This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 857381

Deliverable 4.1: Joint summer school

Practical methods in oncological research

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Workpackage:	WP4
Task:	T4.1
Version:	2.0
Date:	16. 6. 2022

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VISION

Basic information

Project title	Strategies to strengthen scientific excellence and innoVation capacIty for early diagnoSIs of gastrOintestinal caNcers
Project acronym	VISION
Call	H2020-WIDESPREAD-2018-2020
Topic	WIDESPREAD-03-2018
Project type	Coordination and Supporting Action (CSA)
Grant Agreement No.	857381
Nature	**R** (Document, report - excluding the periodic and final reports);
Dissemination level	**PU** (Public, fully open, e.g. web);

Executive summary

This deliverable describes the "Joint Summer School (JSS)" - an educational event organized within the VISION project. The JSS was primarily designed for advanced undergraduate students; however, Ph.D. students and early-stage researchers could also listen to theoretical online lectures if they were interested. The JSS's main goal was to enable young people to improve their knowledge and expertise in oncology by acquiring the latest scientific knowledge and skills in technical innovations. JSS took place from April 7 to 22, 2022, in a hybrid form.

1 Description of work & main achievements

The JSS comprised of two parts. The theoretical part, prepared by VISION partners, consisted of a series of online lectures split into four sessions, each dealing with a different up-to-date topic in cancer research, oncology, and nanobiology. The online presentations were free for all interested registered participants, including Ph.D. students, post-docs, and early-stage researchers. The practical part, held after each session, was intended only for eleven undergraduate students of the Department of Genetics at the Faculty of Natural Sciences (FNS) of the Comenius University in Bratislava, who chose "Practical Methods in Oncological Research" as one of the optional official subjects of the education program at the university. Each participant who attended both the theoretical and the practical parts received credits. In addition, each student prepared and submitted a Final Report to the JSS organizers.

1.1 Session I: Microfluidic system and advanced *in vitro* models

The online lectures for this session were prepared by VISION Partner 2 – FhG-IBMT.

1.1.1 The theoretical part

The participants listened to three lectures:

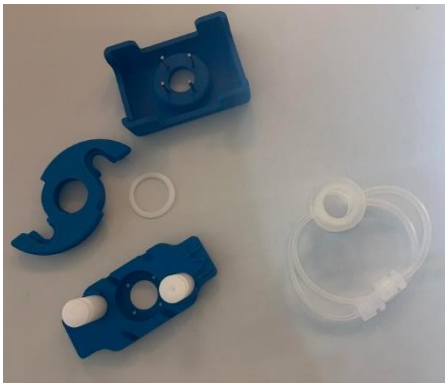
- Microfluidic components and systems for cell applications (Thorsten Knoll, FhG-IBMT)
- Biodegradable nanoparticles for medical applications (Sylvia Wagner, FhG-IBMT)
- Stem cells: Sources, cultivation, differentiation, application (Yvonne Kohl, FhG-IBMT)

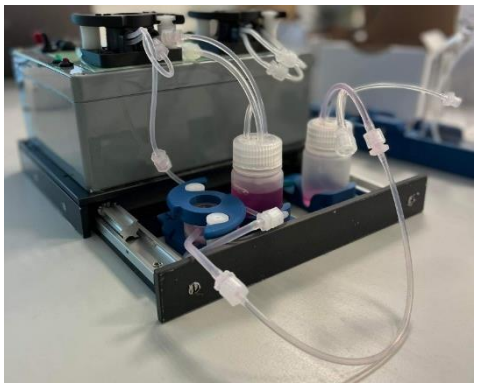
1.1.2 The practical part

The project coordinator covered the practical exercises in the laboratories of the BMC SAV.

