STUDENT’S HANDBOOK

OPTICS

Student’s name: ....

Group: ....

Rules and regulations concerning a course in Biophysics can be found at the webpage https://www.umb.edu.pl/en/s,7120/Rules_and_regulations

CONTENTS

ASSIGNMENTS FOR OPTICS LAB EXERCISES

1.1 Lab Exercise

Determining solution concentrations using a refractometer and a polarimeter

1. Fermat's principle, the law of light refraction, the law of light reflection
2. Phenomenon of the total internal reflection of light
3. Operation principle of the waveguide, endoscopy
4. Operation principle of the refractometer
5. Phenomenon of light polarisation
6. Methods of light polarisation
7. Optical birefringence
8. Optically active substances
9. Optical isomerism
10. Applications of polarimetry in diagnostics
11. Determination of a simple equation by the least square method
12. Types of concentrations: weight to weight, weight to volume, molar and normal

1.2 Lab Exercise

Measurement of focal length and properties of converging lenses

1. Fermat's principle, the law of light refraction, the law of light reflection
2. Thin lenses
3. The lens equation, lens magnification and types of lenses
4. Systems of lenses
5. Focal length and converging properties of the lens and system of lenses
6. Lens aberrations
7. Optical structure of the human eye
8. The lens of the human eye
9. Accommodation of the human eye, accommodation range
10. Resolving abilities of the human eye
11. Energetics of the human vision process
12. Young's model of colour vision

1.3 Lab Exercise

Determining solution concentrations using an absorption spectrophotometer

1. Types and classification of electromagnetic waves
2. Visible light, ultraviolet radiation
3. Young's model of colour vision
4. Mechanism of absorption spectra formation
5. Bouger-Lambert-Beer law
6. Extinction and transmission
7. Determination of simple equation by the least square method
8. Bohr's model of the hydrogen atom
9. Mechanism of hydrogen spectral series formation
10. Structures of the free atom, the atom in a molecule, the atom in a solid
11. Influence of IR, VIS and UV radiation on human organism
12. Mechanism of emission and absorption spectra formation
13. Line, band and continuous spectra
14. Applications of spectral analysis

1.4 Lab Exercise

Weakening of the laser light beam while passing through the solid matter. Determination of the extinction module.

1. The principle laser action
2. Types of lasers
3. Properties of laser light
4. Effects of laser light on tissue
5. Radiation Absorption Law
6. Exponential function, logarithmic function

LITERATURE:

1. Paul Davidovits – “Physics in Biology and Medicine”
2. Roland Glaser – “Biophysics”

1.1 Lab Exercise

DETERMINING SOLUTION CONCENTRATIONS USING A REFRACTOMETER AND A POLARIMETER

EXPERIMENTAL PART

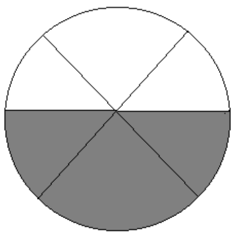
Objective: Determination the concentration of solutions
Materials: a refractometer, a polarimeter
a) Preparation of solutions

- Prepare sugar solutions in water of the following (weight-weight) concentrations 5%,10%,15%,20%,25%,30%, 50 grams of each.
- The students are divided into two subgroups, each of which prepares a 50 gram aqueous sugar solution keeping the concentration value in secret – x_0. Write down the value x_0 of your subgroup here,
- x_0 =

b) Refractometer – measurement of the light refractive index

Spread a thin layer of the solution on the matt surface of the refractometer glass. Next, turn the prism knob to position the refractometer prisms in such a way that the division line between the bright field and the dark one cuts across the intersection point of the crosshairs (Fig.1)

Fig. 1. Image seen in the refractometer eyepiece.



Data and observations

From the scale we read out the value of the light refraction index in the solution for all the prepared solutions and distilled water and enter the results into the table:

Solution concentration (%)	Value of refractive index „n”
0 (distilled water)	
5	
10	
15	
20	
25	
30	

Using a computer program we calculate the linear dependence (simple linear equation and correlation coefficient) for the obtained values of the light refraction index dependent on solution concentration.
Write down here:

- obtained equation: y =
- value of the correlation coefficient R^2 =

Next we carry out the measurement of the light refractive index of the solution prepared by the second subgroup.
Write down here:

- measured value of the light refractive index n =

Making use of the obtained dependence between the value of the light refraction index and the solution concentration we calculate the concentration of solution – x – prepared by the second subgroup.
Write down the calculations here:

Write down here:

- calculated value of concentration x =

c) Polarimeter – measurement of the rotation angle of light polarisation plane

We fill in the polarimeter cell with the solution and place it in the tube. Prior to this, we check the zero point of the polarimeter i.e. on the polarimeter scale we find such a point that corresponds to the image in which all the elements in our field of vision have the same colour (Fig. 2)

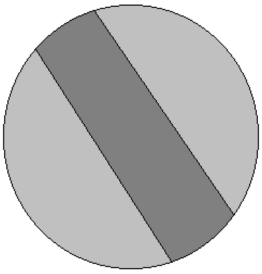


Fig. 2. Image seen in the polarimeter eyepiece with the sugar solution placed inside the tube.

