen. These controls should be quite familiar to you. Note that by clicking on the ‘coarse’ button between the ‘up’ and ‘down’ controls will enable you to toggle your focusing speed between coarse and fine.

6. When using the stage and magnification controls CLICK AND HOLD the button until the desired effect is achieved. DO NOT click the buttons repeatedly, as this can lock up the system.

7. At this time, **all slides must be changed manually** by the lab technician. Up to two slides can fit on the stage at one time, but the technician will only load one slide at a time. For detailed information about the RWSL microscope and its controls, see Appendix B.

8. Before you begin this procedure, it is best to print out the LabPaq pages and also this set of instructions so you can read them side-by-side while performing the steps.

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**EXERCISE 1: MITOSIS IN ANIMAL AND PLANT CELLS**

Once you have logged on to the microscope you will perform the following Laboratory Instructions:

**Procedure:**

1. Request the prepared slide of the whitefish blastula be placed on the RWSL microscope.

2. Locate the cells using 40x objective then increase the magnification to 60x in the following stages of mitosis: Interphase, Prophase, Metaphase, Anaphase and Telophase. Use the “capture image” feature on the RWSL control panel to capture an image of the above stages at 60x. Place your images below.
3. Use the insert and textbox feature on your computer word processing program to label the parts of the cells as outlined in question 3 in the LabPaq instructions.

4. Request the prepared slide of the onion root tip be placed on the RWSL microscope.

5. Locate the cells using 40x objective then increase the magnification to 60x in the following stages of mitosis: Interphase, Prophase, Metaphase, Anaphase and Telophase. Use the “capture image” feature on the RWSL control panel to capture an image of the above stages at 60x. Place your images below.
6. Use the insert and textbox feature on your computer word processing program to label the parts of the cells as outlined in question 3 in the LabPaq instructions.

**ADDITIONAL RWSL ACTIVITY: CALCULATING THE PERCENT TIME SPENT IN EACH STAGE OF ONION ROOT TIP MITOSIS**

At the time when a slide of an onion root tip was prepared, the cells in the region of cell division were arrested at their current phase within the cell cycle. Some were fixed at the time of interphase and others were fixed at some stage of mitosis. The duration of each stage in the cell cycle of the onion root tip can be estimated by determining the proportion of cells arrested at each stage of mitosis and interphase.

Let’s assume that you examined a slide and determined the stage at which about 200 cells were arrested at time of fixation. Table 2 is a summary of your results. It is known that onion root tip cells take about 16 hours to complete the cell cycle. By determining the percentage of cells in each stage of mitosis and in interphase, you can calculate the amount of time spent in each stage. For example, if twenty cells out of 200 were found to be in prophase, the percentage of cells is \( \frac{20}{200} \times 100 = 10\% \). This shows that any one of the hypothetical cells spends 10% of the time in prophase, so they spend \( 0.10 \times 16 \text{ hr} \) or 1.6 hr (1 hr and 36 min) in that stage.

**Procedure:**

Examine the slide of an onion root tip using the 40x objective. Count and record the stages of the cycle of each of the cells in your field of view, then calculate the percentage of cells in each stage, and enter the results in Table 1 (no calculation is required if you have the patience to count exactly 100 cells; just enter the number of cells). Keep in mind that you must count enough cells to make a representative sample. If you count too few your data will likely reflect the hypothetical estimates provided earlier. If you are using the highest power ocular, you may have to record more than one field of view.
Calculate the hours and minutes spent in each stage, assuming the entire cell cycle takes 16 hours (0.1 hr = 6 min).