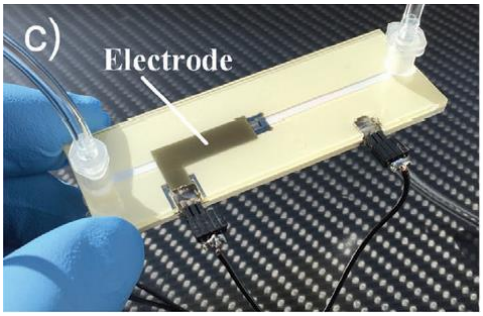
Undergraduate students, Department of Genetics, FNS, Comenius University in Bratislava

In the practical part related to Session I (Organized by Departments of Nanobiology and Molecular Oncology), the students got acquainted with new advanced cell cultivation methods under 3D conditions (spheroids and organoids). Moreover, they could see how to handle cells by seeding on a transwell, microfluidic system, and microchip developed at the FhG-IBMT.





Microfluidic system for cell cultivation on transwells



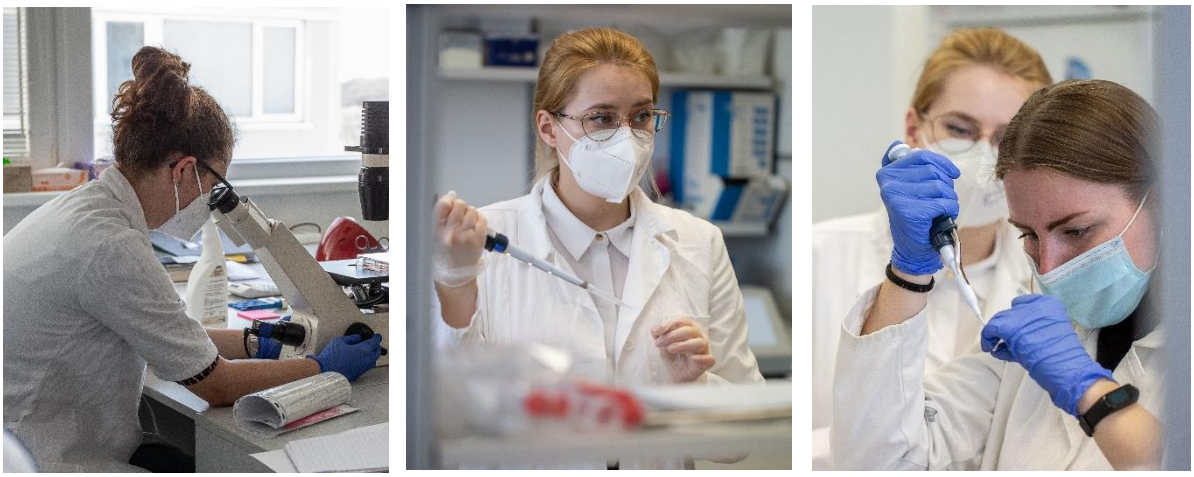
Microfluidic cartridge with connected tubing and cables for impedance measurement (Kohl et al., Small 2021, 17, 2006012, DOI: 10.1002/smll.202006012)

Culturing cells under 3D conditions is a time-consuming process; therefore, a short video for the needs of Joint summer school and other educational activities was prepared to clarify this method. The video is freely available on the VISION website.

Video: 3D models – organoids

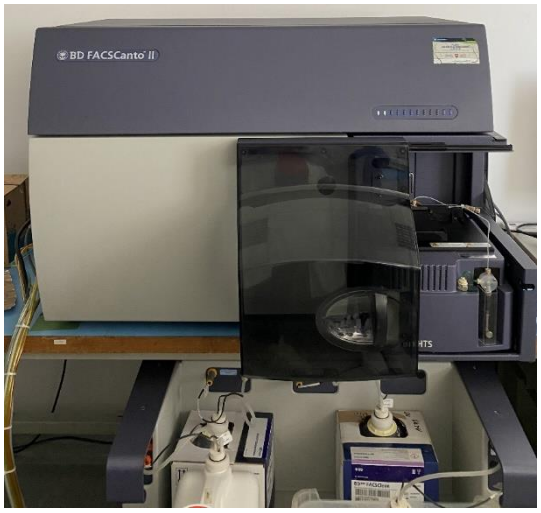
During the practical training organized by the Department of Molecular Oncology, each student prepared cells by himself for cell cycle analysis by flow cytometry.

