Lab Experiment: Mitosis in Onion Root Tip Cells and Mitotic Index

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Objective of the experiment

- To better understand the mitosis and different stages of mitotic processes.
- To better understand the relative length of each stage of mitosis (can be estimated)

Cell division

- Cells are the smallest unit that can live on its own and that makes up all living organisms and the tissues of the body.
- Every cell (eukaryote) contains genetic material as chromosomes (made up of nucleoprotein) residing in the nucleus.
- Every cells (eukaryotes) finite sets of chromosome that undergoes replication to form two sister chromatids attached to centromere from tetrads.
- DNA replication is followed by mitosis (cell division) which ensures that each daughter cell receives one copy of each of the replicated chromosomes in a multistep process.

Mitosis

- Mitosis term was coined by Walter Flemming in 1882. The term mitosis is derived from the Greek word ‘mitos’ meaning warp thread.
- Mitosis is a type of cell division that results in two identical daughter cell with same set of chromosomes as parental cells.
- It results in increase in the number of cells at given time and space, i.e., proliferation.
- It is needed for general growth of living entities and replacement of damaged cells in the case of injuries or wound healing.
- It is a multistep process and progresses through distinguishable 4 stages;
  - Prophase (can be prophase and prometaphase)
  - Metaphase
  - Anaphase
  - Telophase

Stages of mitosis

Prophase

- Chromosomes supercoil.
- Fibers of the spindle apparatus begin to form between centrosomes located at the pole of the cells.
- Nuclear membrane disintegrates.

Metaphase

- Chromosomes come to rest and align at the central plane of the cell.
- Chromosomes are still attached to the centrosomes through spindle fibers.

Anaphase

- Centrosomes split.
- Sister chromatids begin to migrate toward the opposite pole.

Telophase

- Chromosomes begin to cluster together at either end.
- Nuclear membrane begins to form.
- Cytokinesis occurs.