**TABLE 1: DATA ON STAGES IN PLANT CELLS**

<table>
<thead>
<tr>
<th>Cell Cycle Stage</th>
<th>Number of Cells in the Stage</th>
<th>% of Total Cells in the Stage</th>
<th>Hours and Minutes in the Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telophase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>16:00</td>
</tr>
</tbody>
</table>

**TABLE 2: HYPOTHETICAL DATA**

<table>
<thead>
<tr>
<th>Cell Cycle Stage</th>
<th>Field 1</th>
<th>Field 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td>71</td>
<td>101</td>
<td>172</td>
</tr>
<tr>
<td>Prophase</td>
<td>13</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>Metaphase</td>
<td>12</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Anaphase</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Telophase</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total Cells Counted</strong></td>
<td></td>
<td></td>
<td><strong>235</strong></td>
</tr>
</tbody>
</table>

**Discussion Questions**

1. How does your data compare to that of the hypothetical data given in Table 2? If not exactly the same, discuss some of the reasons this might be.

2. Why is the tip of the onion root chosen for this activity?
3. How do the sizes of cells in cytokinesis compare with those in Prophase? Why?
EXERCISE 2: MEIOSIS IN ANIMALS

Procedure:

1. Request the prepared slide of the ovary be placed on the RWSL microscope. Low power is the best for this slide initially. Follow the directions for steps 2 and 3 as presented in the LabPaq. Make sure to capture an image and include it below with labels for the *primary follicle, primary oocyte, secondary follicle and secondary oocyte*.

4. Request the prepared slide of the testis be placed on the RWSL microscope. Low power is the best for this slide initially. Follow the directions as presented in the LabPaq for step 4 and 5. Make sure to capture an image and include it below with labels for the *seminiferous tubules*.
6. Change the magnification to 40X and follow the steps in the LabPaq instructions. Make sure to capture an image and include it below with labels for the mature tailed sperm.
APPENDIX A: CERTIFYING YOUR SYSTEM FOR CONNECTION TO RWSL

Our ideal is that any student/instructor with a standard Internet service should be able to connect to and use RWSL. However, experience has shown that not all computer systems and Internet services work well with RWSL; in some situations the remote system can cause the RWSL server to crash. We hope to rectify this situation, but for now every participant who intends to connect to RWSL must have their system certified by the RWSL techs or it will not be allowed to connect. Certifying your system for RWSL will ensure the best experience for all involved.

Currently RWSL works only on the Microsoft Windows operating system (XP or later). Internet Explorer (version 8 or later), Mozilla Firefox (v. 10 or later) and Google Chrome (v. 17 or later) have all been tested and found to work effectively. Please confirm that your system meets these minimum conditions before proceeding.

To have your system certified, go to this website:
   For CCCS students: http://at.ccconline.org/rwsl/installguide/
   For BCcampus students: http://rwsl.nic.bc.ca/installguide/).

Here you can check your connection speed and download the necessary drivers. This site also has instructions for contacting the RWSL techs should you run into any issues.

If your system cannot be certified you may be asked to go to your local educational institution where a student system has been certified for use with RWSL. Once you have your system certified or have found a system to use that is certifiable, the RWSL Tech will send you the URL you need to access RWSL.
APPENDIX B: THE RWSL MICROSCOPE

The Remote Web-based Science Laboratory (RWSL) is a robotic and software interface designed to enable the student to access and use science lab equipment over the internet and collect authentic real-world data in real-time. This document provides information about using the RWSL microscope.

INTRODUCTION TO THE RWSL MICROSCOPE

Like all microscopes, the RWSL microscope is designed to view objects that are too small to be seen with the naked eye. Most modern light microscopes use an external light source to illuminate a sample placed on the stage, which can then be viewed stereoscopically through a pair of eyepieces. The view of the sample can be altered by manually turning dials to move the stage in three dimensions.

The RWSL microscope is fitted with a camera so that the view of the sample can be captured and transmitted to a digital display. It is also fitted with a touchpad control which can be connected to a computer to enable remote control of the stage movement.