Now we start searching for a new position on the scale to correspond to such an image that would include all the elements of the same colour in our field of vision. We read out the difference between the final and initial position and what we get is exactly the rotation angle of the polarisation plane.

Data and observations

From the scale we read out the value of the rotation angle of light polarisation plane in the solution for all the prepared solutions and enter the results into the table:

Solution concentration (%)	Value of rotation angle „α”
0 (distilled water)	0
5	
10	
15	
20	
25	
30	

Using a computer program we calculate the linear dependence (simple linear equation and correlation coefficient) for the obtained values of the rotation angle of the light polarisation plane dependent on solution concentration.

Write down here:

- obtained equation: y =

- value of the correlation coefficient R^2 =

Now we perform the measurement of the rotation angle of the light polarisation plane in the solution prepared by the second subgroup.

Write down here:

- measured value of the rotation angle of the light polarisation plane α =

Making use of the obtained dependence between the value of the rotation angle of the light polarisation plane and the solution concentration we calculate the concentration of solution – x – prepared by the second subgroup.

Write down the calculations here:

The date	Student's name and surname	Lab assistant signature

1.2 Lab Exercise

MEASUREMENT OF FOCAL LENGTH AND PROPERTIES OF CONVERGING LENSES

EXPERIMENTAL PART

Objective: Determination the focal length of the lenses
Materials: an optical bench, lenses

To determine the focal length of the lenses under investigation we make use of a system which consists of a light source, a lens and screen placed on an optical bench (Fig.1). All the lenses used in our investigation are considered to be thin ones.

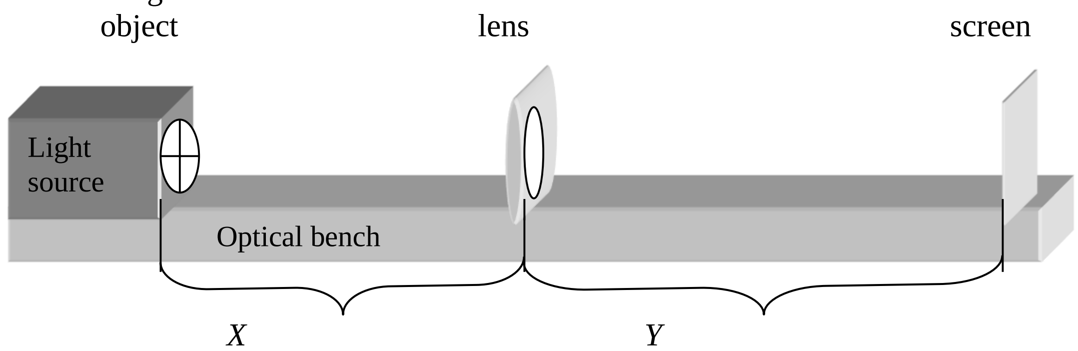


Fig. 1. Drawing of the system used to determine focal lengths.

Having established the distance of the object from the lens, we adjust the distance of the screen from the lens to obtain a clear image of the object. Next we measure the quantities of X an Y on the optical bench and using equation 5 we determine the focal length of the lens. This procedure is repeated at least 4 times changing the distance between the object and the lens by a few centimetres each time. Now keeping the lens under investigation in its position we insert a diverging lens into the frame so that we create a lens system having a converging property. The focal length of the system can be found in the way described for the converging lens above. All the results obtained should be written down in the table prepared according to the arrangement given below:

a) **Measurement of focal lengths (f) and converging properties of the lenses (D)**

	X	Y	f	f-average	D-average
Converging lens					
System of lenses					

b) Data analysis

- Write down the calculations including units for converging lens:

f_1=

f_2=

f_3=

f_4=

f-$_{average}$=…………………… D-$_{average}$=………………………

- Write down the calculations including units for the system of the lenses:

f_1=

f_2=

f_3=

f_4=

f-$_{average}$=…………………… D-$_{average}$=………………………

The date	Student's name and surname	Lab assistant signature

1.3 Lab Exercise

DETERMINATION OF SOLUTION CONCENTRATIONS USING AN ABSORPTION SPECTROPHOTOMETER

a) Solution preparation

- Preparation of aqueous solutions of cupric sulphate ($CuSO_4$) in the following concentrations 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% (10 ml of each).