Cell cycle analysis is one of the most straightforward applications of flow cytometry. This method provides a picture of the analyzed cell population - the current percentage of cells at different cell cycle stages, such as G0/G1, S, and G2/M, and allows the identification of apoptotic cells (sub G0).



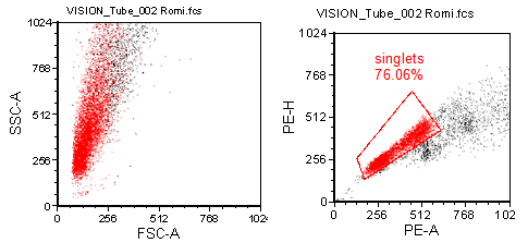
The undergraduate students are preparing samples for cell cycle analysis by flow cytometry

The students then proceeded to the analysis of their samples. First, Miroslava Matuskova, Ph.D., Head of the Department of Molecular Oncology, introduced the flow cytometer BD FACSCanto II and explained how the measurement takes place. After the sample analysis, she explained to the students how to use the software (image analysis) to evaluate the obtained data.



BD FACSCanto II flow cytometer

A



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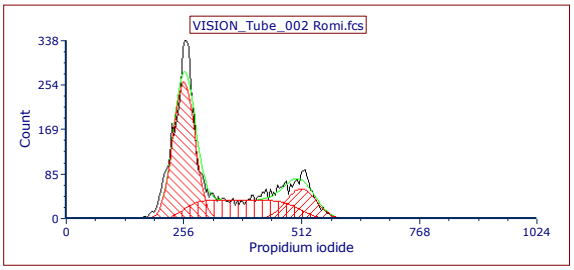


Cell cycle analysis. A. Distribution of cells. B. Cell cycle histogram

Again, the tutorial video by Ph.D. students at BMC SAV, guided by their supervisors, was prepared as a valuable learning tool for students. In this video, undergraduate students can see the process of preparing cells for cell cycle analysis by flow cytometry.

Video: Cell cycle analysis using flow cytometry

In addition, students also had the opportunity to visit the animal facilities of BMC SAV and to learn how to handle animals and the principles of *in vivo* experiments in cancer research, including ethical issues.

The undergraduate students visited the animal facility at the BMC SAV

1.2 Session II: Basic and translational research

The online lectures for this session were prepared by VISION Partner 4 – NKUA.

1.2.1 The theoretical part

The participants listened to three lectures:

- Cancer and senescence (Ioanna Angelioudaki, Ph.D. Student, Medical School, NKUA)

- Translational research: from bench to bedside and back (Anastasia Derventzi, Ph.D., Medical School, NKUA)
- How Genetic engineering can affect cancer research and treatment: CRISPR Technique (Alexandros-Georgios Tzingounis, Ph.D. Student, Medical School, NKUA)

