

Experimental Tasks

Measurement of electromagnetic parameters of the environment – measurement of magnetic field and quantification of external sources of electromagnetic radiation

Task Goal:

- Measure electromagnetic spectrum in the possible range of the analyzer 2399C.
- Quantify observed signals, i.e. determine output in the channel and occupied bandwidth.
- Tune radio signal

Measurement Procedure

1. Make sure that the device is plugged in. It is necessary to turn the device on using the main switch ON/OFF on the back panel. Then you turn it on by pressing button 1. Check correct connection of external antenna to input 7
2. Before you start measuring, it is important to set the signal amplitude using the navigation button AMPL to the max. value –20,0 dBm. The device calibrates itself from time to time. In this case, wait until it finishes, then you can proceed.
3. Pressing button SPAN, further full SPAN, we display the whole spectral range of the device. We can further specify by pressing FREQ, and also START/STOP.
4. Navigation button 6 will take you through the whole signal spectrum. Choose five signals and attempt to determine their origin.
5. Use navigation button 6 to select any signal that you want to quantify (signal should be found in the middle of the display). By pressing button MEAS we select measurement function and further specify using CHANNEL POWER. For better measurement it is recommended to select CH PWR SPAN and turn MAX HOLD to the position ON. In order to achieve a more accurate quantification, we can use buttons INTEG.BW and START/STOP. Measured value can be read directly on the screen. Then calculate the corresponding signal output by applying the following formula

$$P = \frac{10^{\frac{x}{10}}}{1000},$$

where P is signal output with unit watt [W] and value x is the read signal output in dBm. (task no. 2).

6. You will determine the occupied bandwidth in a similar way to item 4, but select Occupied BandWidth. By pressing button OBW SPAN you change the image range and by OBW PWR you indicate the signal. Pressing STOP will help you better read the values (task no. 3).
7. The device enables you to reproduce the measured FM signal and subsequently reproduce. The setting stays the same as in previous items. Using FREQ and button 6 select a signal that corresponds to radio emitting.
8. Pressing button AUX takes you to the menu of signal modulation.. Turn FM DEMOD to the position ON and AUDIO SOUND to ON. Set AUDIO Level to the minimum audible value so that you do not disturb the other students. Set SQUELCH LEV to the level < 70 (task no. 4).

Measurement of blood flow velocity in peripheral arteries.

Task Goal:

Measure blood pressure and then determine blood flow velocity in arteria radialis in the right and the left arm first at rest and then at various time intervals upon defined stress

Measurement Equipment:

Phonendoscope, mercury and digital tonometer.

Device BIDOP, computer with software Smart-V-Link, UZ gel.

Measure in pairs.

Measurement Procedure:

1) Measure blood pressure using digital tonometer – students take each other's blood pressure. Put the inflatable cuff around the arm of the examined sitting person. Make sure the cuff is at the same level as the heart. While measuring, follow instructions that belong to the particular tonometer you are using. Take down the systolic and diastolic pressure. Perform the measurement on both arms.

2) Measurement of blood flow velocity at rest – students in pairs make measurements on each other

a) Turn on the computer and start program Smart-V-Link Ver2.0.

b) Put your first and last name in the field Patient Information and click on "Save."

c) Then click on the field "Setting." Then turn on the measurement device "Bidop" by pressing the middle button (marked in red) and in the software setting click on "Search comm". Confirm startup of the device. As soon as the connection between the device and computer is made, you can start measuring.

d) Click on the button "Main Screen" again, than select item "Upper Limbs Segmentally."

e) First you will measure the so-called "Right Radial." By clicking on the field with the corresponding heading (Right and Radial), an on-line measurement curve will be displayed. Use palpation to find the pulse. Apply a small drop of UZ gel on that area. Then place the probe (in the opposite direction of blood flow) at approx.. a 60-degree angle and very slowly move until you an area with the strongest signal. As soon as the strongest signal is registered long enough (it will no longer change on the screen), press the button on the probe. After the curve can be read, click the field "Resolution/Finding."

f) Repeat item e) for "Left Radial."

g) "Print" the results – separately for each limb – first right click the mouse on the field with the saved record of the left arm, then press print. Create a PDF document and save it in the file "Doppler measurements" under your own name followed by letter L (left). Proceed the same way and save the results for your right arm (your name followed by R for right). Save both documents on your "flash disc."