$CuSO_4$ concentration	$CuSO_4$ volume [mL]	H_2O volume [mL]
1%		
2%		
3%		
4%		
5%		
6%		
7%		
8%		
9%		
10%		

- Each lab group divided into two subgroups prepares an aqueous solution of cupric sulphate (10 ml) of undisclosed concentration – x_0. Write down x_0 value of your subgroup, x_0 =

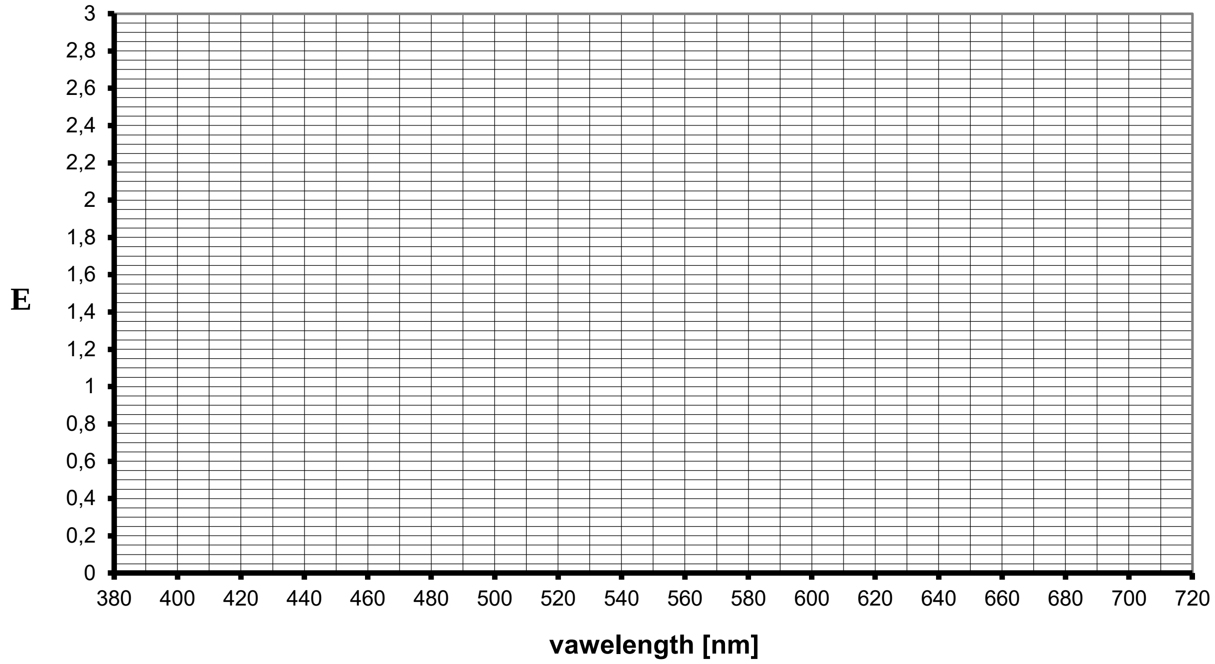
b) Analyzing of $CuSO_4$ absorption spectrum

Find the value of light extinction in 10% solution of $CuSO_4$ changing the wavelength every 10 nm within the visible range of EM spectrum. Write the results into the table below.

λ[nm]	380	390	400	410	420	430	440	450	460	470	480	490
E												
λ[nm]	500	510	520	530	540	550	560	570	580	590	600	610
E												
λ[nm]	620	630	640	650	660	670	680	690	700	710	720	730
E												

- Write down the wavelength at which the extinction reaches its maximum

λ_{max} =…………



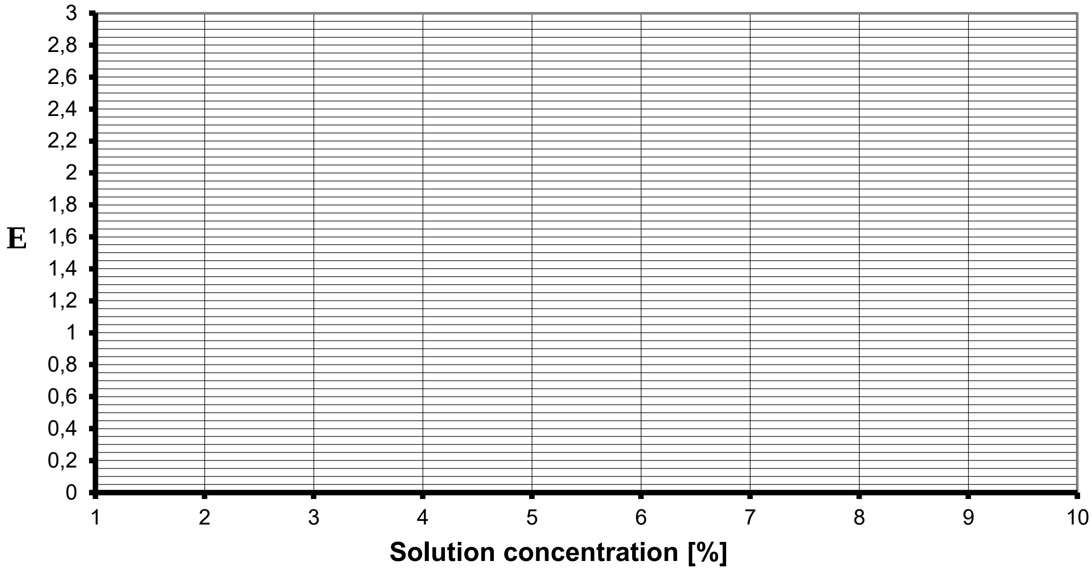
- At λ_{max} measure the extinction for all ten solutions of $CuSO_4$ concentrations from 1% to 10%. Present the result in the table below. Make sure to conduct each measurement in the same experimental conditions i.e. use the same dry clean cuvette at the same wavelength.

c) Extinction measurement

Solution concentration C [%]	E
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

d) Data analysis

- Using a computer program find the linear dependence between light extinction and solution concentration for the values obtained.



