April 14, 2011

PROBLEM 2

Lenses, not only contact ones

General instructions

You have 4 hours to finish all tasks!

Wear a laboratory coat and safety glasses at all times within the laboratory.

Eating and drinking is prohibited in the laboratory.

Disposable gloves are provided and must be worn when handling chemicals.

Use just the pen, pencil and calculator provided.

All paper used, including rough work paper, must be handed in at the end of the experiment.

All results must be entered into your answer sheet.

Your calculations must be handed in along with the answer sheet.

Only the final answer book, and attached sheets, will be marked.

The tasks may be carried out in whatever order you wish.

When you finish your tasks, leave everything on the desk. You are not allowed to take anything from the laboratory.

Introduction

One of the Czech inventions are so called soft contact lenses. They were invented by the Czech chemist Otto Wichterle and his assistant Drahoslav Lím, who also invented the first hydrogel used for their production. These corrective lenses usually placed on the cornea of the eye are now spread worldwide.

A. Optical properties of various lenses

Generally, a lens is an optical device which influences the propagation of light. It can be made out of various materials, glass lenses are very common, however also e. g. water can act as a lens (Nicolas Cage used the bottle with water instead of magnifier in the movie "National treasure", see the figure), or the above mentioned hydrogel.

There are basically two different types of lenses according to the way how they influence the beam – converging lenses and diverging lenses, see Fig 1.

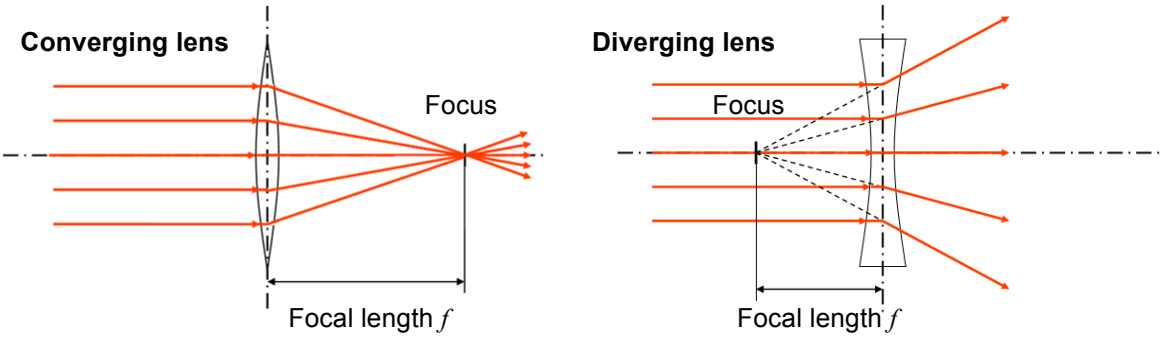


Figure 1 – Lenses

Beware! Do NOT look directly into the laser beam!!!

TASK A.I: THICK WATER LENS WITH VARYING RADIUS

Apparatus and material: Erlenmeyer flask, 4 sheets of cardboard, scissors, ruler with scale, laser pointer, compass

Prepare the set of four cardboards with the circled opening in the middle of them that can be set on the Erlenmeyer flask, see Fig 2.