3) Measurement of blood flow velocity after stress – one student measures (student A),
the other “is measured” (student B)

a) Stress – while standing hold a weight (approx. 5kg) with both hands and extend your arms downward and for about one minute (your colleague will time it) lift the weight as fast as you can all the way up to your chin by bending your elbows.

b) Immediately after you finish lifting, measure the blood flow velocity in the **left** arm and save the result (see item 2). Repeat the measurement of blood flow velocity in your left arm at intervals 3.5 and 10 minutes after stress.

c) Measure pressure according to item 1) both in the left and right arm..

4) According to item 3) student B undergoes stress and student A measures.

5) According to item 3) student A undergoes stress and this time student B measures blood flow velocity in the **right arm!**

6) According to item 3) student B undergoes stress and this time student A measures blood flow velocity in the **right arm!**

7) At the end measure blood pressure according to item 1).

8) Discuss the obtained results, think about the changes in blood flow velocity in various parts of the body, the difference in flow velocity in arteries and veins, the influence of stress on velocity. Compare the results of the right and left arm both from the perspective of blood flow velocity and blood pressure, discuss possibly differences between student A and B. Compare the results of blood pressure measurement by the digital and mercury tonometer. .

Diagnostic Ultrasound – Color Flow Mapping and Spectral Doppler

Goal : Get to know advanced methods of ultrasound imaging

First it is necessary to study theoretical information including the manual and possible uses of the device Logic C5. All measurements are to be carried out under the supervision of the lecturer.

Work will start by demonstration of the device as a whole and its main control elements

Demonstration Procedure:

- Turn on the device by pressing button 1
 - After pressing button 2 type in patient identification data
 - By pressing button 3 (Preset) display preprogrammed types of examination
 - Button 4 serves to select a probe
- The device is equipped with 2 electronic probes – convex and linear.
- By shifting potentiometer TGC (on the upper right side of the panel) show the importance of the correct setting of depth amplification – the whole displayed area must have the same shade of gray

- Show the importance of trackball for various kinds of functions (distance measurement – calipers, after pressing the CF key shifting the color box, shifting individual images by pressing the key CINE and simultaneously freezing the image, etc.)
- Key 16 serves for image doubling while the active image can be shifted using the Left/Right keys.

Independent Measurements

First in flow phantom, then in a volunteer

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- Turn on the device.
- Select linear probe.
- Place the volunteer on an examination table with the head tilted backwards and with a free neck.
- Cover one side of the neck with gel.
- Place the probe in such a way so that the left side of the screen corresponds to the medial plane
- Place the probe above the clavicle and slowly shift towards the head: 2 dark orbicular structures will be visible – medially round, pulsating structure *a. carotis communis*. Laterally from it there will be a rather flattened hypoechogenic structure *v. jugularis int*. While slowly shifting the probe the image of the common carotid artery will split into two when you reach about the upper third of the neck – inner and outer carotid artery. This is an area of carotid bifurcation
- Press the CF key and repeat the above-mentioned procedure while using color-coding.
- Move the linear probe in a longitudinal direction and color image the common carotid artery in the longitudinal section.
- Press the PW key and take the cursor of the sample volume into the middle of the displayed artery using the ball-tracker. In the lower part of the screen you will see the spectral record of flow velocity. If the record has good quality during the whole course, press the key FREEZE. The record will stop, the velocity curve will be automatically circled and in the upper left corner there will be a table containing the measured data: maximum systolic velocity, minimum diastolic velocity, medium velocity, pulsation index and resistance index.
- All this data is diagnostically relevant when evaluating hemodynamics in the particular artery.

Diagnostic Ultrasound – Use of Tissue Phantoms and 3D Imaging

Goal: Get to know advanced methods of ultrasound imaging.

