Energising Cells

Investigating microscopic autotrophs and heterotrophs

Unicellular organisms need to get food for energy, just like any multicellular organism. Have you ever wondered how they get their energy? You will explore that question in this practical.

Euglena gracilis is a unicellular eukaryote classified in Kingdom Protista. *Euglena* usually live in fresh water. They are motile, thus require energy for flagellar function. They are quite adaptable, preferring to live in sunny locations, yet if grown in the dark they can still survive by getting energy by different processes. How does *Euglena* get its energy? You will use the microscope to explore the structures used by *Euglena* to gain energy.

Paramecium caudatum is a large unicellular protozoan, a member of Kingdom Protista.
Most Paramecium species are colourless. They are large motile cells, with many enzyme filled organelles and require a lot of energy to get around. How does *Paramecium* get its energy? In this practical you will feed *Paramecium* and observe the cellular processes used to obtain energy.

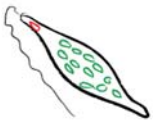
Materials 

- *Paramecium caudatum* culture
- *Euglena gracilis* culture
- Baker's yeast stained with Congo red *SAFETY GLASSES used when dispensing*
- Compound microscope with magnification up to 400x
- Microscope slides
- Coverslips
- 2 transfer pipettes
- Marker pen
- Digital camera to capture microscope images of the cells

Procedure

SPECIMEN 1: Euglena

Prepare a wet mount



1) Using a Pasteur pipette, place a drop of *Euglena* culture onto a slide
2) Add a coverslip
3) View at up to 400x magnification (go to 1000x magnification if available)

Record your observations, including the following points

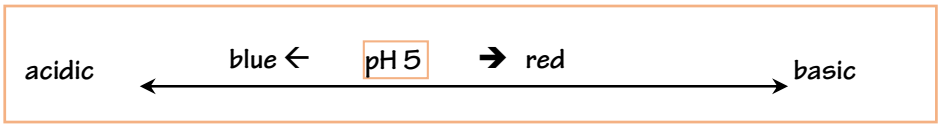
- Name of specimen
- Magnification
- Make an annotated diagram and label all structures.
- If possible photograph the cells under the microscope
- Note colours of cells and organelles. Find the names of the organelles observed
- Describe any motion or cell behaviours observed
- Estimate cell size (if you have previously calibrated the microscope)

SPECIMEN 2: Paramecium.

You have a small dish or tube containing *Paramecium*.

You have a tube containing yeast.

The yeast cells have been stained with Congo red. Congo red is also a pH indicator, appearing blue at < pH 5 (acidic) and appearing red at > pH5.



Feed the Paramecium and Prepare wet mounts

1) Add ONE drop of the red stained yeast cells to the dish of paramecium (take a sample from the bottom of the tube to ensure you collect plenty of cells)
2) Immediately, using a Pasteur pipette take a sample to prepare a wet mount: (*Paramecium* like to gather around the food, so try to take a sample from around the chunky food in the culture)
   - place the drop of the cell mixture onto a slide
   - add a coverslip
3) View at 100x magnification and then at 400x magnification
4) After 10minutes, take another sample for a wet mount
5) After 60minutes or longer, take another sample for a wet mount

Results

Record your observations, including the following points

- Name of specimen
- Magnification
- Estimate cell sizes (based on prior microscope calibration)[CLUE: *Saccharomyces cerevisiae*, baker's yeast, cells are around 7µm in length]
- Make an annotated diagram and label all structures
- If possible photograph the cells under the microscope
- Note colours of cells and organelles – make a table to record changes over time
- Find the names of the organelles observed
- Describe any motion or cell behaviours observed

Laboratory Preparation Notes

Materials

Live cultures; Other		Cost in 2015
L4.40 - Paramecium caudatum, live	Southern Biological	**$18.80**
L1.30 - Euglena, live		**$18.80**
Saccharomyces cerevisiae, Baker's yeast, dry	supermarket	inexpensive
Congo red SIP5.1 - Congo red Care when handling Congo red powder	Southern Biological	**$35.00**