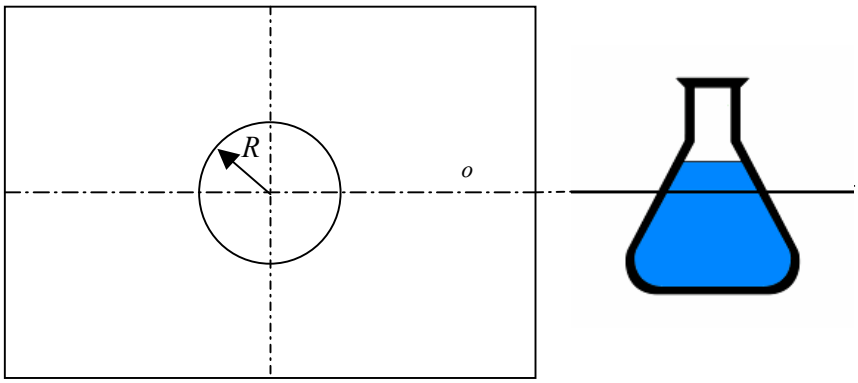


Figure 2 – Preparing of the cardboards

Draw lines dividing the cardboards to two halves in both directions. Use the compass to draw a circle of the radius *R* with the centre in the intersection of both axes, see Fig 2. Choose four different radii – in such a way four different lenses will be measured. Recommended value of *R* are in the interval 2.5 – 5.5 cm. Cut the circle openings in the middles of the cardboards by scissors. The longer axis of the cardboard would be an optical axis (axis *o* in Fig 2). Draw also two half-lines on each cardboard parallel to the optical axis starting in the openings. The half lines should be on different side from the axis in the distance not longer than 50 percent of the radius, see Fig 3. Set the cardboard on the flask and place the laser pointer in such a way that it makes a "light ray" on the prepared half line. Bend the cardboard in a suitable way so you will be able to see a light ray even behind the flask. Find the point where the ray intersects the optical axis, see Fig 3. The light beam diffracts also in the vertical plane while propagating through the flask, see Fig 4. That is the reason why you have to bend the cardboard to find the correct position of the focus. Draw the intersections of the rays with the optical axis for both prepared parallel rays on each cardboard and draw also the trajectories of the rays. **Add your cardboards to the answer sheet.**

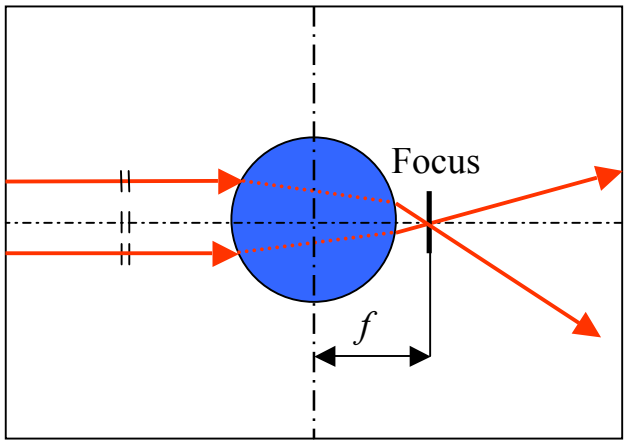


Figure 3 – Focal length measurement

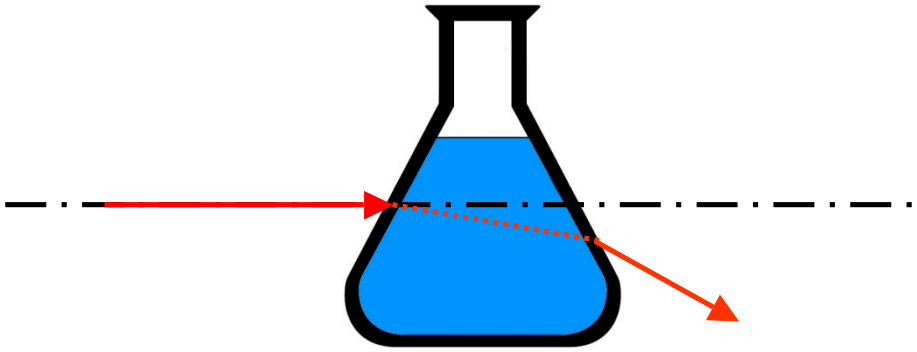


Figure 4 – The diffraction in the vertical plane

A.I.1 Measure the focal lengths f_1 – f_4 for the radii R_1 - R_4 and record them to the Answer sheet.

Draw the graph of the dependence of *f* on *R on the graph paper.* Denote your graph by "GRAPH A1" and do not forget to add it to the Answer sheet.

A.I.2 What is the pattern of your graph? Choose one of the following possibilities.

a) $f = ke^{qR}$, $q>0$

b) $f = ke^{qR}$, $q<0$

c) $f = kR + q$, $k>0$

d) $f = kR + q$, $k<0$

e) $f = kR^2 + qR$

A.I.3 Estimate the values of the parameters *k* and *q* from your graph and record them to the Answer sheet including the correct units.