First it is necessary to study instruction and possible uses of the device Logic C5 based on theoretical information.

Tissue phantom 1

Procedure:

- Open the plastic case with soft tissue phantom
- Cover the surface of the phantom by a continuous layer of gel
- Apply the linear probe and slowly slide over the phantom while keeping the probe at an angle.

- You will see images of spherical structures of various sizes and various degrees of reflectivity
- Take this opportunity to explain nomenclature used in ultrasound examinations. It is based on processing reflections of the ultrasound signal from tissue interfaces, i.e. on reflectivity – echogenicity.

We differentiate between

structures with the same reflectivity as its environment: **isoechogenic**

structures with decreased reflectivity: **hypoechoogenic**

structures with increased reflectivity: **hyperechoogenic**

and areas that do not contain any reflective structures: **anechoogenic**

Reflectivity can be: **homogeneous or heterogeneous**

- By pressing the key HARMONICS, show the difference between basic and harmonic imaging (higher contrast, clearer structure borders in the phantom).
- By pressing the key MEASURE above the ball-tracker, measure the displayed phantom structure. End each measurement by pressing the SET key (bottom right).

Tissue phantom 2

This phantom contains solid and hollow inclusions located longitudinally. They are supposed to simulate arteries and longitudinally located nerve structures.

- Turn on the device.
- Cover phantom 2 with a layer of gel.
Select a linear probe.
By shifting the probe perpendicular to the long axis of the phantom, image simulated structures in a cross section. Print out the frozen image. Repeat the same by placing the probe parallel with the long axis of the phantom. Press the key MEASURE to measure the diameter of the hollow structure that simulates both the artery and solid structures, simulating nerve stems. simulující cévu i solidních struktur, simulujících nervové kmeny.
- Select a convex probe.
Repeat the preceding steps using a convex probe. Print out the frozen image of the cross section and compare the images obtained by using both probes (of the same area).

Procedure to create a 3D image:

1. Optimize B- image of the examined area
2. Cover the examined area with a sufficient amount of gel.
3. Press the function key F7 that will open a submenu for 3D
4. In order to start acquisition, press the left button (L) of the doubled image (above the FREEZE button).
5. Shift the probe evenly in order to obtain a series of parallel images.
6. 3D VOI (Volume Of Interest) will be displayed in the right part of the doubled image

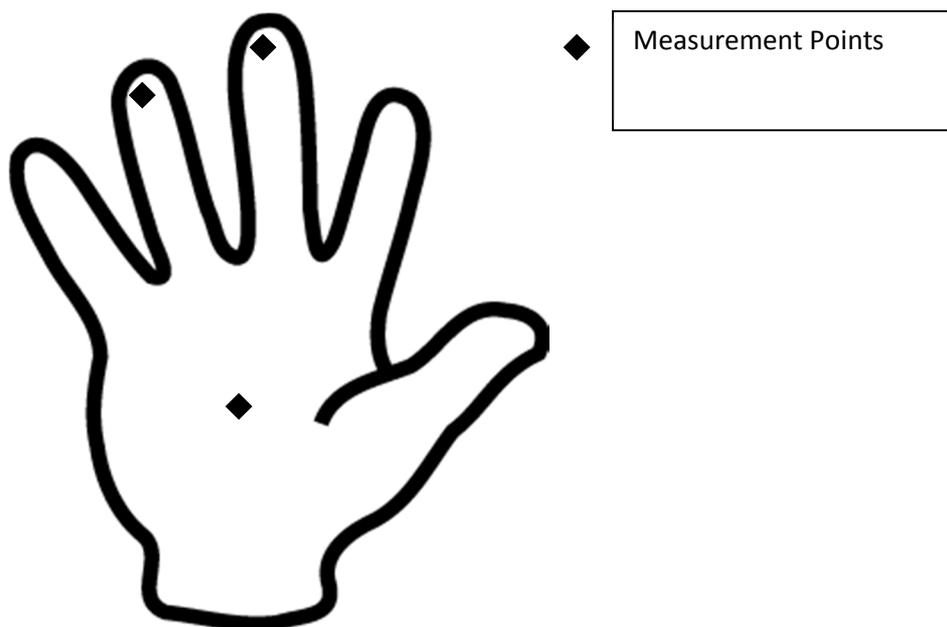
7. Press the right button (R) to finish 3D scan. It is also possible to finish the process by pressing FREEZE. In this case it is necessary to press the 3D button again to obtain the volume image.

