
Mitosis

Mitosis is a process during which a parent nucleus produces two daughter nuclei, each having the same number and kinds of chromosomes as the parent nucleus. Mitosis is a nuclear division (versus division of the cytoplasm and its contents into daughter cells, a process called cytokinesis).

Objectives:

1. Identify the various stages of mitosis ("mitotic figures") in prepared slides of plant and animal cells.
2. Estimate the duration of each mitotic stage.
3. Prepare "squash" preparations of onion (*Allium*) root tips to observe mitotic stages in fresh plant material*.

*if the materials are available

Materials:

Microscope

Forceps

Microscope slides

Cover Slips

Razor Blade (single edge)

Ruler

Prepared Slides

Allium (onion) root tip

White Fish Blastula

Ascaris (round worm)

Reagents

Hydrochloric Acid (HCl)

Toluidine Blue

Carnoy Solution

Methods:

Part 1. Mitotic Stages. Recognizing the *principle stages of mitosis* in representative cells.

1. Obtain a prepared slide of an onion root tip (*Allium*) longitudinal section (l.s.). Using the microscope locate the region of dividing cells. It is located slightly above the root tip and will have smaller cells with mitotic figures within them.
2. Using the high dry objective lens identify cells containing the different stages of mitosis, i.e. *prophase, metaphase, anaphase and telophase* (see Figure 1), as well as cells in *interphase* (not part of mitosis).
3. Repeat steps 1 and 2 with prepared slides of Whitefish blastula (Whitefish mitosis). Be patient! Locating the stages is often a little more challenging in animal cells.
4. Using the prepared slides make drawings of each of the stages (a.k.a. mitotic figures) as observed in the representative plant cells (onion) and animal cells (Whitefish). Label your drawings with the name of the specimen, the total magnification at which it is being observed, and the name of each stage, respectively. Labels should always be complete, neat and readily legible.

Part 2. Estimating the *duration* of the respective stages of mitosis in an onion root tip. One *basic assumption* of this exercise is that *the more cells there are in a particular stage, the longer the duration of that specific stage* in the process of mitosis. A second *assumption* is that the duration of mitosis *from start to finish (including all five stages) is 24 hours (1440 minutes)*.

Procedure:

1. Use the *Allium* root tip slide from the previous section. Scan the root tip using the scanning (low power, 4X) objective and locate a zone of cell division. Change to the high power objective (40X) and select a field of view in which to count the various stages of mitotic figures. *Once a field has been selected it is important that the slide NOT be moved* and only the cells (viz. nuclei or mitotic figures) in that field are to be counted. Do not include cells in which no chromosomes or nucleus are visible.
2. In this field *count the total number of cells* (i.e. nuclei) and the *total number of cells that are in prophase, metaphase, anaphase and telophase*, respectively. Record these numbers in table 1.

Total number of Mitotic figures = _____
Total number of cells – Total number of mitotic figures = total number in interphase

3. The duration of each mitotic stage can now be calculated using the following equation:

Duration of Mitotic Stage = $\frac{\text{Number of cells in a stage}}{\text{Total number of cells}} \times 1440 \text{ minutes}$

Calculate the duration of each mitotic stage and enter in Table 1.

4. The instructor will pool the numbers for the class and provide the data for you to complete Table 1. Compare your data and that pooled from the class to those reported in the literature* for mitosis occurring at 25°C.

* Reported durations of mitosis and interphase occurring at 25°C

Prophase	42 min.
Metaphase	12 min.
Anaphase	6 min.
Telophase	30 min.
Interphase	1,350 min.

Which data are in greater agreement with that reported in the literature, yours, or those pooled from the class? _____

Suggest a few reasons for any discrepancies that might be observed in the data collected.

Part 3. Observation of mitotic figures in fresh plant cells* using *Allium* root tip.

*if the materials are available

-CAUTION- Acid used for part of this exercise. Observe all safety precautions. Eye Protection Required, use safety glasses or goggles.

1. Obtain two small cups from the lab supplies cart. Label one cup "HCl" (for Hydrochloric Acid) and the second cup "Carnoy" (for the staining solution).
2. To the first cup carefully add enough HCl to cover the bottom.
3. To the second cup carefully add enough Carnoy to cover the bottom.
Use forceps to transfer an onion root tip into the cup of HCl. Avoid crushing the delicate specimen with too much pressure. Leave the root tips in the HCl for

FOUR MINUTES.

4. Transfer the root tips to the Carnoy solution, and leave them in the Carnoy for FOUR MINUTES.
5. Transfer the root tip to a clean microscope slide, and using a single edge razorblade, cut off the last 1 to 2 mm of the tip (where mitosis is occurring). Discard the remainder of the root tip.
6. Cover the root tip with a few drops of toluidine blue and allow to stand for TWO MINUTES.
7. Absorb the stain away from the root tip with a piece of tissue or paper towel, being careful to not touch the root tip.
8. Cover the root tip with one or two drops of water then gently lower a cover slip down over the specimen.
9. Cover the slide with a folded paper towel or absorbent paper and firmly press down on the cover slip WITHOUT TWISTING. This pressure will "squash" the root tip spreading the cells into a single layer.
10. Examine the "squash" slide preparation with the low power objective of the microscope to determine whether the cells have been separated sufficiently for observation of the chromosomes in the individual cells. *If not, carefully* squash the cells again.
11. Using the low power objective scan the "squash" preparation for mitotic stages. Change to the high dry objective lens and draw the respective stages of mitosis, as they are located. Label each drawing with the specimen, preparation (i.e. "squash"), stage of mitosis and magnification at which it was observed. Keep in mind that you are observing intact, whole chromosomes rather than thin sections as with most of the commercially prepared slides.

Table 1. Duration of Respective Mitotic Stages*

Mitotic Stage	Group / Individual Totals		Class Average Counts	
	Number of Cells	Duration (min.)	Number of Cells	Duration (min.)
Total Nuclei				
Prophase*				
Metaphase*				
Anaphase*				
Telophase*				
Interphase**				

**Total number of cells – Total number of mitotic figures = total number in Interphase

Cell Cycle and Mitosis

- A. Interphase
- B. Prophase
- C. Metaphase
- D. Anaphase
- E. Telophase

Match each of the phases of the *Cell Cycle* and *Mitosis* listed above with the cell activities listed below. Write the letter of the best match in the space provided to the left of each activity.

- ___ 1. Chromosome replicates
- ___ 2. Chromatids separate; the centriole divides
- ___ 3. Chromosome condenses, thickens; chromosomes become visible
- ___ 4. A cleavage furrow forms in animal cells
- ___ 5. Chromosomes are lined up on the cell's equator
- ___ 6. The centriole pairs begin to move toward the poles of the cell
- ___ 7. A cell plate forms in plant cells
- ___ 8. The nuclear membrane and the nucleoli reappear in the forming daughter cells
- ___ 9. The daughter cells begin normal metabolic functions
- ___ 10. Spindle formation is complete
- ___ 11. Chromosomes exist exclusively as chromatin
- ___ 12. Chromatids separate and migrate to opposite ends of the cell
- ___ 13. The cell carries out protein synthesis
- ___ 14. Astral rays begin to form in animal cells
- ___ 15. Centromeres begin attachment to the spindle fibers