Write down here:

- Equation obtained: y =…………………………………………………………….
- Value of the correlation coefficient R^2 =…………………………………………..

e) Determination of solution unknown concentration

Next we measure the light extinction value in the solution with unknown concentration

- Measured extinction value E =

Making use of the obtained dependence of light extinction value on the solution concentration, calculate the solution concentration „x”.

Write down the calculations here:………………………………………………………………………………………
……………………………………………………………………………………………….

- **Calculated value of concentration "x" = …………….**

The date	Student's name and surname	Lab assistant signature

1.4 Lab Exercise

WEAKENING OF THE LASER LIGHT BEAM WHILE PASSING THROUGH THE SOLID MATTER.
DETERMINATION OF THE EXTINCTION MODULE.

Theoretical part

LASER

The word **LASER** is an acronym, which describes the principle of operation of this device: LASER - **L**ight **A**mplification by **S**timulated **E**mission of **R**adiation. The word "light" here is slightly misleading, because it originally stands for the electromagnetic waves of 380-720nm wavelength, i.e. visible to human eye, while modern lasers can also emit infrared radiation (of 760nm -2000μm length), as well as the ultraviolet and X-radiation, which we are not capable of seeing.

Amplification

Laser is a radiation generator and as each generator it transforms provided energy into electromagnetic wave energy. In the process it uses the effect of amplifying radiation in the gain medium, as well as the process of feedback achieved through the resonator. (Fig.1)

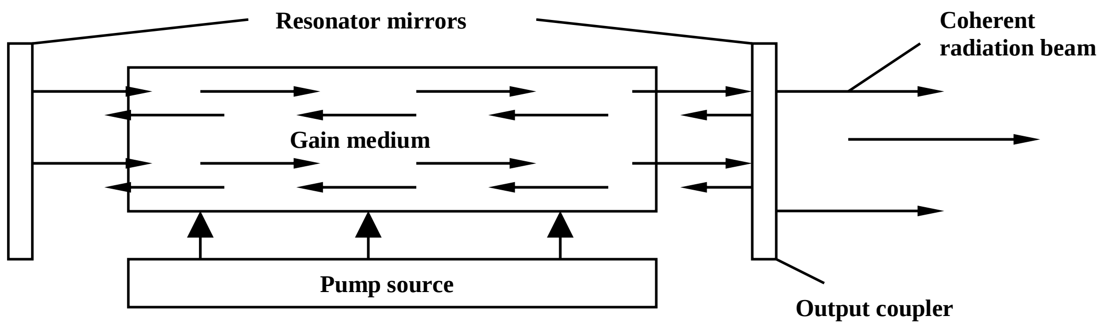


Fig. 1. Schematic diagram of a laser.

Stimulated emission

The spectrum of each substance consists of the series of more or less separated spectrum lines. These lines indicate the quantum (discontinuous) nature of matter. Each chemical substance can absorb and emit radiation of strictly determined frequencies – wavelength. They correspond to the differences of the energies characteristic for the quantum states of the given substance. In the process of absorption a particle absorbs a quantum of energy and is raised from a lower (E_1, Fig.2) to a higher-energy quantum state (E_2 state). In the process of emission the excited particle emits a quantum of radiant energy and returns from the higher (E_2) to the lower-energy quantum state - E_1.

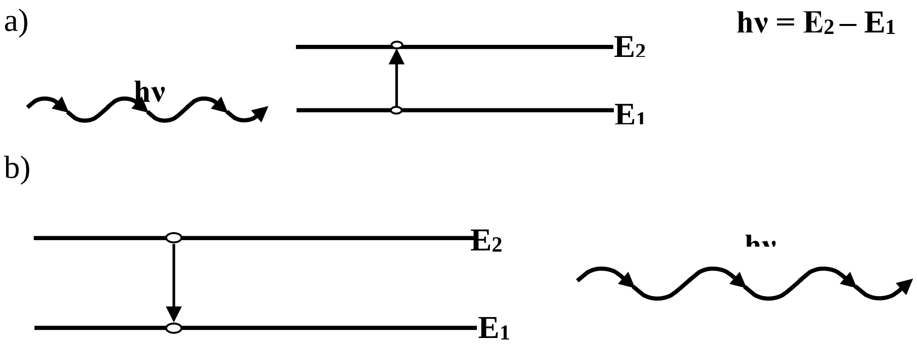


Fig. 2. Absorption (a) and spontaneous emission (b) process.