Thermography – Cooling Test

Requirements – ice bath, thermal camera/imager, thermometer, PC, and software.

Be careful to avoid touching and imprints on measured surfaces of the limbs before and during the experiment! Perform the thermographic measurement of the cooling test of upper limbs.

1. Record the surface temperature of upper limbs (ventral side, both limbs together).
2. Immerse limbs in the ice bath for a long enough time (30+ s).
3. Take surface temperature every 30 s for 10 minutes.
4. Save the thermo images and perform the cooling test analysis (mean value, maximum, minimum, etc.).
5. Plot a graph depicting dependence of temperature on time for measured points – point temperature – first and last finger parts, three point on the palm according to the enclosed schema.
6. Comment the development in time, compare individual points. Compare the beginning and final state and both limbs with each other.



Thermography - Determination of Surface Emissivity of a Model (Object)

Requirements – plate (an object) made of unknown material (shiny/matte surface), thermal imager, thermocouple with a multimeter, PC and software ...

Procedure:

Determine the value of emissivity of the “plate/object” with a thermal imager (on its matte surface and also on the shiny surface in the area of the thermal imager control panel)

1 - Determine the surface temperature of the “plate” by using a thermistor sensor (in defined positions - shiny surface, matte surface). Determine the ambient temperature and its humidity and the temperature of the examined subject’s skin.

2 – Obtain a thermal image of the “plate” using a thermal imager. The thermal image should be recorded from such a distance from the “plate” so that the whole image area is filled by the scanned object. Attention! The mutual position of the thermal imager axis and the “plate” surface must be perpendicular; also reflection of the measuring person on the surface of the “plate” is necessary. Save the image using the device memory.

3 – Transfer the saved thermal images from the device memory to a computer and analyze emissivity and surface temperature of the object.

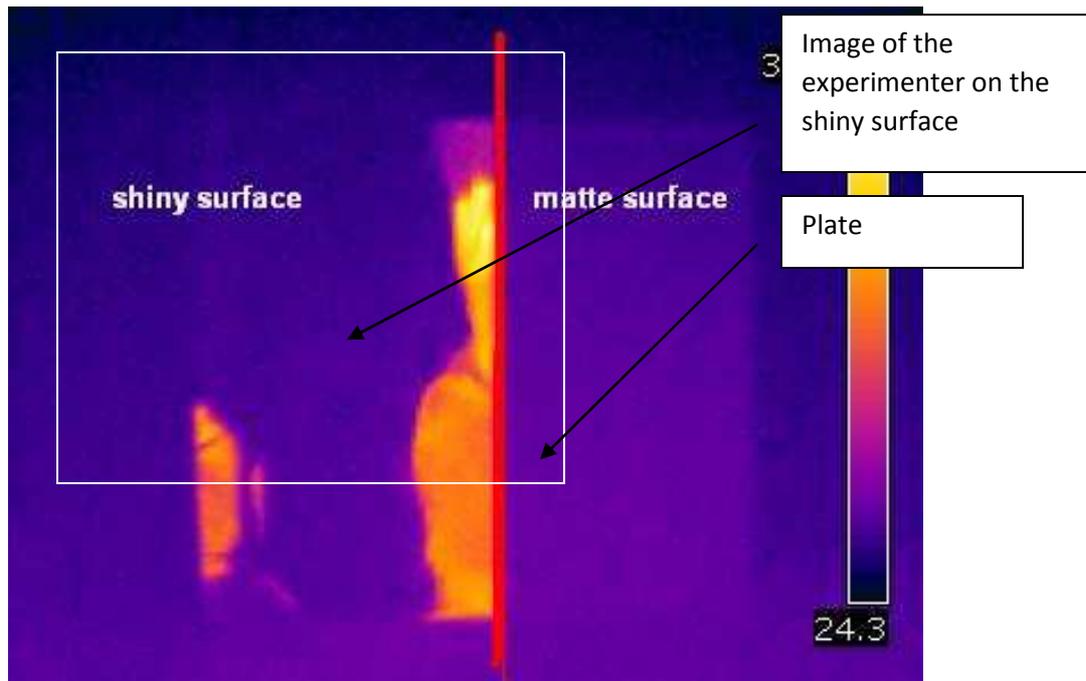
4 - Open the FLIR QuickReport software.

