Mechanisms of Mitosis
Practicing the Experimental Procedures

To discover how chromosomes move in a dividing cell, your team will 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 learn and practice the techniques you must master in order to analyze mitotic cells.

I. Preparation for Lab Procedures
Before you begin, you must complete important preparations.

Before you begin...
1. Don your Personal Protective Equipment (PPE)!
   - Gloves
   - Lab coat
   - Safety goggles
   - Other protective gear

2. Label all materials (beakers, onion plants, microscope slides) appropriately. *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.

II. Chromosome Squash Procedure
For our practice run, onion root tips have been incubated in plain water. To visualize chromosomes in the phases of mitosis, you will prepare and stain them in a procedure known as a chromosome squash.

Because some reagents we will use may be somewhat caustic, you must wear the nitrile gloves provided and your own safety glasses while you perform the chromosome squash. Wear a lab coat or lab apron to protect your clothes from staining.

Onion bulbs will sprout roots if they are placed in water for several days (Figure 1). The onions you will use today had all old roots removed approximately three days before your lab session. The bulbs were then immediately placed in plain water and allowed to sprout new roots to ensure the presence of fresh, growing root tips.
The onion root tip cell cycle is about 24 hours. Thus it may take approximately 24 hours of incubation with any particular reagent before one can expect to see any effect on mitotic cells.

Plant mitosis occurs in meristem cells at the tips of roots and shoots. These 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, composed of dead cells) (Figure 2a).

For safety reasons, students will not cut roots. Your lab instructor will give them to you.

1. Obtain an onion root from your lab instructor.
   The root tip is delicate, and desiccates easily.
   
   - **Keep the onion root wet at all times!**
   - **Do not leave onion roots out of the water or lying on the lab bench.**

![Figure 1. Sprouting green onions (scallions), Allium sp.](image)

![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.](image)

![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.](image)
2. Place the root on an appropriately labeled slide.

3. Using the dissecting scope, identify the **root tip**.
   - Long, rectangular cells above the root tip are no longer undergoing mitosis.
   - Do not include non-mitotic cells in your squash or counts.
   - With a sharp razor blade, *cut off only the meristematic region* of the root tip.

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. **LABEL THE TUBE** with a Sharpie marker.

7. Close the tube and place it 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).

8. Carefully remove the tube from the hot bath.

9. 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!

10. Add 2 drops of 0.5% toluidine blue to the tube.

11. 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.**

12. **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

13. Add one drop of DI water to the root tip on the slide. Gently drop a coverslip over it.
14. 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!**
   Keep the pages clean and uncontaminated for your future slide preps.

15. Remove and discard the bibulous paper.

16. 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.

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

**III. 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.**

See Figure 4 for an example of what you should expect to see in your slides.

| Figure 4a. Allium root tip cells undergoing mitosis (acetocarmine stain). [http://upload.wikimedia.org/wikipedia/commons/d/d3/Onion_root_mitosis.jpg](http://upload.wikimedia.org/wikipedia/commons/d/d3/Onion_root_mitosis.jpg) | Figure 4b. Your preparation will probably look something like this. Yellow arrows indicate cells in various stages of mitosis. (preparation and photo courtesy of Linda White) |
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).

Record
- the total number of cells you can identify
- the total number of cells in any stage of active mitosis
- the total number of cells in EACH stage of the cell cycle
  1) interphase
  2) prophase
  3) metaphase
  4) anaphase
  5) telophase

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.

IV. Data Analysis: Mitotic Indices
A Mitotic Index (M) is a measure of the proportion of mitotic cells in a sampled cell population.

\[ M = \frac{n_m}{N} \]

\( n_m \) = total number of mitotic cells in the sample
\( N \) = total number of cells counted in the sample

For each of your 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.

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.

\[ M_p = \frac{n_p}{n_m} \]

\( n_p \) = # 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.)
Enter your data in a table such as the one shown below, and provide an appropriate legend that clearly describes the source of the data and contents of the table.

Table 1. __________________________________________________________

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Mitotic Index (M)</th>
<th>Prophase Index (M_p)</th>
<th>Metaphase Index (M_m)</th>
<th>Anaphase Index (M_a)</th>
<th>Telophase Index (M_t)</th>
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When your team collects data from treated and untreated (control) onions for your research project, you will perform essentially the same protocols you have practiced here. You will also decide which indices to calculate and report to best reflect your observations.

When you are completely finished with a slide preparation, place it in the **Broken Glass Disposal Container** at the front of the lab room.

Upon completion of all of today's exercises, notify your lab instructor, who will then inspect your station for cleanliness. If the station is not properly cleaned and restored to its original condition, you must correct that before you leave the lab.

**Teams leaving an untidy lab station, including undisposed slides, trash, or other materials, will be docked 5 points.**