Now, what happens if a resonance radiation of a quantum energy $E = E_2 - E_1$ falls onto an excited particle in E_2 state? The particle emits another “twin” quantum (photon), leaving the excited state and moving back to E_1 state. This process is called stimulated emission.

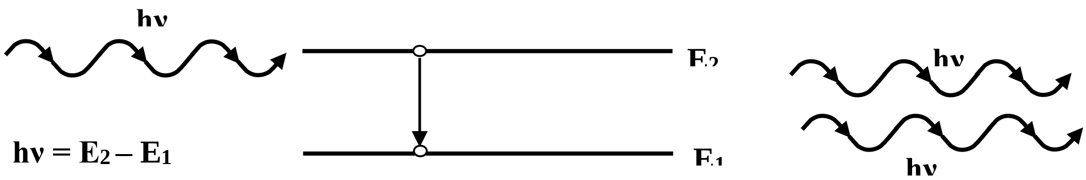


Fig. 3. Stimulated emission process.

Radiation

Laser is a generator of coherent electromagnetic waves. The radiation generated as a result of stimulated emission process characterizes with specific features which differentiate it from the radiation resulting from spontaneous processes. They can be generally described as “twin” features with the ones of the stimulating signal. From the medical perspective the important aspects are:

- **low beam divergence;** the laser radiation is emitted in a single direction specified by the resonator axis, and the diameter of a beam grows very slowly in the process of increasing distance from the resonator window. Due to the low beam divergence sending a ray to great distances is possible, as well as intense beam focusing by the use of optic systems. Achieving density power of 10^2-10^6 MW/cm^2 allows for material ionization and their evaporation as a result of interaction with plasma.
- **monochromaticity** – laser radiation characterises with an extremely narrow spectrum range (even 10^{-7} nm) in comparison to other natural sources of radiation: stars, lamps, etc.
- **coherence** – electromagnetic waves which are generated in the laser maintain the same phase throughout the whole time of spreading, which differentiates them from the completely incoherent spontaneous radiation.

Pumping, Population inversion

The effect of amplification, which is essential in the operation of lasers occurs in the systems where the number of particles in excited E_2 state exceeds the number of particles in E_1 state. It is a state of non-equilibrium particle system and it determines the existence of so called **population inversion**. Under normal conditions, in the state of thermodynamic equilibrium, the number of particles in lower-energy state E_1 exceeds the number of the excited state E_2.

The process of unbalancing a system, commonly called pumping, relies on exiting the gain medium (e.g. with electrical discharge in case of gas lasers or optically in solid-state lasers) as well as on the correct manipulation and proper selection of relaxation processes, i.e. processes of returning to equilibrium. It the relaxation process the particles go through various quantum states of excitation approaching the lowest energy state – ground state. If at that time they achieve a state, whose lifetime (the time of staying in the certain state) is relatively longer then those of other states, accumulation (a significant population) of particles in this state occurs and the **population inversion** appears, under condition that the time of staying in the lower-energy states is considerably shorter. If the inversion is sufficiently high to compensate for optical system losses, the device starts to amplify the noises and the optical generator is created – the laser.

Gain medium

Interaction between light and matter can be defined on the basis of three phenomena: photon absorption (absorption), spontaneous emission and stimulated photon emission. Photon, which is emitted as a result of stimulated emission has the same polarization as the photon causing the emission. The stimulating photon must possess specific energy, which equals the medium excitation energy. Atoms in the ground state absorb the stimulating photons (the emitted ones as well). For a laser to function, the stimulated emission process must exceed the absorption. It occurs when the excited atoms outnumber the ground atoms in the medium. Achieving the state where the higher energy levels are populated more frequently than those of lower energy is difficult due to the spontaneous emission phenomenon, which causes the excited atoms to stay in the higher states for an extremely short period and quickly return to the ground state.

Pumping system

The task of this system is to move possibly highest number of electrons in the active substance to the excited state. The system must be productive enough to cause population inversion. Pumping can occur through a flash of a flash lamp, other laser, electrical discharge in a gas, chemical reaction, atom collision as well as shooting a beam of electrons into a substance.

Optical system

As far as the gain medium is treated as a generator of electromagnetic waves, the optic system acts as feedback (influence of the result of a certain phenomenon on its cause) for selected frequencies. Thanks to this laser generates light of a single frequency (with minor deviations). Optical system normally consists of two precisely produced and properly set mirrors (at least one of which is partially transparent) and creates a resonator for a selected frequency and determined direction of the wave movement, so that only these photons which resonate with it will move through the gain medium causing the emission of other coherent photons. The rest of the photons fade in the gain medium or optical system. Thanks to this the laser emits almost parallel beam of highly coherent light.

