You separated cell components using cell fractionation and made wet mounts to observe certain cell organelles under the compound light microscope in order to distinguish between them. You also tested for the presence of mitochondria using Tetrazolium Assay.

**Part I: Cell Fractionation**
- Cell fractionation starts by breaking open cells without causing damage to the organelles.
- Mechanical disruption was done by shearing pea cells in a blender containing a cold buffered isotonic sucrose solution.
- The sucrose solution is buffered to protect the organelles from pH changes, and it is isotonic so that the organelles are not damaged by gaining or losing water. Adding cold sucrose also prevents the cells from heating up during blending.
- Since mechanical disruption tears open the cell walls & membranes, why it doesn’t it also damage the organelles?
  - Organelles can withstand greater shear forces and grinding since they are much smaller than the cell
  - In general, plant cells require greater shear forces or grinding than animal cells to break cell walls.
- **Differential centrifugation** uses different amounts of centrifugal force (different spinning speeds) to separate cell components based on size and density. You centrifuged at 1500 and 3500 RPM.
  - **At low speed:** larger particle settle down such as starch grains forming a **pellet** at the bottom of the tube
  - The smaller components that do not settle down will **float in the supernatant**
  - **At higher speed:** nuclei & chloroplasts that were floating in supernatant will settle down in pellet

**Part II: Separating cell organelles**
- You prepared 90 ml of pea homogenate which was distributed among 3 tubes.
- Exact volumes were needed to ensure that the centrifuge was completely **balanced out** (spin 6 identical tubes at a time)
- You used increasing speeds of centrifugation to separate components as follows:
  - Layer 1: white pellet at **1500 RPM** = starch grains in **Amyloplasts**
  - Layer 2: beige pellet at **3500 RPM** = nuclei
  - Layer 3: dark green pellet at **3500 RPM** = chloroplasts
  - Layer 4: supernatant = mitochondria
Part III: Identifying cell organelles using the light microscope

A drop of dilute iodine solution was added to each of these samples:
- Mount # 1: contained starch + iodine and should turn purple
- Mount # 2: contained nuclei (grey pellet)
- Mount # 3: contained chloroplasts (green pellet); very hard to see as they are very small but shiny green
- Mount # 4 contained supernatant with smaller organelles that cannot be observed under light microscope.

- Iodine solution was used to dilute the above samples. Some samples still had a high concentration of organelles and required further dilution. Dilution prevented the crowding of organelles, so that they form a single or very few layers on the slide.
- Starch grains were commonly observed in most mounts since the layers were not pure due to cross contamination during the preparation, centrifugation and transfer of samples.

Part IV: Tetrazolium Assay for mitochondria

– You used the pellet & supernatant from spin # 2 to test for mitochondria by adding Tetrazolium solution
- You set up 3 samples from spin # 2: supernatant, pellet remixed with cold sucrose & negative control (sucrose only)
- You added equal volumes of Tetrazolium slowly down the side of the tube of these 3 samples, so that Tetrazolium can form a separate layer that floats on top.
- You Incubated at 37 C for 55 min.
- Positive result: pink band will form at the interface of the sample and tetrazolium indicating presence of mitochondria in tube A only. This tube contained the supernatant sample with mitochondria.
  - The pink color results from Tetrazolium reacting with NAD, which is produced only by mitochondria. NADH is a critical component of cellular respiration (you will hear a lot about it in lecture)
  - Tube B yielded negative test result since the pellet does not contain any mitochondria (they are too small/light to form a pellet at this speed)
  - Tube C yielded negative test results as it is a negative control that does not contain any cell organelles. It contained sucrose only.
Study Guidelines:

- Review the concepts and methods involved in this lab using the lab manual and the PowerPoint presentation.
- Review this document to summarize and check your understanding and to review the new terms.
- Reflect on your own data:
  - Did you see all the organelles as expected?
  - How did your data compare to class data?
  - Did you observe any cross contamination?
  - Why did the starch grains seem to predominate?