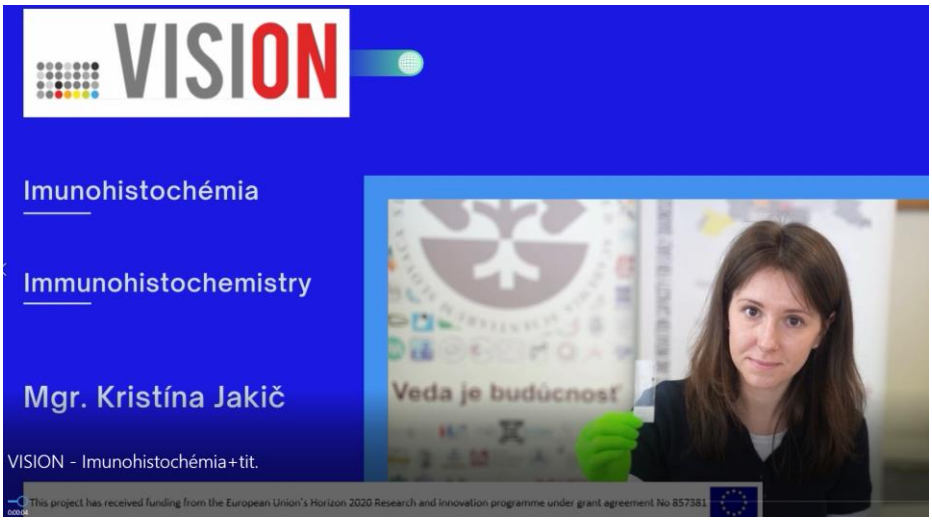
1.2.2 The practical part

The practical training for Session II was organized by the Department of Nanobiology. The students acquired basic knowledge in histochemistry and immunocytochemistry, two important techniques frequently used in oncology. These allow visualizing changes in tissue structures or the presence of specific protein/antigen in cells by special dyes or antibodies. After staining, the samples are examined under light or fluorescent microscope.

Ph.D. student Kristína Jakic, MSc., explained to students the principles of histochemistry/immunohistochemistry technique. They started with the preparation of tissue samples, continued with fixation, sample embedding in paraffin, sectioning by microtome, and finally staining. Next, the three-micrometer thin sections were examined by light microscopy.

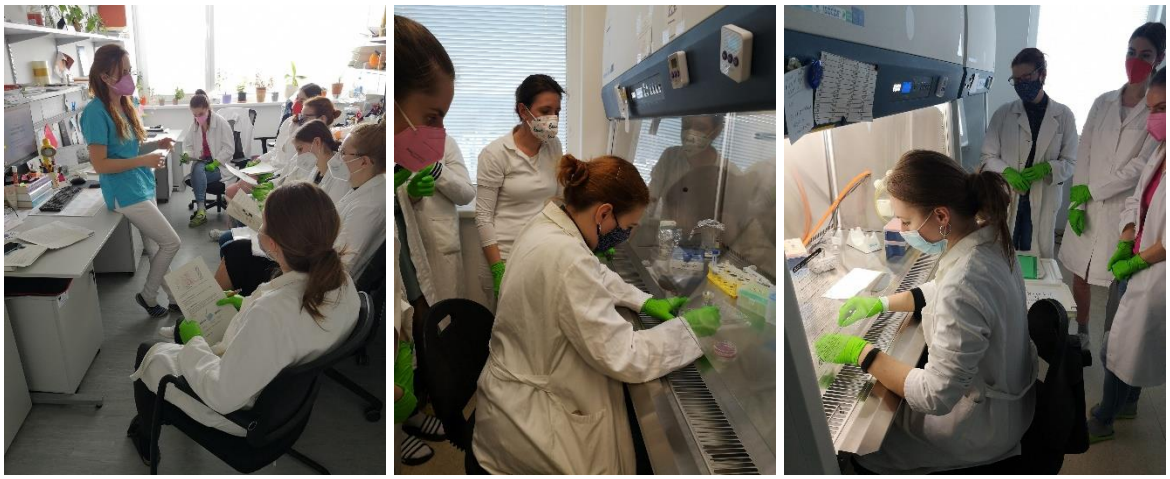
Preparation of tissue sample for histochemistry

Undergraduate students appreciated the tutorial video prepared for this purpose by the Ph.D. student from the BMC SAV. The video shows the individual steps of this technique and highlights the critical points in the procedure. The procedure is time-consuming because it takes several days to prepare a sample for analysis. However, the samples can be stored for several years with eosin-hematoxylin staining.



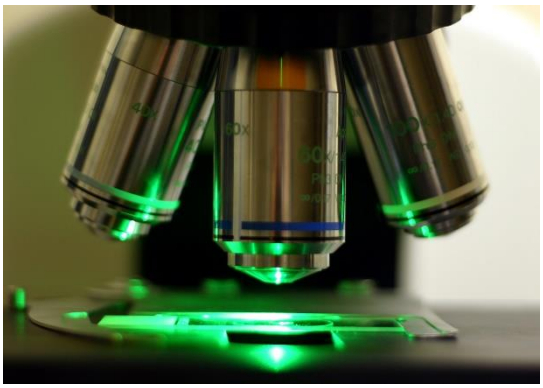
Video: Tutorial video on histochemistry/immunohistochemistry