Types of lasers

According to the type of lasing substance we classify lasers into: gas, liquid, solid-state, molecular and semiconductor lasers.

Considering the ways and types of the electron movements between the levels of lasing medium we divide lasers into: triple- and quadruple-layer lasers. The diversity of the emitted wavelength divides lasers into devices emitting visible, ultraviolet, infrared, microwave and X-radiation. For a proper eye-protection determining the type of work and power of the emitted radiation which could cause specific effects of interacting with matter (e.g. biological tissue) is absolutely essential.

Lasers are classified according to the physical state of the gain medium used:

- solid
- gas
- dye

From the point of view of a radiation power value we divide into:

- low power (4-5 mW),
- medium power (6-500 mW)
- high power (above 500 mW)

Laser influences with heat

Energy carried through s laser light stays in the tissue and the significant amount of it is transformed into heat. Biological effects resulting from this depend on the radiation wavelength and its intensity, the time of exposure and features of the influenced tissue. According to the amount of the provided tissue and the time of its exposure to laser influence photochemical, thermal, photoablation and electromechanical mechanisms can be differentiated.

- Photochemical reactions are used for biostimulation in the photodynamic method.
- Thermal influence depends on the temperature of the tissue. For the temperatures lower then 60^oC we observe permanent damage of the cell membrane structure. Temperatures higher then 60^oC cause tissue necrosis as a result of coagulation. It results in blood and lymphatic vessels closure. After exceeding 100^oC water contained in the skin evaporates and the tissue is damaged.
- Ablation effects (ablation – resection, exfoliation) occurs as a result of laser radiation influence of a short span impulse and of a very small penetration depth. This effect is obviously a result of thermal influence.
- Electromechanical influence, also described as photodestruction, is observed during the use of extremely high laser radiation power. It is a mechanic destruction of tissue structure resulting from the exposure to laser radiation. An example would be laser wrinkle, tattoo or skin lesion removal, always connected with resection of the surface layer of the skin. In this process the ablation and thermal effects play the main role.

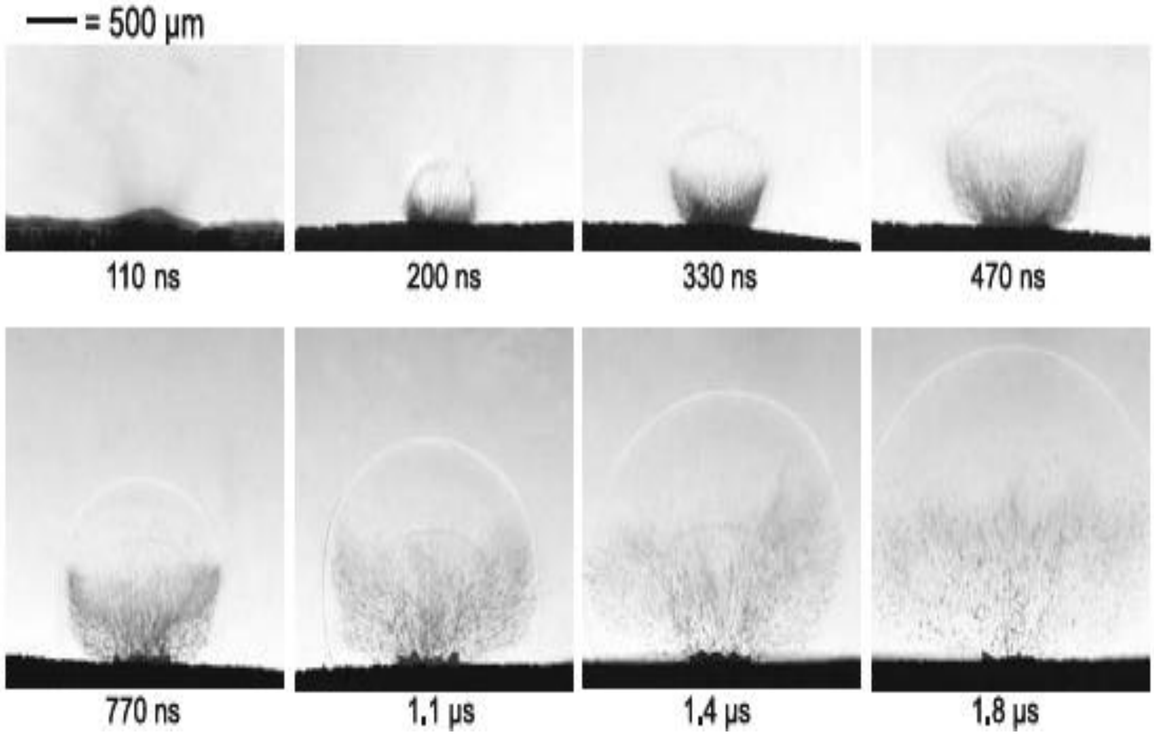


Fig. 4. In this picture we can see the effect of the influence of a laser radiation (intensity 5,4 W/cm^2 and beam diameter 0,5 mm) on the human skin. Initially the visible plume contains only gas – the steam and tiny biomolecules. After 120 ns (ns – a nanosecond is one billionth of a second) the skin fragments are thrown in the air.

