BIL 151 - Mechanisms of Mitosis
PART III. Collecting Data

To discover how chromosomes move in a dividing cell, your team has chosen to examine the outcome of treating a rapidly dividing tissue (onion root tip) with a substance that either **promotes** (Indole-3_butyric acid) mitosis or **inhibits** (trifluralin) mitosis. Today you will count the number of cells in each stage of mitosis in the control and treatment onion root tips your team requested.

Data Collection Procedures

Use the same materials and equipment you used for practicing chromosome squashes. Chromosome squash instructions are on laminated cards at your lab stations.

Onion root tips have been incubated in either

- plain water (control)
- indole-3-butyric acid in phosphate buffer (treatment)
- trifluralin in water (treatment)

In each of your two data collection lab sessions, your team will squash and count cells from

- **four control onions**
- **four treatment onions**
- **(one treatment and one control squash per team member)**

for a total of eight treatment and eight control samples by the end of Session 4.

EACH TEAM SHOULD COUNT CELLS FROM ONLY ONE ROOT TIP PER ONION.
A single organism is considered a single replicate.
Counting multiple roots from the same organism would create false replicates.

Before you begin…

1. Don your PPE!

- Gloves
- Lab coat
- Safety goggles
- Other protective gear

2. Label all materials (beakers, plants, microscope slides) appropriately.
Microscope slides should be labeled

- as **treatment** or **control**
- with **incubation start time**

Your team may have 10-20 slides out at any given time.
It is critically important to label everything properly.

3. For best results and ease of counting, *clean microscope slides well*.

- Place 1-3 drops of 95% ethanol on the slide
- Wipe well with a Kimwipe.
- Do this on both sides of the slide
- Repeat, as necessary, until the slide is very shiny and clear.

Chromosome Squash Procedure

Control and treatment roots (as requested by your team) will be available at the front desk. For safety reasons, students will not cut roots. Your lab instructor will give them to you.

1. Obtain an onion root tip from your lab instructor.

   - **Keep the onion root wet at all times!**
   - ***Do not leave onion roots out of the water or lying on the lab bench.***

2. Each onion will have an **identification code**. **Label the microscope slide** with your **onion's ID code** and then place the root on the labeled slide.

3. Using the dissecting scope, identify the **root tip**.

   Plant mitosis occurs in **meristem** cells, which can differentiate into any other type of cell. The **apical meristem** is about one millimeter from the apparent tip of the root (the **root cap**) (Figure 2).

   If you cut off too much of the root, you will see long, rectangular cells. These cells are no longer undergoing mitosis. Do not count them.
   Carefully cut off *just the meristem region* of the root tip with a sharp razor blade
   Count only meristem cells when collecting data.

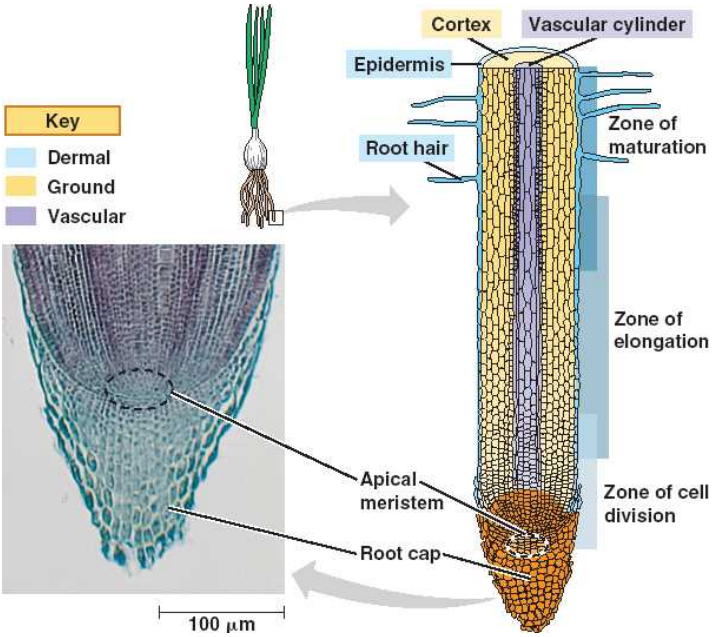

Figure 2a. Onion root tip anatomy. Only the cells at the very tip of the root (Zone of Cell Division) are undergoing mitosis. These are visually distinct in a fresh root tip, appearing more round or square than the elongated cells in the Zone of Elongation above it.

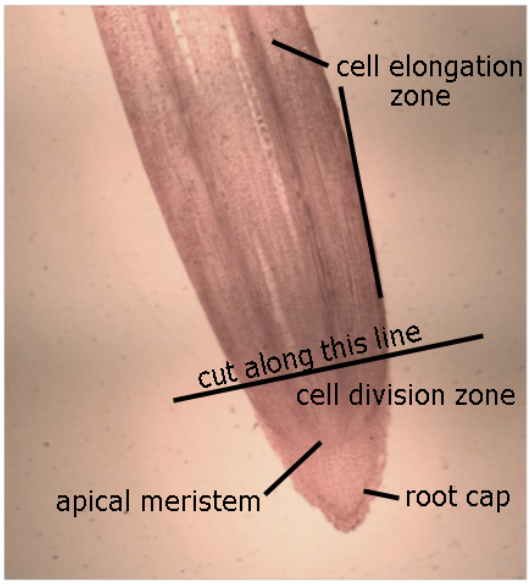

Figure 2b. Root tip of corn (Zea mays). Note the clear appearance of the root cap. Just above it is the apical meristem and the Zone of Cell Division. The darker, longitudinal lines above the cell division zone mark the newly formed vascular cambium.