Stages of mitosis

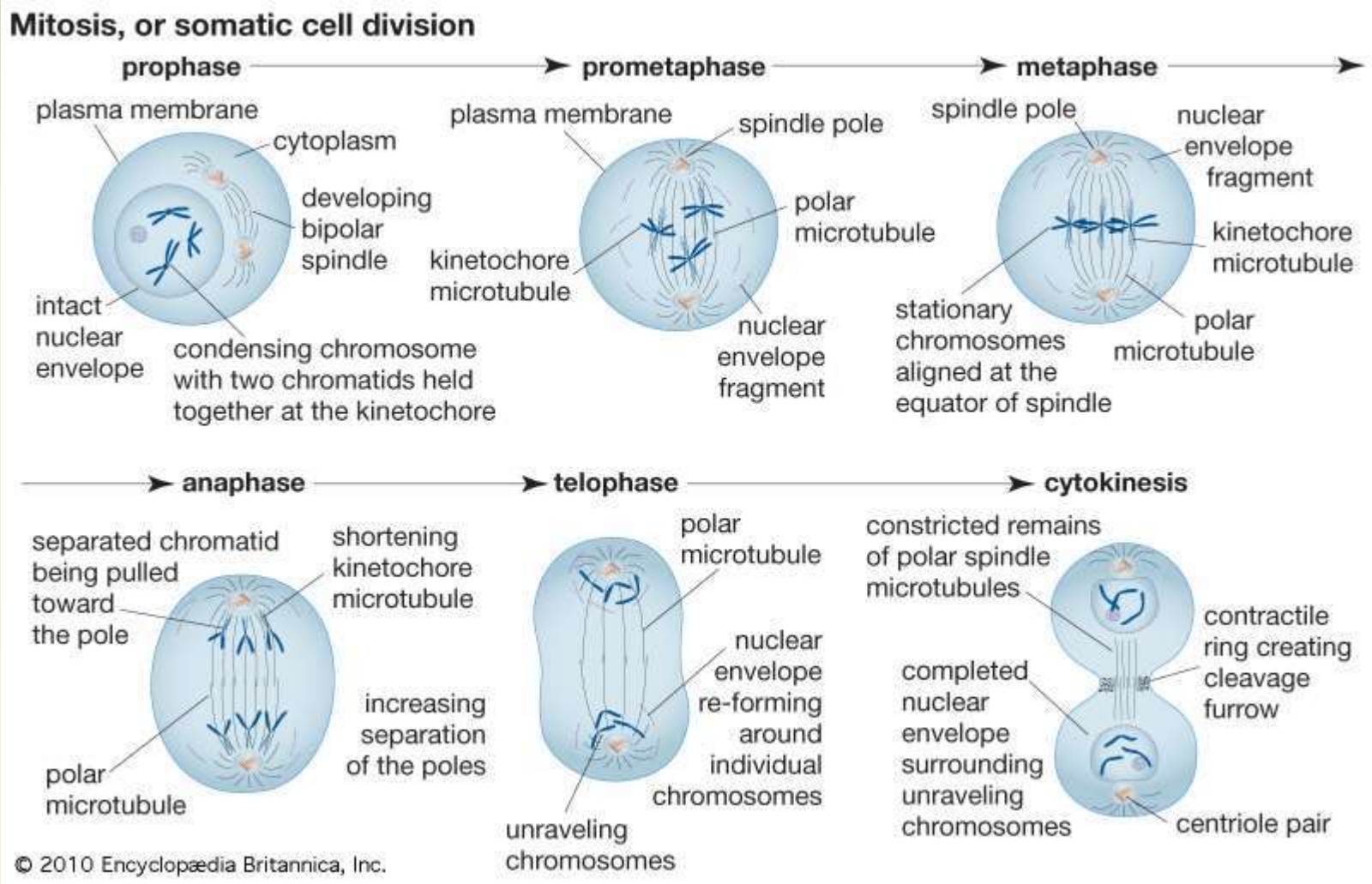
Interphase

- *Chromosomes are thread like chromatin fibers.*
- *Nuclear membrane and nucleolus are distinct.*

Prometaphase

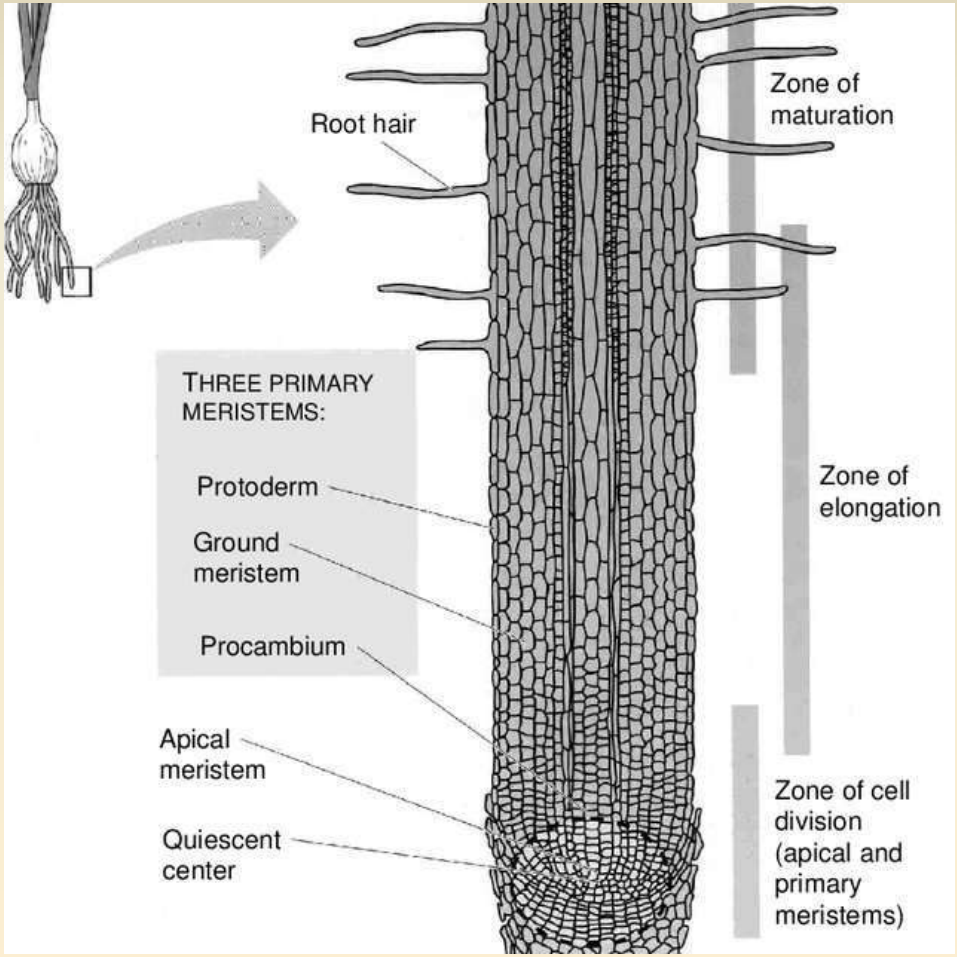
- *Chromosomes supercoiling taken place.*
- *Some of the fibers of the spindle attach to the centromere of each pair of sister chromatids.*
- *Sister chromatids begin to move toward the centre of the cell.*
- *Nuclear starts disintegrating.*