Figure 2: the N-800D Motorized Autofocus Microscope

The specific model used for RWSL is an N-800D motorized autofocus microscope, incorporating a 4-motor drive, and an objective nosepiece/stage enabled with coarse and fine motion in all three dimensions. A progressive digital dimmer controls the illumination of the halogen lamp and allows for 100 steps of brightness.
USING THE RWSL MICROSCOPE

Slide preparation
The RWSL microscope does not have an automatic slide loader (at this time); consequently, all slides must be placed on the stage by the RWSL technician. This, of course, limits the number of samples you can view during a single experimental session. However, the microscope stage can accommodate up to 2 slides at once. If 2 (or even 3) samples are fixed on each slide, you can arrange for up to 6 samples to be viewed during a single session.

APPARATUS

Fig. 2: the N-800D Microscope set up for RWSL

The RWSL Microscope is a precision instrument with 4 objective lenses: 04X/0.10, 10X/0.25, 20X/0.40, 40X/0.65 and 60X/0.85. The stage can be moved left, right, backward, forward, up, and down. The illumination of the sample can be changed, and it has an automatic focus feature. A Nikon camera is
mounted over the microscope so that the sample can be viewed and transmitted digitally. The camera (a Nikon DS-L2 stand-alone control unit with 8.4" LCD screen) is capable of capturing 5 MP (megapixel) images which can be downloaded for data and discussion in lab reports.

EXPERIMENTAL SETUP

The RWSL Microscope Virtual Instrument (VI)

When you first access the RWSL Microscope you will see a view similar to the following:

![Fig. 3: RWSL Microscope Start VI](image)

The right side of the VI shows the live feed from the Nikon camera (directed through the microscope) and camera controls for both the microscope camera and the picture-in-picture camera with which you can view the actions of the microscope itself. The left side of the VI contains the microscope controls.
CAMERA CONTROLS

Fig. 4: RWSL Microscope Camera Controls

The top part of the Camera Control sub-VI shows the real-time video feed from the Nikon camera, ‘looking through’ the microscope. You can also initiate a real-time video feed from a second camera which has been focussed on the outside of the microscope, showing the objectives and stage. To bring up this second video feed, click the “Picture-in-Picture” button. You will see something like the following:
You will have no control over this second camera other than the ability to turn it on and off, but you’ll be able to watch the operation of the objective lens selection and (if you look very carefully) the movement of the stage. Basically it adds a sense of ‘being there’ to the operation of the microscope. You can use the PIP camera controls to look around and zoom in or out to get a better view of what you are interested in seeing.

On the left side of the camera controls, you will note a column of 5 buttons. These buttons allow you to apply special effects to the image generated by the Nikon camera so that you can accentuate various aspects of the image.

This is a sample view of an image viewed with “Normal” (or no) special effects:
Here is the same image viewed with “Negative” special effects:

Figure 7: Microscope Negative Image

Here is the same image viewed with “Blue-Black” special effects:

Figure 8: Microscope Blue-Black Image

Here is the same image viewed with “Black & White” special effects:

Figure 9: Microscope Black & White Image
And finally, the same image viewed with “Sepia” special effects:

![Microscope Sepia Image](image)

**Figure 10: Microscope Sepia Image**

The “White Balance” button works just like the white balance on your home digital camera. The White Balance option is used to get the colours in the images as accurate as possible. You can see quite a difference if you capture an image, then do a white balance and then take a second image for comparison. You will notice that the second image will have truer colours.

The “Automatic Exposure” button activates the Nikon camera’s automatic exposure feature, so that it can automatically adjust for varying light levels. Its default setting is “on,” but if you want to control the light level manually then this button needs to be turned off (dark green).

Finally, when the “Capture Image” button is pressed, a 5 megapixel image will be copied into a new tab in your browser. To download that image you need to right-click on it, select “Save” from the pop-up menu, and then give the image a name and select a location on your computer where you want this image to be saved.

### EXPERIMENTAL OPERATION

**Gaining control of the microscope**

To gain control of the RWSL microscope, right click anywhere on the grey area of the VI and choose “request control” from the dialogue box that appears. After you request control of the microscope, you may have to wait several minutes before you actually receive control. Be patient and occasionally click on the ‘Automatic Focus’ button. You’ll know that you have achieved control when this button lights up.
and you see the image go out of focus and come back into focus. If it seems that you have been waiting for control for too long, right click on the VI and (if it is not greyed out) click on “request control” again. If this option is greyed out, you’ll know that you already have control.