Suppose that the lens are made from homogeneous material called "waterglass", the following equality will hold true

$$k = \frac{n}{2(n-1)},$$

where n is the refractive index of "waterglass".

A.I.4 Determine the refractive index of waterglass. Write your answer to the Answer sheet.

TASK A.II: OPTICAL BENCH

Apparatus and material: optical bench, glass lens, laser pointer with two LED sources of light, screen, scale, screw driver

Put togehter the optical bench according the attached instruction manual and collimate the laser beam. Put the lens approximately 30 cm from the light source. Switch on the LEDs and focalize their image on the screen. Denoting by a the distance of the light source to the lens and by a' the distance of the image to the lens, the following equation is satisfied

$$\frac{1}{f} = \frac{1}{a'} + \frac{1}{a}$$

where f is a focal length.

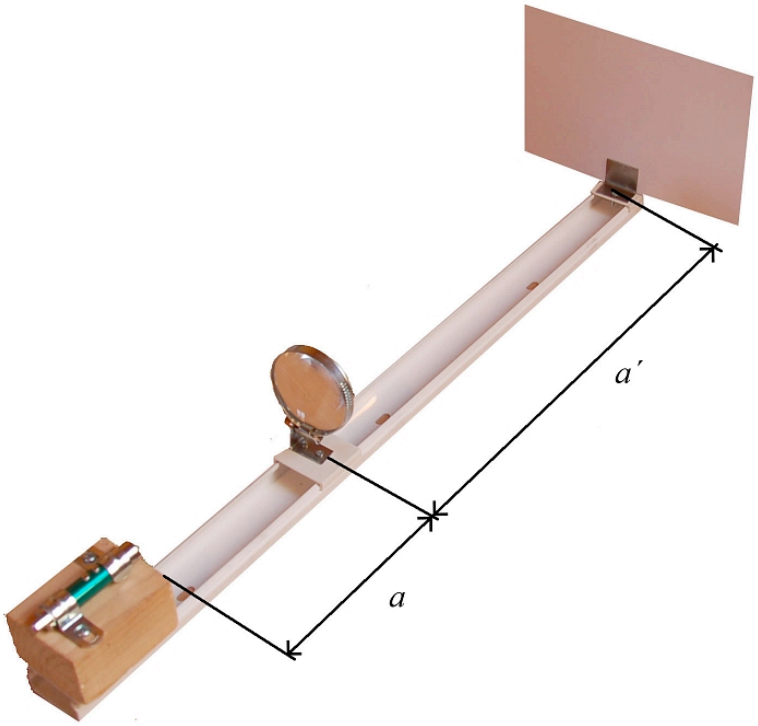


Figure 5 – optical bench

Another quantity suitable for the description of the lens properties is called magnification

$$Z = \frac{y'}{y}$$

where y denotes the distance of two points on the source in the plane perpendicular to the optical axis and y' is the distance of these two points on the screen.