Stages of mitosis



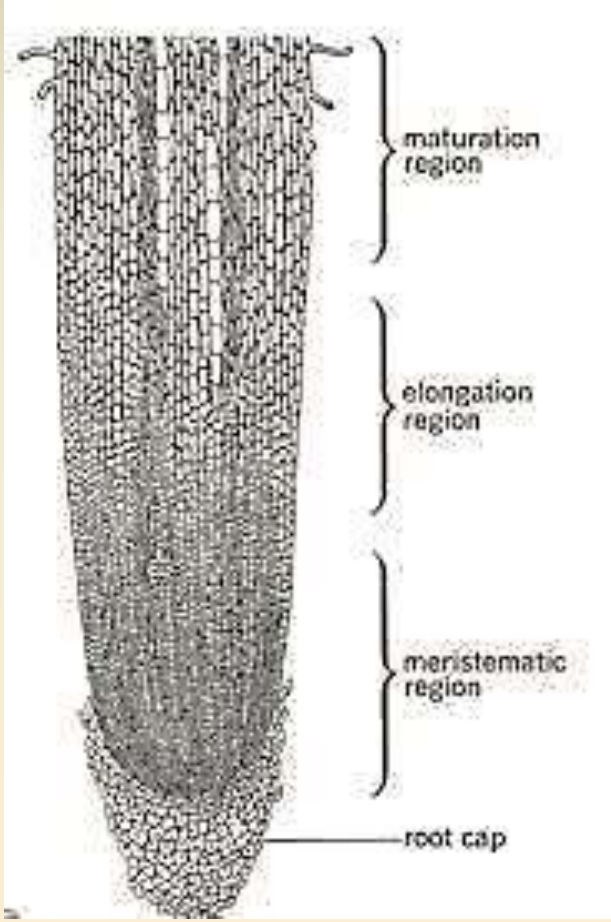
Why onion root tip for lab experiment?

- The onion roots grow rapidly in large number.
- The cells at the tip are actively dividing and therefore all cell cycle stages are easy to visualize in single preparation.
- The onion tips are easy to prepare for squashing on microscopic slide.
- The slide preparation can be stained to make them more observable.



Thies & Grossman, 2006;
DOI: 10.1201/9781420017113.ch5

Why onion root tip for lab experiment?



L.S. of root apex showing terminal root cap and sub apical meristem.

There are 3 zones (regions) near the tip of the onion root.

Root cap: *contains cells that cover and protect the underlying growth regions under the soil.*

I. ***Region of cell division:*** It is also called meristem, where size is not significantly increasing.

II. ***Region of elongation:*** Cells are increasing in size, but not dividing. Due to cell elongation, roots are lengthened.

III. ***Region of maturation:*** It is also called as the region of differentiation or root-hair zone. Cells mature into the various types of primary tissues.

Principle

Somatic growth in living entity requires increase in the number of cells, which takes place by dividing a cell mitotically to give rise to two daughter cells with similar number and sets of chromosomes to the parental cells. Plants show such rapid cell division in meristematic tissues found in the root and shoot apices where it can be easily observed, whereas in animals, such rapid division can be viewed easily in bone marrow tissue, in the epithelial cells of fish gills and in the tail of frog tadpole. Onion root tips are easily available and can be grown in the laboratory as well. Therefore, it is preferred for visualizing mitosis.

