## TP 3. MICROSCOPIC STUDY OF PLANT CELLS

**OBSERVATION OF AN ONION SKIN (*ALLIUM CEPA*L.)**

1. **INTRODUCTION**

Observing a fragment of “inner” epidermis of the onion bulb allows us to understand the essential structure of the plant cell.

The onion bulb shows, when cut vertically, a very short stem called a tray which carries a bundle of adventitious roots and scales nested one inside the other. The outermost ones are dried out, the others are full of reserves. Axially, the central bud is enveloped in thin scales.

**Dry scales**

**Fleshy scales Central bud**

**Very short stem (plateau)**

**Youth roots**

1. **MEQUIPMENT AND REAGENTS**

Microscope, slides, coverslips, 1 onion, 1 knife, fine tweezers, 2 watch glasses for each bench, neutral red solution at 1g/L (Dissolve 0.1g of neutral red in

100 ml of buffer

phosphate at pH 6.5: the penetration of neutral red into the cells is only possible at this pH), iodo-iodide or Lugol water solution (4g of iodine, 8g of KI in 1L of distilled water) , crystallizer with bleach for used slides and coverslips

1. **PBLADE REPAIR**
* We prepare 2 dyes simultaneously in 2 watch glasses:
	+ Neutral red
	+ Iodo-iodide water solution
* Using fine forceps, we take small shreds of epidermis from the concave side of an onion scale.

We immediately place them in the colored solutions (2 or 3 in each watch glass).

* On a first slide, place 1 drop of the neutral red solution and place 1 or 2 flaps on it. Cover

of a slat.

* We do the same with the 2nd slide and the iodine solution.



**Installation of the slat**

**Preparation complete**

1. Observations (objectives x10, x40)

## Neutral red coloring (vital coloring)

Neutral red penetrates the cell without killing it: it is a vital dye.

* Look for the lowest objective, a clear image of the preparation and place a cell

in the center of the microscope field.

* Switch to high magnification (400X)
* Draw, put a title and a legend and describe your observations: coloring, shape

cells, presence of vacuoles, nucleus and nucleoli, pecto-cellulose wall etc.

## Iodine staining (post-vital staining)

Iodine kills the cell by causing coagulation of the cytoplasm and nucleus (it is a fixative) and by tinting certain elements yellow.

Proceed as in 4.1

## TP 4. MICROSCOPIC STUDY OF ANIMAL CELLS

* + 1. **OBSERVATION OF LIVER CELLS (HEPATOCYTES)**
	1. **GGENERAL**

Practical equipment for the microscopic observation of animal cells consists of fresh liver. Sheep, veal or chicken liver can be used indiscriminately. The only constraint that must be taken into account is that the liver must not have been

frozen.

* 1. **MEQUIPMENT AND REAGENTS**
	+ Piece of liver, microscopes, slide and coverslips, spatula, paper towel, glycerol, demethylene blue 0.01% (Dissolve 100 mg of powdered methylene blue in 100 mL of distilled water).
	1. **MOPERATIVE ODE**
	+ Cut a small piece of liver and scrape the surface of the section with a spatula so as to Place a sample no larger than a lens on a microscope slide.
	+ Dissociate the cells as best as possible with the spatula then cover with a drop ofblue [of](http://www.snv.jussieu.fr/bmedia/ATP/#Solutions)

[methylene .](http://www.snv.jussieu.fr/bmedia/ATP/#Solutions)

* + Leave to act for approximately one minute.
	+ Place a drop of glycerol and mix well with the spatula.

# Glycerol makes it possible to observe the preparation for a long time without risking evaporation of the mounting medium.

* + Place a cover slip on the sample and place the whole thing on a sheet of paper towel.
	+ Use another sheet to press firmly on the coverslip so as to dissociate the cells into

taking care not to break the lamella.

# The paper is used to wring out the excess liquid that escapes during pressing.

* + Carefully wipe the surface of the coverslip and observe under a microscope.
	+ Look for regions of the preparation where cells are dissociated and

sufficiently colorful to facilitate their observation.

* 1. **RRESULTS**

Draw, put a title and a legend and describe your observations:

* + Shape and size of hepatocytes, shape and number of nuclei per cell, presence of nucleolus, presence

granulations etc.

## OBSERVATION OF ORAL EPITHELIUM CELLS

* 1. **MEQUIPMENT**

Microscope, slides, coverslips, hematoxylin-eosin or methylene blue, wash bottle with ordinary water, dropper, staining tank, crystallizer with water

Bleach for used blades and coverslips, Bunsen burner.

* 1. **PRELIEF AND COLORING**
* Rinse first, mouth with water
* Rub the inner wall of the cheek with a clean finger.
* Place the collected product on a slide and place it on the support of the staining tank.
* Cover immediately with a few drops of hematoxylin-eosin or methylene blue
* Rinse with water after 5 minutes.
* With absorbent paper, wipe the underside and around the edge of the blade.

- Cover the preparation with a coverslip and place the slide on the microscope stage.



* 1. **OBSERVATIONS**

Draw, put a title and a legend and describe your observations:

**High magnification** : coloring of different cellular compartments, presence of granulations, appearance and size of cells, appearance of the cell membrane, etc.

## Remarks

* If the preparation is good, one or two shiny grains can be observed in

the core. These are the**nucleoli**(set of fibrils and granules, producer of ribosomes).

* In reality, these cells constitute a tissue (they are joined). We can observe them isolated, because upon friction, they have been detached from this tissue.

## Finally, draw up a summary table of the distinctive morphological and structural features of animal and plant cells