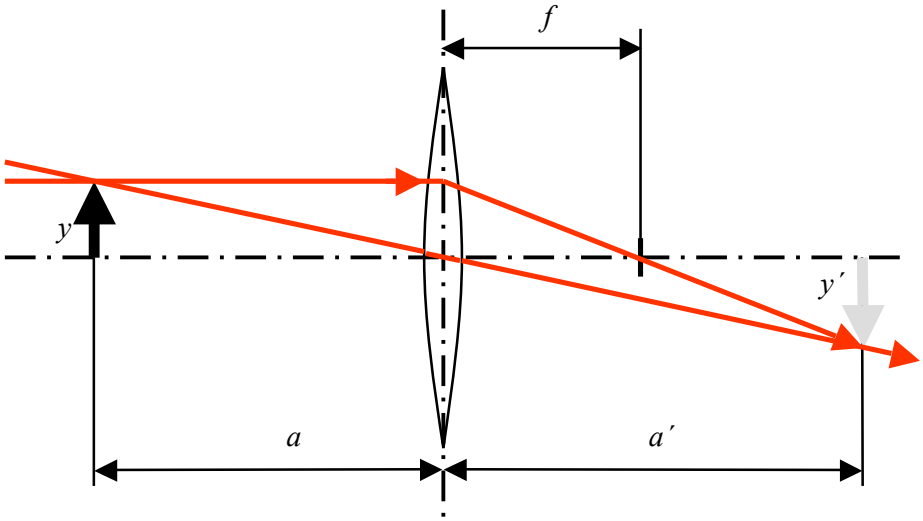


Figure 6 – For deriving the formula for the maginification

A.II.1 Use the Fig 6 to derive the formula for the magnification in terms of the distance of the light source to the lens *a* and the distance of the image to the lens *a'*.

A.II.2 Measure the distance of the source to the lens and the distance of the image to the lens. Repeat the measurement for 5 different values of the distance of the source to the lens. Write your measurements to the table in the answer sheet.Calculate the magnification for each measurement. Write your results to the table A.II.2 in the Answer sheet.

Draw a graph on the graph paper of the dependence of the magnification on the distance of the image to the lens. Denote the graph by "GRAPH A2" and do not forget to add it to the Answer sheet.

A.II.3 Derive the formula for the magnification in terms of the focal length and the distance of the image to the lens.

A.II.4 Use the Graph A2 to determine the focal length of the lens. Indicate in the graph the way how you have obtained the focal length and write your result to the Answer sheet.

TASK A.III: CONTACT LENS

Apparatus and material: laser pointer with two LED sources of light, screen, contact lens

Carefully place the contact lens to the light source on the optical bench. It is necessary that the bench is collimated according to the instructions! Use the screen and move it slowly from the distance of 10 cm from the laser beam source to 3 m from the laser beam source. Study the trace of the laser beam on the screen.

A.III.1 In the Answer sheet circle the correct word on each line.

A. The trace is getting **bigger** **smaller** with the distance from the source.
B. Given contact lens is **converging** **diverging**.
C. Is it possible to see the image of any object displayed by the contact lens on the screen? **YES** **NO.**

DO NOT FORGET TO ADD YOUR GRAPHS AND CARDBOARDS TO THE ANSWER SHEET!

B. Residual formaldehyde measurement

Formaldehyde is a colourless gas with a characteristic pungent odour. It is an important precursor to many other chemical compounds, especially for polymers. In the history of contact lens preparation, formaldehyde was used as a part of polymerization mixture. The reason, why formaldehyde based polymers were banned was residual formaldehyde content, which irritated the lens and lead to the contact allergy responses. The only complication concerning formaldehyde today is a fact that permeable contact lenses may absorb formalin and cause irritation to the eyes. Those working with concentrated formalin (aqueous formaldehyde solutions) should remove contact lens to prevent eye irritation. I our case we will work with highly diluted formalin solution and measure concentrations relevant to the residual formaldehyde content in industrial polymers, including resins originally tested as a contact lens material.

Iodometric determination of formaldehyde

Iodometry is one of the most important redox titration methods. Iodine reacts directly, fast and quantitively with many organic and inorganic substances. Thanks to its relatively low, pH independent redox potential, and reversibility of the iodine/iodide reaction, iodometry can be used both to determine amount of reducing agents (by direct titration with iodine) and of oxidizing agents (by titration of iodine with thiosulfate). In all cases the same simple and reliable method of end point detection, based on blue starch complex, can be used.

Reversible iodine/iodide reaction mentioned above is:

$$2\ I^- \leftrightarrow I_2 + 2\ e^-$$

and obviously whether it should be treated as oxidation with iodine or reduction with iodides depends on the other redox system involved. Second important reaction used excessively in iodometry is reduction of iodine with thiosulfate:

$$2\ S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2\ I^-$$

In the case of both reactions it is better to avoid low pH. Thiosulfate is unstable in the presence of acids, and iodides in low pH can be oxidized by air oxygen to iodine. Both processes can be source of titration errors.