Requirement

Sample

- Onion root

Equipment

- Compound microscope
- Glass Jar
- Spirit lamp
- Slide
- Cover slips
- Blotting paper

Sample & Reagents

- Onion bulbs
- Glacial acetic acid
- Ethanol (70%)
- Acetocarmine/acetoorecin stain
- HCL (N/10)
- Molten wax/nail polish

Reagents

- ***Fixative:*** *Aceto-alcohol solution (1:3:: glacial acetic acid : ethanol)*
- ***Stain:*** *Acetocarmine (*1% solution of carmine in 45% acetic acid**;** Dissolve 10 g carmine (Fisher C579-25) in 1 L of 45% glacial acetic acid, add boileezers, and reflux for 24 h. Filter into dark bottles and store at 4°C) or *aceto-orecein (*1% solution in 45% acetic acid; prepared by pouring 55 ml boiling glacial acetic acid over 1 g orcein powder, cooled, 45 mL of distilled water added, and filtered).

Procedure

- **Growing of onion root:**
  - Take only freshly grown onion root (dried or old roots are not good for the experiment). For this, take onion bulb carefully removed dried roots and place on glass jar filled with water for 3 to 6 days to grow.
  - Cut 1 cm long freshly grown roots and transfer them to freshly prepared aceto-alcohol fixative. Keep it for 24 hrs.
  - Transfer root tips to 70% ethanol for use (root tip is preserved).

- **Preparation of root tips:**
  - Soften the root tips so that they are easily spread on the slide.
    - For this, fill the tube about 2/3 full with 1N HCl.
    - Place the tube in 60 °C water bath, and incubate for 12 minutes.
    - Remove the tube from water bath.
  - Rinse the root tips in water 3 times.

Procedure

- **Preparation of slide:**
  - Take rinsed root tips (1 or 2) on a clean slide.
  - Place one drop of 1/10N HCl on the root tip followed by 2-3 drops of acetocarmine or aceto-orecein stain on it.
  - Leave the slides for 12 minutes.
  - Blot the excess stain carefully using blotting paper and rinse the root tips thrice.
  - Cut 2-3 mm tip portion of the root tip using razor blade and discard the rest.
  - Put a drop of water and mount a cover slip carefully by avoiding any air bubble.
  - Squash with thumb using slight pressure.

- **Observation of slide:**
  - Observe the slide under compound microscope.

Observation

All the stages of mitosis can be observed under the microscope. However some cells are observed as quiescent or not in any phase, hey are considered as cells in interphase (most of the cells in a view field are in interphase). Following relatable observation can be made:

Interphase: *Rectangular, oval or even circular in shape; densely stained homogenously granular nucleus; distinct nuclear boundary; nucleolus may also be observed.*

Prophase: Intact nuclear boundary; chromatin appears as a network of fine threads (chromosomes); nuclei may or may not be visible.

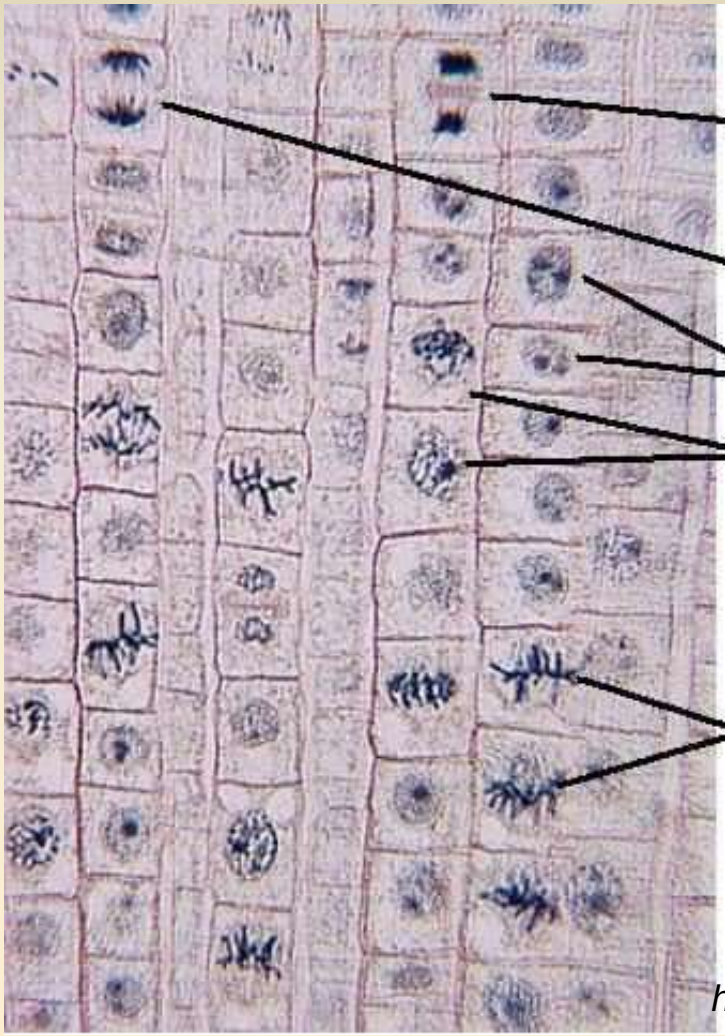
Metaphase: Nuclear boundary is not seen; chromosomes are thick and arranged at the equatorial plane of the cell (chromosome has two chromatids that can be seen by observing carefully); nucleolus is not observed.