5 - Following the user manual of the software, display the appropriate thermal image from the directory and define the positions of measured temperature points (areas). Positions: area where the experimenter is reflected in the glossy area of the material and in the matte area of the material.

6 – Gradually evaluate the temperature of individual defined points (areas) at various setting of the following parameters: reflected temperature, atmospheric temperature, humidity and emissivity. Set the emissivity values in the following order 0.1, 0.25, 0.4, 0.5, 0.7, 0.9 and 1.

7 - Plot a graph of the obtained values of temperature (for glossy and for matte area) in relation to the value of emissivity.

8 – Based on the graph, specify the value of emissivity in the area of measured points – by comparing the thermal image temperatures and the temperatures obtained from the thermocouple readings (see item 1 under Procedure).



Additional (optional) Task:

Measurement of distances using a thermal imager

Requirements – measured object at a higher temperature than the environment, thermal imager, thermometer, PC and software

In the environment of air there is attenuation of infrared radiation, which means that with an increasing distance between the measured object and the scanner, the attenuation of detectable IR also increases.

1. Turn on the thermal imager and set it on areal thermal imaging with searching for the highest temperature in the measured field.
2. Record maximum temperature of the measured object at a distance of 10 cm, 30 cm, 1 m, 2 m, 3 m, 4 m.
3. Measure the temperature of the identical object at an unknown distance X (determined individually by the lecturer)
4. Map the dependence of object temperature (maximum) on the detection distance
5. Using the graph determine the unknown distance X determine object temperature at measurement from “zero” distance, i.e. the maximum possible closeness to the object.

Evaluation of X-ray Parameters

Study of x-ray spektra of Mo and Cu anodes

Task:

1. Plot a graph depicting the dependence of energy and radiation wavelength on the angle of diffusion on the LiF crystal. Use the table values h a c , consider the grid crystal constant to be $d = 201$ pm. Put $n = 1$.
2. Using the manual control of x-ray device PHYWE measure the spectrum of x-rays of a copper of molybdenum anode at the voltage of 35 kV. Select position changes of the G-M computer by 1 degree (rotation of crystal connected to goniometer), current .0,8–1,0 mA. Plot a graph depicting the number of photons recorded by the G-M computer in relation to energy or wavelength.
3. Using the software controls and data reading, measure the spectra of copper and molybdenum anodes for accelerating voltage 15 kV, 19 kV, 22 kV and 30 kV. Select position changes of the G-M computer by $0,25^\circ$ or less, select the highest possible current. Mark the maxima of characteristic radiation for both materials, calculate their energy and compare them with table values. Plot the measurements for all voltages on a given anode in a graph in relation to energy and wavelength. .
4. Using the measured spectra, determine the approximate value of the Planck's constant. Bragg's equation results in $\sin \theta = \frac{hc}{2dE}$, where E is energy of the incident radiation. Because we know what maximum energy is, which can be found in the spectrum (E_{\max}) and we can determine the angle at which the spectrum ends, value h can be calculated if we know the value of other constants. The above given equation can be adjusted $\sin \theta = \frac{hc}{2deU}$, where U is accelerating voltage. If we plot into a graph the values of $\sin \theta$ in relation to $1/U$, we get points that should theoretically lie in a bisector/straight line. If we put a line through the plotted values, then from its direction (směrnice) $\sin \theta = a \frac{1}{U}$, $a = \frac{hc}{2dc}$ we can calculate the value of Planck's constant as $h = a \frac{2de}{c}$. Compared the found constant h with its table value.