Iodine is very weakly soluble in the water, and can be easily lost from the solution due to its volatility. However, in the presence of excess iodides iodine creates I_3^- ions. This lowers free iodine concentration and such solutions are stable enough to be used in lab practice. Still, we should remember that their shelf life is relatively short (they should be kept tightly closed in dark brown bottles, and standardized every few weeks). Iodine solutions are prepared dissolving elemental iodine directly in the iodides solution. Elemental iodine can be prepared very pure through sublimation, but because of its high volatility it is difficult to weight. Thus use of iodine as a standard substance, although possible, is neither easy nor recommended. Iodine solutions can be easily normalized against arsenic (III) oxide (As_2O_3) or sodium thiosulfate solution.

Formaldehyde, which is basic part of phenol-formaldehyde resins, is possible to determinate by iodometric titration method. In this method the sample is added to an excess of hypoiodite (IO^-), formed from standard iodine solution making the solution alkaline. Part of the hypoiodite is reduced by the formaldehyde in the sample, and the unreduced part is converted to iodine by acidifying the solution (iodine is then titrated with sodium thiosulfate using starch indicator) according to the following reactions:

$$I_2 + 2\ OH^- \rightarrow IO^- + I^- + H_2O$$

$$HCHO + IO^- + OH^- \rightarrow HCOO^- + I^- + H_2O$$

$$IO^- + I^- + 2\ H^+ \rightarrow I_2 + H_2O$$

These reactions are possible to summarise into the following one:

$$\mathbf{I_2 + HCHO + 3\ OH^- \rightarrow HCOO^- + 2\ I^- + 2\ H_2O}$$

Apparatus and reagents:

- *Sample:* Formaldehyde (in volumetric flask 100 mL)
- *Tools*: 2× Erlenmeyer flask (250 mL)
  2× Titration flask (250 mL)
  1× Pipette 10 mL
  2× Burette 25 mL
  1× Funnel
  2× beaker 150 mL
  1× Graduated cylinders 10 mL
  1× Plastic wash bottle (with distilled water)
- *Chemicals*: 0.1 MSodium thiosulfate ($Na_2S_2O_3$) solution - real concentration declared by organisers written on the board
  approx. 0.05 M Iodine (I_2) solution
  Starch indicator
  Hydrochloric acid (HCl) – diluted 1:4 with distilled water
  2 M Sodium hydroxide (NaOH)

TASK B.I: STANDARDISATION OF APPROX. 0.05 MIODINE SOLUTION

- Put 10.0 mL of standard iodine solution (from burette) into a 250 mL titration flask.
- Add appropriate volume of distilled water (approx. 50 mL) and 5 mL of HCl (1:4) by the graduated cylinder.
- Titrate immediately with standardized sodium thiosulfate ($Na_2S_2O_3$) to a light yellow colour.
- Add 5 mL of starch indicator from a graduated cylinder and continue the titration to the disappearance of the blue colour.

- **B.I.1** Record to the answer sheet the volume of standardized 0.1 M sodium thiosulfate in the start position, end position and the difference. Perform the analysis at least twice (three times if necessary).
- **B.I.2** Calculate the concentration of I_2 solution (mol/l). Write your calculations and result to the answer sheet.

TASK B.II: ANALYSIS OF FORMALDEHYDE SAMPLE

- Fill the sample inside 100 mL volumetric flask up to the mark by distilled water.
- Pipette 10.0 mL of sample into a 250 mL Erlenmeyer conical flask.
- Add 15 mL of 2 M sodium hydroxide (NaOH) and accurately 25.0 mL of 0.05 M standard iodine solution (from burette).
- Stopper the flask, swirl the contents, and allow it to stand approximately 5 min.

- At the end of this time period, add 20 mL of HCl (1:4) by the graduated cylinder (solution must get brown according to the iodine creation; otherwise the next portion of acid is required).
- Titrate immediately with standardized 0.1 M sodium thiosulfate ($Na_2S_2O_3$) to a light yellow colour.
- Add 5 mL of starch indicator from a graduated cylinder and continue the titration to the disappearance of the blue colour.

- **B.II.1** Record to the answer sheet the volume of standardized 0.1 M sodium thiosulfate in the start position, end position and the difference. Perform the analysis at least twice (three times if necessary).