Anaphase: Chromatids separation can be seen; chromosomes appear to be moving apart and look like the shape of alphabet ‘V’, ‘J’ or ‘I’ depending upon the position.

Telophase: Chromosomes observed at two poles as chromatin mass; nuclear membrane appears to form on both the chromatin mass at the poles.

Cytokinesis: *The cell plate begins to form at the middle after telophase, which are extending outwards to ultimately reach the margin of the cells and divide the cell into two.*

Observation



Mitosis, Teaching biology, Biology classroom
https://www.pinterest.com/pin/747456869374725427/

Observation

Observation should be tabulated as following:

Features	**Interphase**	**Karyokinesis**				**Cytokinesis**
		Prophase	Metaphase	Anaphase	Telophase	
1. Cell morphology						
2. Nuclear morphology						
3. Chromosomes						

Mitotic index

Mitotic Index (MI) is defined as a ratio of the total number of dividing cells (n) and the total number of cells (N) in a particular focus chosen randomly under the microscope.

Therefore,

$$\boldsymbol{MI = \frac{n}{N}}$$

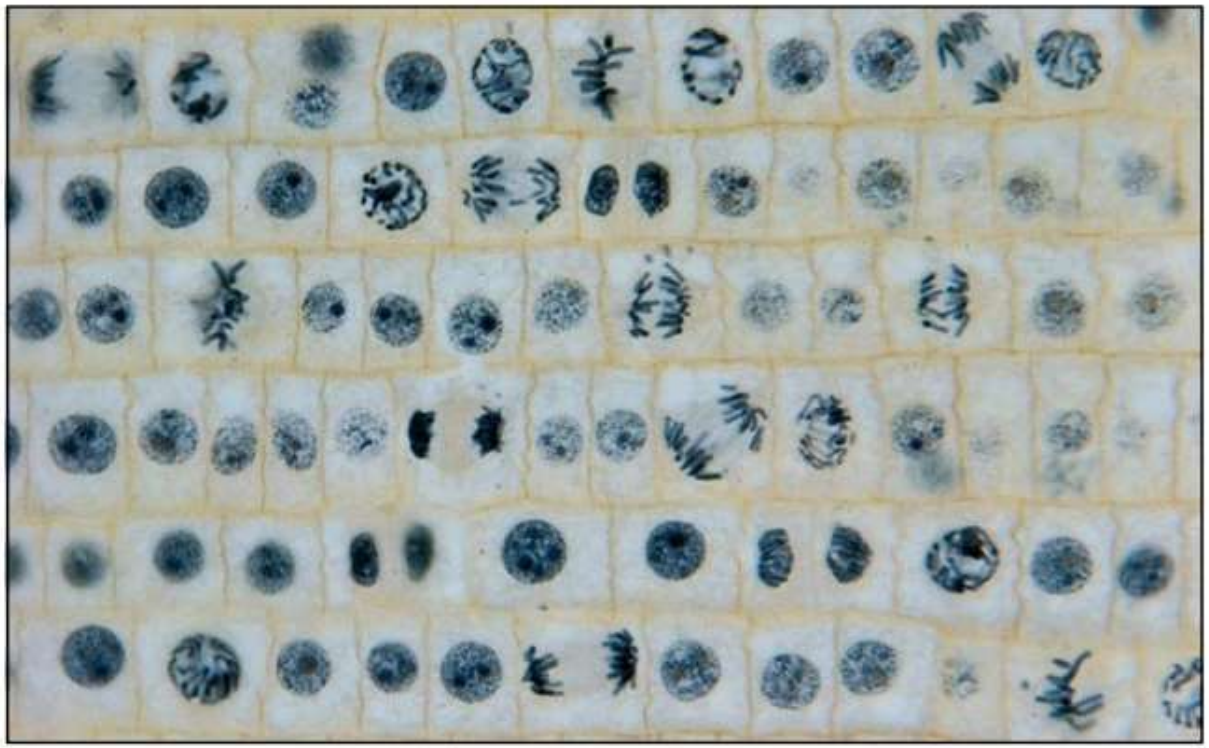
By randomly selecting 5 to 10 foci, mitotic index can be estimated.