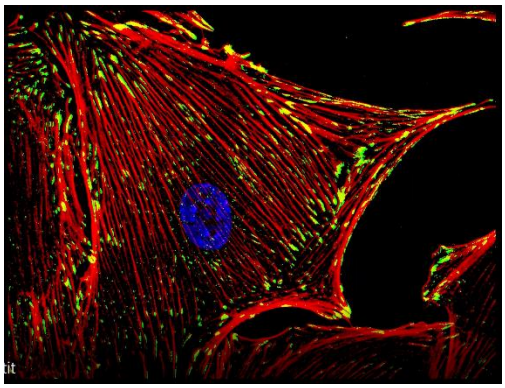
Barbora Svitková, Ph.D., introduced basic principles of immunocytochemistry. Then, students moved to the sterile room, where they seeded the cells on special round glasses for cell cytoskeletal analysis in a laminar flow cabinet. After cell fixation and labeling with specific fluorescently labeled dyes, samples were analyzed by fluorescent microscopy.



Cell seeding for immunocytochemistry

Images of cells were obtained with a fluorescent microscope (Axio Imager, Zeiss) using ISIS software from MetaSystems GmbH (Altlussheim, Germany).





Analysis of cytoskeleton of mouse podocytes by immunocytochemistry
(Blue- nucleus, red- actin fibers, green-vinculin, 630x magnification)

The tutorial video, prepared at the BMC SAV, describes a detailed immunocytochemistry procedure. The video is available on the VISON website.

Video: Tutorial video on immunocytochemistry

1.3 Session III: Clinical oncology research

The online lectures for this session were prepared by VISION Partner 3 – SERMAS/IRYCIS.

1.3.1 The theoretical part

The participants listened to three lectures:

- The fundamentals of translational research: Ethics, Informed consent, sample and data collection, and storage (Julie Earl, IRYCIS)

- Liquid Biopsy methods: Analysis of circulating free nucleic acids (Emma Barreto, IRYCIS)
- *In vitro* Tumor Models: From monolayer cultures to 3D and organoid cultures (Jesús Frutos Diaz-Alejo, IRYCIS)

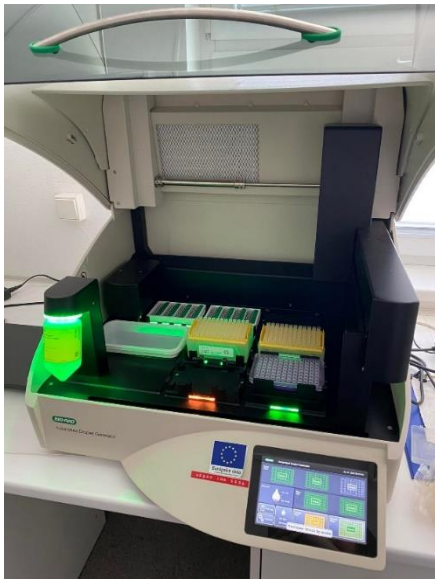
1.3.2 The practical part

The practical training for Session III was organized by the Department of Molecular Oncology BMC SAV. The application of digital droplet PCR (ddPCR), based on water-oil emulsion droplet technology, which is one of the crucial liquid biopsy methods due to its high sensitivity, was demonstrated to the students. The advantage of ddPCR compared to standard TaqMan probe-based assays is that the sample is fractionated into 10,000-20,000 droplets, and PCR amplification of the template molecules occurs in each individual droplet. That means that a single sample can generate thousands of data points rather than a single result, bringing the power of statistical analysis. Moreover, ddPCR technology enables high-throughput digital PCR, using lower sample and reagent volumes. The technique was trained using QX100™ Droplet Digital™ PCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Božena Smolková, PhD., prepared samples for ddPCR analysis and explained the principle and advantages of this new technology to undergraduate students.

Preparation of patient samples for ddPCR

The device consists of two integral parts, a droplet generator, and a droplet reader.



