Mitosis Study
Adapted from AP Biology Laboratory Manual
Reference: Neil 9e, Ch. 12

Goals: At the end of this experience, the student should be able to:

- Recognize growing cells (those in interphase) and dividing cells (those undergoing mitosis & cytokinesis)
- Compare the events of mitosis & cytokinesis in plant cells with those in animal cells,
- Calculate and display a mitotic index.

Part 1. Recognizing interphase cells and dividing cells

Materials per team: 4 prepared onion root tip slides; 4 prepared whitefish blastula slides; 4 compound microscopes. These prepared slides are fragile and expensive, please be careful with them!

Vocabulary: in plants, the process of forming new cells is restricted to tissues called meristems. There are meristems at the tips of the shoot and root. In animals, an embryo is a good subject for cell division. A "blastula" is an embryonic stage in fish characterized by a rapidly dividing ball of cells.

Goal: To recognize cells in IPMAT (interphase, prophase, metaphase, anaphase, and telophase/cytokinesis) in meristems and embryos.

Directions:
1. Start with the onion root tip slide. Each team member needs to locate the meristem on 40X. This is important - we've got to look at the correct part of the root tip! See Fig. 1, right.

2. Switch to 100X, then to 400X.

3. On 400X, have each team member find a cell that is in interphase. MOST cells will be in interphase! Interphase cells have a round nucleus that looks grainy inside. It may be possible to see nucleoli.

4. Now have each team member find a cell in mitosis: prophase, metaphase, anaphase, or telophase/cytokinesis. If you have 4 team members, that's perfect: each person needs to find either P, M, A, or T. If the microscope has a pointer, use it! Consult Figure 12.11 in Campbell 9e, page 236.

5. Get out of your chairs & confirm each other's choices. When ready, ask your teacher to check your work!

6. IF TIME PERMITS, have each team member view a prepared whitefish blastula slide. As before, try to find examples of cells in interphase, then in PMAT. Because of a variety of planes of section in relation to the mitotic spindle (side views, end-on views, oblique views, etc.) it can be difficult to identify all the phases of mitosis in whitefish embryos, but try!
Part 2. Estimating the Mitotic Index of Onion Root Tip Meristem

Materials per team: 4 prepared onion root tip slides; 4 compound microscopes.

Goal: To estimate the mitotic index (MI), the percentage of cells dividing at any given time (i.e., the number of cells in mitosis divided by the total number of cells observed, x 100), from observing cells in four high power field of views. (Note: It is common for researchers to use ten FOVs to estimate MI.)

Why this matters: In plants, MI can reflect a plant’s growing conditions. For example, the meristems of plants grown in zero gravity in space have a higher mitotic index than those of control plants grown on Earth. In animals, MI has been demonstrated to be a strong predictor of outcome (both overall survival and response to chemotherapy) for several human and canine cancers.

Directions:
1. Go back to the prepared onion root tip slides. Each team member needs to locate the meristem on 40X as s/he did in Part 1. See figure 1 on the front page of this handout!
2. Switch to 100X, then to 400X.
3. On ONE 400X field of view, have each team member estimate the number of cells in each phase of the cell cycle, and record in Table 1. Share data within your team.

<table>
<thead>
<tr>
<th>Cell Cycle Phase</th>
<th>FOV 1 Initials:</th>
<th>FOV 2 Initials:</th>
<th>FOV 3 Initials:</th>
<th>FOV 4 Initials:</th>
<th>Total number of cells</th>
<th>Percentage of cells in I or PMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Prophase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaphase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Anaphase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>This is the mitotic index!</td>
</tr>
<tr>
<td>Telophase/cytokinesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Construct a pie chart with Excel (or other) graphing software to display the percentage data, and include the printout of the graph when you turn in this assignment. Be sure your graph is informative: give it a title and label both pie slices with their names (nondividing or dividing) & their percentages.

1. FILL IN’s. Identify the appropriate phase of the Cell Cycle (G₁, S, G₂, P, M, A, T, Cytokinesis)

   a. _________ Formation of two daughter nuclei as nuclear membranes reappear.
   b. _________ Sister chromatids separate and chromosomes move apart.
   c. _________ Mitotic spindle begins to form.
   d. _________ Cell plate forms (or, cleavage furrow pinches cells apart)
   e. _________ Phase during which chromosomes are replicated.
   f. _________ Chromosomes line up on a plane in the middle of the cell.
   g. _________ Phase after DNA replication.
   h. _________ Chromosomes become visible with a light microscope.
   i. _________ The nuclear envelope begins to fragment.
   j. _________ Cell is growing; chromosomes have not yet replicated.
   k. _________ Chromosomes uncoil into chromatin again.

2. In this photomicrograph of an onion root tip (figure 2), identify the cell cycle phases for the five indicated cells:

   A = __________________________
   B = __________________________
   C = __________________________
   D = __________________________
   E = __________________________

3. Fill out Table 2 at the right, based on your understanding of the cell cycle.

<table>
<thead>
<tr>
<th>Cell Cycle Phase</th>
<th># of Chromatids per Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td></td>
</tr>
<tr>
<td>G₂</td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
<td></td>
</tr>
<tr>
<td>Metaphase</td>
<td></td>
</tr>
<tr>
<td>Anaphase</td>
<td></td>
</tr>
<tr>
<td>Telophase/cytokinesis</td>
<td></td>
</tr>
</tbody>
</table>
4. Read the problem below:

Problem: A researcher observed a live cell under the microscope, about to divide. This cell contained 18 chromosomes. Some time later, the researcher observed the resulting two cells and noted that one of them had 19 chromosomes while the other had 17 chromosomes. Apparently, a malfunction had occurred during the mitotic process. Which of the following is the most likely explanation of this malfunction?

Write a clear, convincing justification for accepting one of the possible answer choices as the best (most appropriate) choice. You must also give your reason for rejecting each of the 4 other choices. Your reasons for rejecting choices are just as important as your reasons for choosing the best answer!

a. Cytokinesis failed to occur at the end of telophase.

b. Two of the chromosomes failed to line up in metaphase.

c. Metaphase preceded anaphase in this cell division.

d. In one chromosome, the chromatids failed to separate in anaphase, but separated at some later time.

e. The spindle apparatus on one side of the cell was not functional.