By multiplying with 100, one can calculate for percentage of cells undergoing mitotic cell division.

$$\boldsymbol{Percent\ mitotic\ cell\ population = \frac{n}{N} \times 100}$$

Mitotic index

Calculate MI:



Mitotic Index

$$\frac{\text{Cells in mitosis}}{\text{Total number of cells}}$$

Cells with visible chromosomes:

?

Cells without visible chromosomes:

?

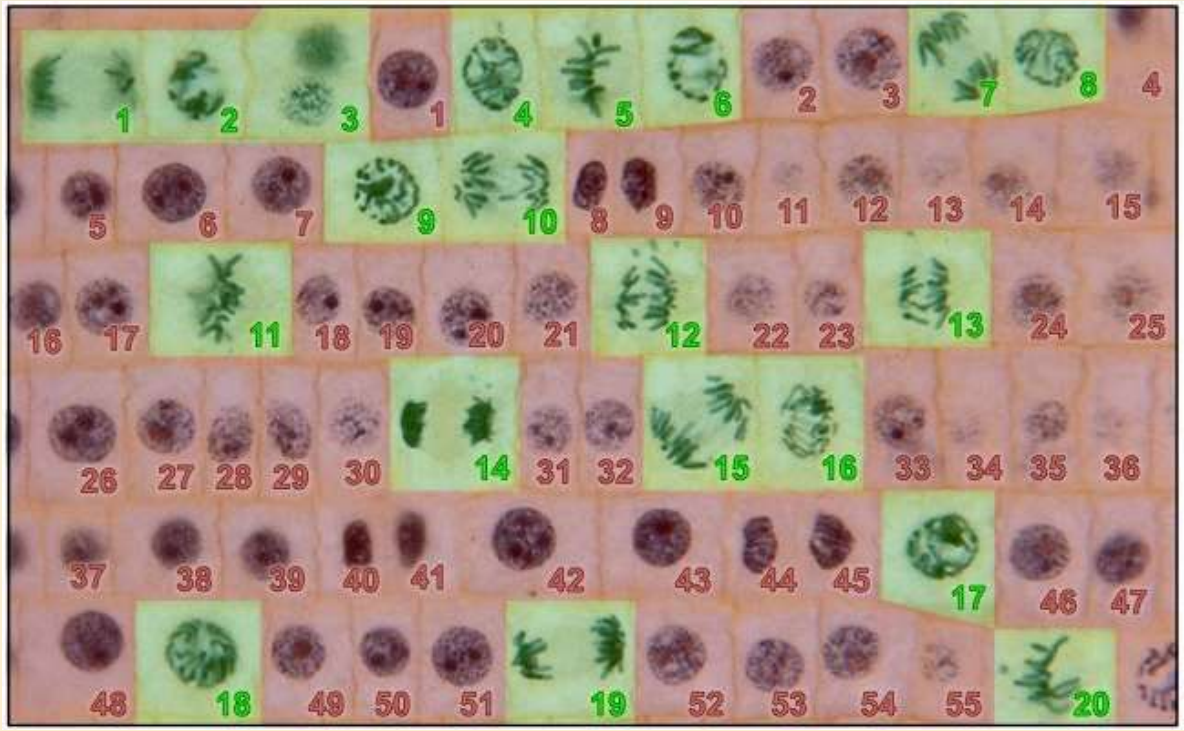
Mitotic Index:

?

https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/16-cell-division/mitotic-index.html

Mitotic index

<u>Calculate MI</u>: ***Result from previous slide***



Mitotic Index

Cells in mitosis

Total number of cells

Cells with visible chromosomes:

20

Cells without visible chromosomes:

55

Mitotic Index:

20 ÷ (20 + 55) = 0.267

https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/16-cell-division/mitotic-index.html

Thank you!