Weakening of a laser light beam

Laser light beams passing through crystal substances weakens in accordance to the following dependence (Radiation Absorption Law):

$$I = I_o\, e^{-\alpha d} \qquad (1)$$

where:

I_o – initial light intensity,
I – light intensity after going through a material,
e – base of natural logarithms (irrational number, e=2,718281828.....,),
d – layer thickness,
α – extinction module.

Value of α module depends on the light wavelength and the type of the material through which the light passes. In this exercise we are using a monochromatic light of a wavelength of 670nm, thus the evaluated value of the extinction module depends exclusively on the type of the material.

After dividing both sides of the equation (1) by I_o we get:

$$\frac{I}{I_o} = e^{-\alpha d} \qquad (2)$$

We take logs of both sides of the equation (2) and get:

$$\ln\frac{I}{I_o} = -\alpha \cdot d \qquad (3)$$

After transforming:

$$\mathbf{\ln I = -\alpha d + \ln I_0} \qquad (4)$$

Knowing the value d we determine α module value.
With a specified wavelength and constant α (the same material) we test the dependence of the intensity of light passing through the system from the thickness of the layer which weakens the beam.

ATTENTION!

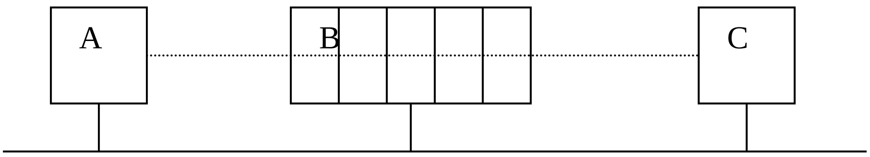
1. Revise the material about natural logarithms.
2. To solve this exercise you will need a calculator which possesses the ability to calculate natural logarithms.

<u>Practical part</u>

Necessary equipment and tools:

Laser, instrument for measuring laser light, samples of crystal materials.

There is a semiconductor laser placed on an optical bench, a laser light intensity detector (it shows light intensity in relative units) and a special support stand to hold the tested substances (see the picture below).



A- laser B – absorbent plates C - detector

Performing the experiment

a) Determination of absorption coefficient α

In the first part of the experiment we test the value of α module for various substances. To achieve it you need to:

- measure the intensity of the laser light without the absorbing substance.

a. measure the intensity of the laser light after placing an absorbing plate on a support stand,

b. measure plate thickness and deriving values of I i I_o assess α value from the equation. (3).

material	**d 10^{-3} [m]**	**I_0**	**I**	**$\ln I/I_0$**	**α [m^{-1}]**

Write here the calculations of absorption coefficients α (include units):

b) Measurement of light intensity depends of thickness of the absorbing layer

In the second part of the experiment we test how the intensity of a light passing through the system depends on the thickness of the absorbing layer. To do this you need to:

c. choose a set of plates prepared from the same material, measure their thickness with a micrometer
d. measure the intensity of the laser light without the absorbing substance,
e. place a higher number of plates in the support stand (1, 2, 3, 4, etc.), each time read the value of light intensity that reaches the detector and fill it into a table below,
f. illustrate the results graphically on two graphs: on the first one we put values of „I" and „d"; and „lnI" and „d" on the second one. After taking logs equation (1) transforms into $\ln I = \ln I_o - \alpha \cdot d$)

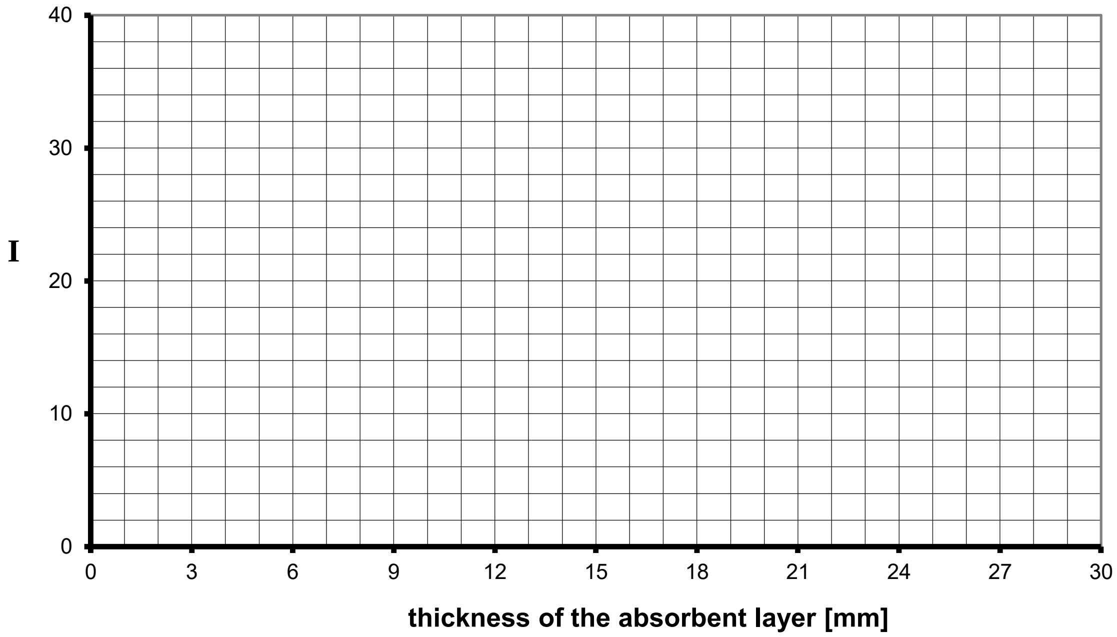
From the second graph read α value for the tested material (how?), and compare the received value with the value from the first part of the experiment.