- **B.II.2** Calculate the mass of formaldehyde in the sample. Result should be expressed in miligrams (mg) of formaldehyde in the original sample provided.
  M(HCHO) = 30,03 $g \cdot mol^{-1}$

TASK B.III: SUPPLEMENTARY QUESTIONS

B.III.1 Use equations for describing the reaction of iodine with the following ions:

a) SbO_3^{3-} (antimonite)

b) SO_3^{2-} (sulfite)

c) $S_2O_3^{2-}$ (thiosulfate) in neutral environment

d) $S_2O_3^{2-}$ (thiosulfate) in alkaline environment

B.III.2 Which compounds (present at least two for each example) are used for the standardisation of following solutions?

a) Thiosulfate ($S_2O_3^{2-}$)

b) Iodine (I_2)

B.III.3 How many grams of $Na_2S_2O_3.5\ H_2O$ are necessary for the preparation of 500 mL solution at concentration of 0.05 M ($mol \cdot l^{-1}$)?

A_r(Na)=23.0

A_r(S)=32.1

A_r(O)=16.0

A_r(H)=1.0

C. Eye and vision

TASK C.I: VISION

Eyes are organs that detect light and convert it to electro-chemical impulses in neurons. In higher organisms the eye is a complex optical system which collects light from the surrounding environment; regulates its intensity; focuses it through an adjustable assembly of lenses to form an image; converts this image into a set of electrical signals; and transmits these signals to the brain. The first proto-eyes evolved among animals 600 million years ago, about the time of the Cambrian explosion. In most vertebrates and some molluscs, the eye works by allowing light to enter and project onto a light-sensitive panel of cells, known as the retina, at the rear of the eye.

Evolution of the eye

C.I.1. Indicate in the Answer sheet if the following statements are true or false and circle the right answer.

A [TRUE] [FALSE] While photoreception and photoreceptive pigments are phylogenetically very old, eyes developed independently many times in animal kingdom.

B [TRUE] [FALSE] Photoreception, photoreceptive pigments as well as all animal eyes are phylogenetically very old and have a common origin.

C [TRUE] [FALSE] While eyes are phylogenetically very old and of common evolutionary origin, different animal groups co-opted many different photoreceptive pigments.

Adaptation of the vision to life requirements

C.I.2. Indicate in the Answer sheet if the following statements are true or false and circle the right answer.

A [TRUE] [FALSE] Animals living in dark developed pigments sensing ultraviolet light

B [TRUE] [FALSE] Birds of prey posses higher concentration of neural elements such as rods and cones, therefore have much greater visual acuity than humans.

Perception of colours

C.I.3. Indicate in the Answer sheet if the following statements are true or false and circle the right answer.

A [TRUE] [FALSE] Vision in mammals is restricted to a small range of electromagnetic spectrum; this varies from creature to creature, but is mainly between 400 and 700 nm.

B [TRUE] [FALSE] Vision in organisms covers substantial part of the electromagnetic spectrum, varies from creature to creature, in majority invertebrates spans from ultraviolet to infrared wavelengths (100 – 1500 nm)

Adjusting the focus

C.I.4. Indicate in the Answer sheet if the following statements are true or false and circle the right answer.

A [TRUE] [FALSE] The curvature of the human lens can be adjusted to "tune" the focus depending upon the object's distance

B [TRUE] [FALSE] Human lens have fixed shape, focusing is achieved by moving the lens forwards or backwards within the eye.

Colour vision

C.I.5. Indicate in the answer book if the following statements are true or false and circle the right answer.

A [TRUE] [FALSE] Mammals, except for primates, are colour-blind

B [TRUE] [FALSE] Most mammals posses dichromatic colour vision, they can distinguish blue from yellow-green but not red from green; they are red-green colour-blind

C [TRUE] [FALSE] Sub mammalian vertebrates are colour-blind

TASK C.II: CORNEA

The cornea is the transparent front part of the eye that covers the iris, pupil, and anterior chamber. Together with the lens, the cornea refracts light, with the cornea accounting for approximately two-thirds of the eye's total optical power. In humans, the refractive power of the cornea is approximately 43 dioptries. While the cornea contributes most of the eye's focusing power, its focus is fixed. Important functions of the cornea are mechanical resistance and translucence. This is linked to the corneal morphology, which is composed from several distinct layers.