Yeast stained with Congo red

- Store dry yeast in the refrigerator. Warm to room temperature before opening.
- For this practical, older yeast can be used as they are not needed for growth, just as a food supply for *Paramecium*.
- Mix approx. 0.25g dry baker's yeast with 20mL tap water. mix into a homogeneous suspension
- **Kill the yeast by boiling or placing in the microwave until boiled – important because**
  - Live yeast will grow and potentially produce a froth that releases Congo red-contaminated aerosol
  - Prevents it going very smelly if stored for later use
- Cool to room temperature
- In the fume hood, or wearing a face mask, use a small (micro) spatula to add a very small amount of Congo red powder to the yeast suspension until the colour is a deep red. The amount is too small to weigh out, so you add directly to the yeast. Mix thoroughly.
- Dispense into 0.2-0.5 mL aliquots for student benches.
- The killed Congo red-stained yeast can be stored in the fridge for weeks. The yeast cells eventually disintegrate, but are still eaten by *Paramecium*.

Paramecium

For each student group:

- Place 1-2mL paramecium culture into a small dish, e.g. 35mm Petri dish, or a clean test tube
- The *Paramecium* culture also contains *Chilomonas* – these are much smaller than *Paramecium;* they may eat disintegrated red-stained yeast cell debris or other red-stained particles in the culture.
- Dispense ~2mL Paramecium per student group. Try to get a high density of cells – dispense with some of the chunky food from the original culture bottle – the paramecium like to congregate around the food.

Euglena

Dispense into small volumes, approx. 0.1-0.2 mL, for each student bench.

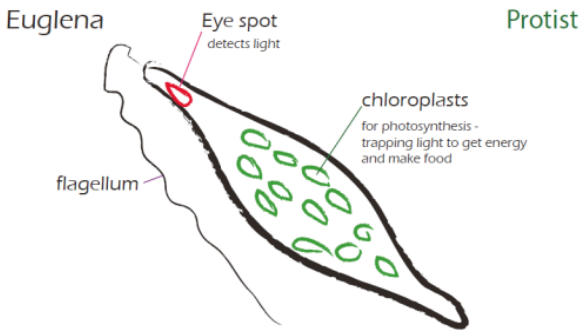
Alternatively, arrange the lab with one station for students to come and collect a drop of Euglena for a microscope slide.

Cultures of *Euglena* from SB are usually high cell density; one drop of cells is plenty to see.

Background information

This practical provides direct observation of functioning organelles in living cells. The focus is on how the cells use their organelles to gain or produce food and energy. The role of digestive organelles, lysosomes, is directly observed by feeding paramecium with stained yeast.
Relevant theory to cover before & in conjunction with this prac: prokaryotes/eukaryotes, autotrophs & heterotrophs cell structure, organelles & functions - especially mitochondria, chloroplasts, lysosomes, flagella/cilia.

Euglena is a unicellular protist about 10µm long, with chloroplasts, a red stigma (eyespot) for detecting light and flagella for movement; the flagella are located at the 'front' of the cell next to the eyespot. It has chloroplasts when grown in the light, i.e. it is a photosynthetic autotroph, and stores excess glucose as starch. When grown in the dark it loses chlorophyll and uses its stored starch, i.e. it behaves as a heterotroph. Because it changes between autotrophic and heterotrophic lifestyles it is hard to classify as an alga (usually photosynthetic autotrophic protists) or a protozoan (usually heterotrophic protists). They are motile, roll around and change shape.



Paramecium is a unicellular protozoan about 200-250µm (very big for a cell), with millions of cilia all around its membrane for motion. They are heterotrophic, i.e. do not have chloroplasts and have to eat smaller cells to survive. Cilia around the oral groove sweep in food cells, taken in by phagocytosis into food vacuoles, which subsequently fuse with lysosomes for digestion of the material. Lysosomes have digestive enzymes and acidic pH. We are looking for lysosomal action by feeding yeast cells (pre-stained with Congo red) to the live paramecium. Soon after mixing paramecium & yeast, red yeast cells are seen in food vacuoles near the oral groove. After a little more time some vacuoles have turned blue because the lysosomal acid changes the colour. Congo red is a stain and a pH indicator (red at >pH5, blue at <pH5). After a few hours the colour in the vacuoles changes to blue: phagosomes (the vacuole/vesicles containing the consumed yeast) fuse with lysosomes, the digestive enzymes and acid condition in the lysosomes cause Congo red to change colour to blue.