	Absorbent layer thickness $[10^{-3}m]$	Light intensity value I	ln I
No absorbent	-		
1 plate			
2 plates			
3 plates			
4 plates			
5 plates			
6 plates			
7 plates			
8 plates			
9 plates			

c) Data analysis

GRAPH 1:

On the **Graph 1** present the dependence I(d) of the intensity of a laser light after passing through absorbent from the thickness of the absorbent layer.



Graph 1. Dependence I(d)

Using Microsoft Excel determine an equation of dependence I(d) as well as correlation module value R^2. Write it below:

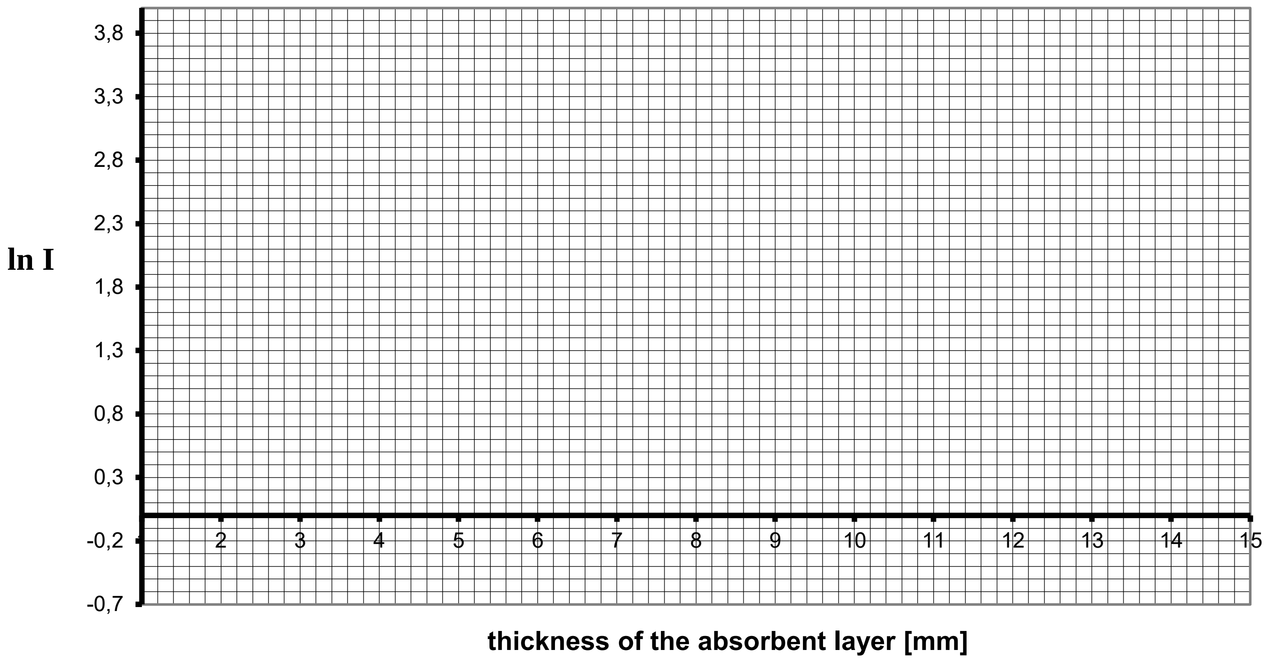
- y = ………………………… R^2 =……………………………………

From the received equation read the value of module α.

α = ………………..

GRAPH 2

On **Graph 2** present the dependence lnI(d) – of natural logarithm of the laser light intensity after passing through the absorbent from the thickness of the absorbent layer.



Graph 2. Depedence lnI(d)

Using Microsoft Excel determine an equation of dependence lnI(d) as well as correlation module value R^2. Write it below:

- y = ………………………… R^2 =……………………………………

From the received equation read the value of module α.

α = ………………..

Date	Name and surname of the person performing the experiment	Signature of the person conducting the class