Your task will be to stain cornea, which was briefly fixed with formaldehyde, saturated with sucrose, frozen and cryocut to the thickness 10 micrometers.

- put glass with the corneal cryosection inside the staining chamber
- use plastic Pasteur pipette for the transfer of liquids on top of the glass slide
- please wash the Pasteur pipettes properly with the distilled water after each transfer
- using Pasteur pipette cover the cryosection with approx. 1ml haematoxylin solution, incubate for 5 min
- wash excess of the staining solution with distilled water
- using another Pasteur pipette cover the cryosection with approx. 1ml eosin solution, incubate for 5 min
- wash excess of the staining solution with distilled water
- remove the excess of the water from the glass by touching the filtration paper
- put a droplet of water (10 microliters) on the top of the cryosection and cover with the cover glass
- microscope the specimen

C.II.1. Draw with a pencil a schematic picture of the histological crossection to the Answer sheet and using following characteristics identify distinct cellular layers. Highlight them in the drawing using pencils with different colours. With the arrow mark the direction of the light entering the eye.

A. **Corneal epithelium (shade the area in RED)**: a thin epithelial multicellular tissue layer (non-keratinized stratified squamous epithelium). It is composed of about 6 layers of cells which are shed constantly on the exposed layer.
B. **Corneal stroma (shade the area in BLUE):** a thick, transparent layer, consisting of regularly-arranged collagen fibers along with sparsely distributed interconnected keratocytes.
C. **Corneal endothelium (shade the area in GREEN)**: a simple squamous or low cuboidal monolayer of cells responsible for regulating fluid and solute transport between the aqueous and corneal stromal compartments.

C.II.2. One of the layers described in C.II.1. does not regenerate. Remnant cells stretch to compensate loss of the dead cells. The overall cell density of the particular layer therefore reduces with age. Which one of the 3 cell layers does not regenerate? Encircle in the Answer sheet the right answer.

A
B
C

C.II.3. Based on your observation of the histological specimen and your experience select the right answer/answers. Which of the following tissue types are localized in the cornea? Indicate in the answer book if the following statements are true or false and circle the right answer.

A **[TRUE] [FALSE]** epithelial tissue
B **[TRUE] [FALSE]** connective tissue
C **[TRUE] [FALSE]** muscle cells
D **[TRUE] [FALSE]** sensory neurons

C.II.4. Based on your observation of the histological specimen and your experience indicate in the answer book if the following statements are true or false and circle the right answer (suppose that we are talking about healthy people).

A **[TRUE] [FALSE]** The cornea has no blood supply; it gets oxygen directly through the air. Oxygen first dissolves in the tears and then diffuses throughout the cornea to keep it healthy.
B **[TRUE] [FALSE]** The cornea is highly vascularised; it gets oxygen directly from the capillaries. Atherosclerosis leads to the loss of the corneal translucence called glaucoma, with causal treatment - corneal transplantation.

TASK C.III: NON-KERATINIZED STRATIFIED SQUAMOUS EPITHELIUM

In your body you can find similar tissues to the corneal non-keratinized stratified squamous epithelium. One of them is buccal epithelium localized inside your mouth.

- gently scrape surface of your mouth with toothpick

- resuspend cellular material in the Eppendorf tube in the 200 microliters of the 140 mM NaCl
- pipette 30 microliters of the cell suspension to the edge of the microscopy glass
- prepare 4 smear specimens using 30 microlitre of the cell suspension
- let the smears dry
- put glasses with the smears inside the staining chamber
- using Pasteur pipette cover the glasses with approx. 2ml ethanol solution and incubate for 5 min
- please wash the Pasteur pipettes properly with the distilled water after each transfer
- wash excess of the fixation solution with distilled water
- using Pasteur pipette cover the glasses with approx. 2ml staining solution (use 4 different staining solutions (A – Acridine orange, B - Haematoxylin, C – Eosin and D - Toluidin blue) and incubate for 10 min
- wash excess of the staining solution with distilled water
- put a droplet of water (10 microliters) on the top of the stained smears and cover with the cover glass
- microscope the specimen

C.III.1 Which dyes (A-D) stain basophilic structures (binds to acidic molecules, in the cells stain mostly nuclei)? Encircle in the Answer sheet the right answer(s).