4. With fine-tipped forceps, place the root tip with apical meristem into a 1.5 ml microcentrifuge tube. (Forceps tips are fragile. Handle with care.)

5. Fill the microcentrifuge tube **halfway** with 1M HCl (dropper bottle on your lab bench) This will soften the connection between the cells.
   Use caution:HCl is a strong acid.

6. Close the tube and **place in a hot 60°C water bath for exactly 8 minutes**.
   (Too long in hot acid yields a soggy mass of cells that will disintegrate when you rinse).
7. **Remove the tube carefully from the hot bath.**
8. To remove the 1M HCl, fill the tube with deionized (DI) water, and then suction it out with a plastic squeeze pipet. **Repeat this procedure for a total of three rinses.**

   Place all removed waste water into the container at your station labeled "WASTE SOLUTIONS". Nothing goes into the sinks or trash cans!

9. Add 2 drops of 0.5% toluidine blue to the tube.
10. Incubate at room temperature for 5 minutes
    Gently flick the tube with your fingernail about once per minute to distribute the stain.
    Make sure the root tip stays in the stain.
11. Rinse the excess toluidine blue as you did for the HCl.

    a) Fill the tube with DI water, then remove it with the plastic squeeze pipet.
    b) Repeat a total of three times
    c) Remove almost all of the last rinse.
    d) Use a dissecting probe to gently push the root tip onto a clean, labeled slide.

    By the time you have removed the last bit of rinse water, you should be able to see your blue root tip clearly

    Always place waste solution in the "WASTE SOLUTIONS" container. Not in the sinks or trash cans!

12. Add one drop of DI water to the root tip on the slide. Gently drop a coverslip over it.

13. Place a sheet of bibulous paper (booklet supplied on your tray) over the coverslip.

    - ***Gently* press straight down** onto the coverslip with root tip underneath.
    - Be careful not to break the coverslip, or you'll have to start over.

    DO NOT PLACE YOUR SLIDE INSIDE THE BIBULOUS PAPER BOOKLET!
    Please keep the pages clean and uncontaminated for your future slide preps.

14. Remove and discard the bibulous paper.
15. Place the slide on your compound microscope stage.

    ALWAYS BEGIN MICROSCOPE OBSERVATIONS ON LOW POWER.

    a) Find and focus on your root tip cells in the viewing field on **low power**.
    b) Swivel the objective to the next higher objective, and focus again.
    c) Do this until you are properly focused with the **40X objective**, which you will need to use to see nuclear material clearly.

16. Examine your squash. You should be able to see cells in various stages of mitosis.

Data Collection

Choose a properly squashed area and count all of the cells you can see (~ 50-200 cells).
The cells you count should be **round** or **cuboidal** and flattened into a single cell layer.
Do not count long, rectangular cells, as these are no longer undergoing mitosis.

From among the counted cells, record how many cells—each—are in

(1) **interphase**
(2) **prophase**
(3) **metaphase**
(4) **anaphase**
(5) **telophase**.

Count cells in four different fields of view for each root tip.
This will give you a good sample from an individual onion (about 100-300 cells per root tip, depending on its size and quality).

one sample = all the cells counted in one root from one onion

Avoid Pseudoreplication

- **Do not take multiple roots from the same onion**
- **Do not count multiple fields of view as separate experimental samples.**

All cells counted from a single onion plant comprise one sample.
A single individual onion's root tips are all part of the same organism.
Counting them as separate samples creates false replication.

1. Count the number of cells you can identify in each stage of mitosis.
2. **When you are completely finished with a slide preparations, place it in the Broken Glass Disposal Container at the front of the lab room.**
   Teams leaving slides or other materials at their station will be docked 5 points.
3. **Each team member** should perform a squash and cell count on **ONE TREATED** root tip and **ONE CONTROL** root tip.
4. Thus, on *each* of your two data collection days, your four-member team should squash and count at least (if your team has more members, you can collect more samples of each).
   - **4 control** root tips
   - **4 treatment** root tips

   for a **total of**
   - **8 control** root tips
   - **8 treatment** root tips

   at the end of your two weeks of data collection.

Preliminary Data Analysis: Mitotic Indices

A **Mitotic Index** (**M**) is a measure of the proportion of mitotic cells in a sampled cell population.

$$M = n_m/N$$

n_m = total number of mitotic cells in the sample

N = total number of cells counted in the sample

For each of your treatment and control samples, calculate and record a Mitotic Index, and record these values in a table like the one shown. Provide an appropriate legend for the table.

Table _____. _____

_____

Treatment Sample #	Mitotic Index (M)	Control Sample #	Mitotic Index (M)

A **Mitotic Phase Index** (**M_P**) is a measure of the proportion of cells in a particular phase of mitosis in a sampled population of mitotic cells.

$$M_P = n_p/ n_m$$

n_p = # of cells in prophase in the sample

n_m = total number of mitotic cells in the sample

(The equation above shows the index for prophase, but it can be used for any phase.)

Consider your overall hypothesis.

- Do you think you need to calculate Mitotic Phase Indices for all phases of mitosis?
- If not, which phases would be most informative in a comparison of treatment and control groups?

Calculate the appropriate M_P of your treatment and control samples.
This will allow you to determine whether treated onion root tips have been affected in a particular stage of mitosis. Tables for recording mitotic indices are linked in the online syllabus.

Polyploidy

A polyploidy cell has more than two complete sets of chromosomes.
Did you observe any **polyploid cells**?

What might be the significance of the presence of polyploid cells?
(Do a quick search on "plant polyploidy" for some ideas.)
HINT: some commercially grown plants are intentionally cultivated as polyploids.
How might this be done? And why?