Perimetry and corneal topography.

Goal: Get acquainted with the function and significance of computer perimeter in optics and optometry and perform a simple examination of the visual field using this device.

Introduction and measurement of cornea with corneal topographer Easygraph – **it will be performed by the lecturer.**

Requirements: Examined subject, examiner, computer perimeter, and laptop with installed software „Centerfield“.

Procedure:

1. Have the examined subject sit in a correct position and inform him/her about the course of the examination
2. Connect a PC (laptop) with installed software for the given tests to the perimeter and find the corresponding program..
3. Fill in the basic information about the examined subject (name and surname, date of birth, etc.) on the title screen.
4. Start the selected program (e.g. examination of the visual field for a driver's license)
5. At the sound of the signal, the examined subject will simultaneously see a test mark, to which he should react by pressing a corresponding button.
6. Test marks are screened randomly so as not to influence adaptation of the retina. First a lower intensity of the mark is used. If not noticed by the patient, it is increased until the patient sees it.
7. As the examination is quite time-consuming, we will select a program with lower demand for time (e.g. test for a driver's license).
8. Attach the measurement results to the report.
9. We will focus closely on developing discussion, in which we will particularly pay attention to the overall evaluation of the applied method, to its significance and to processing measurement results.

Spectrophotometry and electrophoresis of nucleic acids.

Goal: Get acquainted with measurement with semi-automatic spectrophotometer Genesis 6 in a UV area. Get acquainted with agarose electrophoresis of nucleic acids, including fission by restriction endonuclease.

Material and devices: DNA from herring sperm dissolved in TE buffer in native form; plasmid DNA (pGL from company Promega) in TE buffer in native form; agarose; TAE buffer (50x concentrated) restriction endonuclease BamHI including buffer; application (loading?) buffer (15% ficoll a bromophenol blue) staining solution ethidium bromide. Water bath; spectrophotometer Genesis 6 with 3 UV permeable cuvette; pipettes; electrophoretic bath; voltage source; transilluminator; gloves; safety goggles; digital camera.

(rem. the task is quite time-consuming, it will take place only once a semester)

Spectrophotometry Procedure:

1. Denature 2ml DNA solution of herring milt in a water bath - 2 minutes at 90°C, then rapidly cool in an ice bath.
2. Measure absorption spectra in the range from 220 nm to 340 nm; TE buffer is a reference solution (blank), sample no. 1 is denatured DNA, sample no. 2 is undenatured DNA.

3. Compare the recorded spectra from the perspective of shape and the extent of absorption in absorption maximum. Discuss reasons of these differences.

Electrophoresis Procedure (work in gloves!!):

1. First add 2 microliters of buffer for BamHI, 1 microliter of restriction endonuclease BamHI (it has one sensitive spot on the used plasmid, therefore during diffusion there is linearization of scDNA into a linear form) to 18 microliters of plasmid DNA. Incubate at 37°C for about 30 minutes.
2. Put the prepared gel (1% agarose in TAE buffer) into the electrophoretic bath and cover with a layer of TAE buffer.
3. Mix the DNA samples (18 microliters of undiffused DNA and a sample after diffusion with BamHI) with 5 microliters of loading buffer.
4. Pipette DNA samples to the wells in the gel.
5. Cover the electrophoretic bath with a lid and connect to a source (the lecturer will start that)
6. Observe the movement of bromophenol blue in the gel. As soon as it reaches the distance of at least 2 cm from the start (approx. after 60 minutes), the lecturer will turn off the source. Transfer the gel into a dye bath for about 30 minutes, then onto a trans-illuminator with a UV lamp. Photograph the gel and attach the picture to the report.
7. Discuss differences in electrophoretic mobility of native and linearized DNA samples from the perspective of acting forces.