A **[YES] [NO]**
B **[YES] [NO]**
C **[YES] [NO]**
D **[YES] [NO]**

C.III.2 Which dyes (A-D) stain acidophilic structures (bind basic molecules, in the cell stain mostly cytosol)? Encircle in the Answer sheet the right answer(s).

A **[YES] [NO]**
B **[YES] [NO]**
C **[YES] [NO]**
D **[YES] [NO]**

C.III.3. How 96% ethanol fix the tissue sample? Indicate in the answer book if the following statements are true or false and circle the right answer.

A **[TRUE] [FALSE]** Covalently modify macromolecules in the sample.

B **[TRUE] [FALSE]** Dehydrate and therefore denaturize - in that way in non-water environment cellular components, mostly proteins dramatically change conformation.

C.III.4. Identify and draw in the Answer sheet a cell covered with the bacteria, indicate which dye(s) (A, B, C or D) was(were) used for the staining of the specimen, where bacteria were easily visible. Indicate the bacteria with an arrows.

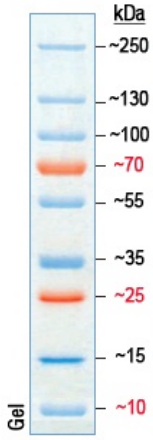
C.III.5 The size of the buccal cell is about 100 [ ] – add appropriate metric system units in the box.

TASK C.IV: LENS

The lens is a transparent, biconvex or spherical structure in the eye that, along with the cornea, helps to refract light to be focused on the retina. Adjustment of the lens is known as accommodation. The lens is flatter on its anterior side. In humans, the refractive power of the lens in its natural environment is approximately 18 dioptres, roughly one-third of the eye's total power. Size and shape can change due to accommodation and because the lens continues to grow throughout a person's lifetime

In a Petri dish you can find polyacryamide gel with separated proteins obtained from the mammalian lens along with the molecular weight standards.

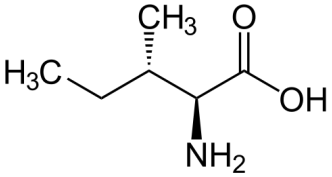
C.IV.1 Draw in the Answer sheet (along with the molecular weight standards) position of the bands corresponding to the 4 major lens protein components named crystallins - soluble proteins that compose over 90% of the protein within the lens.
Indicate corresponding estimated m.w. of individual crystallins.



Ladder of molecular weight standards used in the experiment with corresponding molecular weights in kDa (1 Da=1.66x10^{-27} kg=unified atomic mass unit).

C.IV.2 Identify in the drawing (using an arrow) the edge of the gel where samples were loaded.

C.IV.3 What is the approx. number of amino acids in the biggest crystalline? Below is a model structure of an amino acid with molecular weight close to the average amino acid molecular weight.



C.IV.4 Amount of the protein in the crystalline band with highest molecular weight is about 10 micrograms. Sample loaded to the gel corresponds to the 1/500 of the total protein amount from one mouse lens. How many of these crystalline molecules contain visual system of a single mouse?

C.IV.5. Indicate in the Answer sheet if the following statements are true or false.

A [TRUE] [FALSE] Lens proteins must last in a human for his/her entire lifetime

B [TRUE] [FALSE] Important factor in maintaining the transparency of the lens is the absence of light-scattering organelles such as the nucleus, endoplasmic reticulum, and mitochondria within the mature lens fibers

C [TRUE] [FALSE] Glucose is the primary energy source for the lens. As mature lens fibers do not have mitochondria, majority of the glucose is metabolized via anaerobic respiration.

GOOD LUCK !!!