[At this time, there is no graphical indicator to confirm that you have control of the VI: your only confirmation that you actually have achieved control of the microscope is when the buttons respond to your clicks. It can take a couple minutes to connect, so be patient. If it takes more than a couple of minutes, contact the RWSL tech on the communications ‘back channel’ (Mumble, Skype, Google Plus, MSN, telephone) that was agreed upon ahead of time.]

**Microscope Control**

![Microscope Controls](image)

*Figure 12: Microscope Controls*

The controls for the RWSL microscope are very similar to controls you have used with on-site microscopes, and they behave the same way. The microscope controls consist of:
Stage Controls:

As with any precision microscope, the position of the RWSL microscope stage can be controlled in a number of ways. Six buttons control the direction of movement and each button is labelled with its function: “Forward”, “Backward”, “Left”, “Right”, “Up” and “Down”. The button located between the “Up” and “Down” buttons will toggle the speed of the stage motion (for any direction) from Coarse to Fine and back. In this image, the setting currently reads “Coarse”, but clicking on the button will cause it to become bright green and the setting label will change to “Fine”. When in Coarse mode the stage will move up or down more quickly than when it is in Fine mode.

Use these controls to manually focus the microscope. Note that when you click on one of the “Forward”, “Back”, “Left”, or “Right” buttons the image of your sample will appear to move the opposite direction. This is the way the same buttons work on most microscopes so the ‘feel’ of the microscope is preserved over the web. The round button situated in the centre of the stage controls indicates when the stage is active/moving.

Keep in mind that because the stage is quite large, if students are assigned a large number of samples they may start to feel lost while scanning back and forth across the stage. For less experienced students you may provide a “map” indicating the relative position of multiple samples. You may require more experienced students to develop their own map of the sample layout before continuing with the study.

Objective Control:

The Objective Control determines which objective lens is used to view the slide. If you have activated the ‘Picture-in-Picture’ option, you will be able to see the objective move when you press one of these buttons. You can select from four different objective settings: 04X/0.10, 10X/0.25, 20X/0.40, 40X/0.65, and 60X/0.85.
Automatic Focus:

Figure 15: Microscope Autofocus Control

Clicking the “Automatic Focus” button will (not surprisingly!) cause the microscope to focus automatically. You will see the image of your sample go out of focus and then come back into focus. At times you may have to press the button several times to get the best focus. If you wish to focus on different ‘planes’ within your sample, then it would be better to use the “Up”, “Down”, and “Coarse/Fine” stage controls rather than relying on the auto-focus.

Luminance:

Figure 16: Microscope Luminance Control

The “Luminance” Control determines the brightness of the light illuminating the sample and can be set between 0% and 100% of brightness. For this control to work properly you must have the “Automatic Exposure” button turned off; otherwise, the Nikon Camera will automatically adjust the exposure and defeat your efforts.

If you would like to brighten the image, begin with the “Automatic Exposure” button turned on and turn down the “Luminance” control until it reads close to zero. Wait for the Nikon Camera to automatically adjust the exposure. Then turn off the “Automatic Exposure” and adjust the “Luminance” control upward until you get the illumination you want.

To reduce the illumination, begin with the “Automatic Exposure” turned on and adjust the “Luminance” control up. Wait for the Nikon Camera to adjust automatically. Then turn “Automatic Exposure” off and adjust the “Luminance” control downward until you get the desired illumination.

WRAPPING UP THE EXPERIMENTAL SESSION

If you are using the RWSL microscope with other participants, be sure to release your control so others can have a turn. Simply right-click on the VI and select “Release the VI”. Other group participants will not be able to get control of the spectrometer until the VI is released. If you are done with the lab exercise then “Release the VI” and close your window.