Droplet generator

Droplet reader

ddPCR technology uses reagents and workflows similar to those used for most standard RT-PCR. For standard quantitative RT-PCR, which is the most commonly used molecular method, was also prepared tutorial video, freely available on the VISION website.

Video: Tutorial video on qRT-PCR

1.4 Session IV: Nanosafety research in regulatory context: Challenges and opportunities

The online lectures for this session were planned to be prepared by VISION Partner 5 – NILU.

1.4.1 The theoretical part

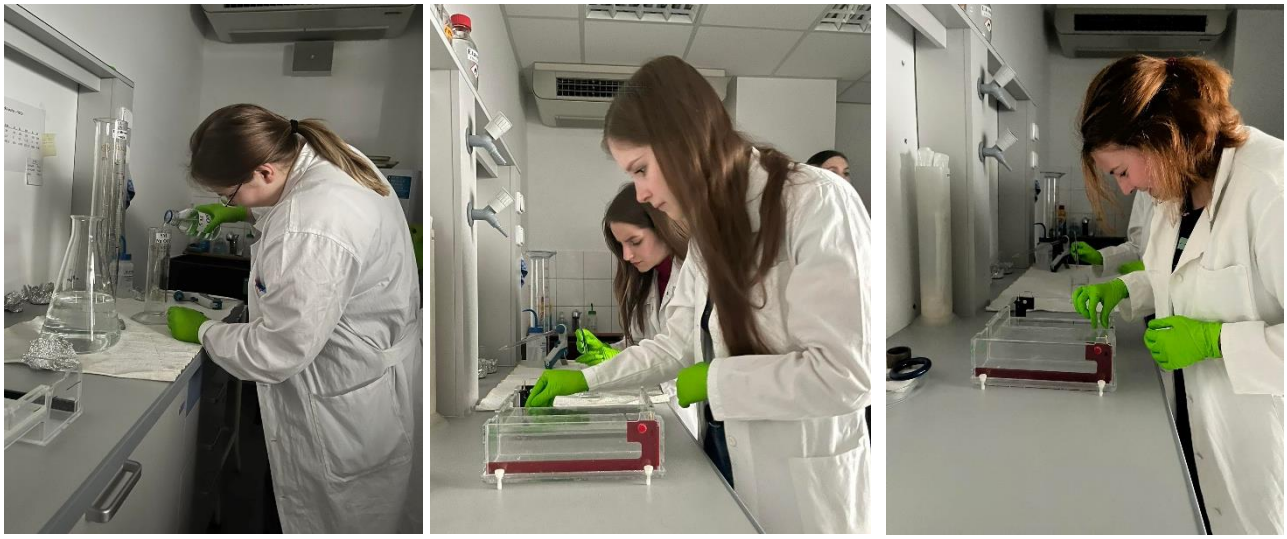
The participants should listen to three lectures:

- Hazard and risk assessment of nanomaterials: Historical perspective and current status (Maria Dusinska, NILU)
- Smart, safe, and sustainable by design approach in nanotechnology (Elise Rundén Pran, NILU)
- Implementation of new advanced approaches into the regulatory landscape (Maria Dusinska, NILU)

Unfortunately, due to the persisting severe health problems of the main speaker assoc. Prof. Mária Dušinská, DS.c., the lectures of session IV could not take place during JSS.

1.4.2 The practical part

Despite this fact, the Session III of practical training took place and was organized by the Department of Nanobiology BMC SAV. The undergraduate students had the opportunity to learn the widely used microscopic electrophoretic method – single-cell gel electrophoresis (also known as the comet assay). Under the guidance of Ph.D. student Lucia Balintová, MS.c., each undergraduate student was given one plate with cells. They treated the cells with hydrogen peroxide, a known DNA damaging agent causing strand breaks and oxidative DNA lesions. Then each student prepared the agarose gels (embedded cells into agarose) for electrophoresis. Finally, they went through all the steps of the comet assay.



Preparation of solutions for the comet assay and inserting the samples into the electrophoretic solution

The principles of the image analysis using automated slide scanning and imaging system Metafer (MetaSystems GmbH, Altlussheim, Germany) were explained. The students could see

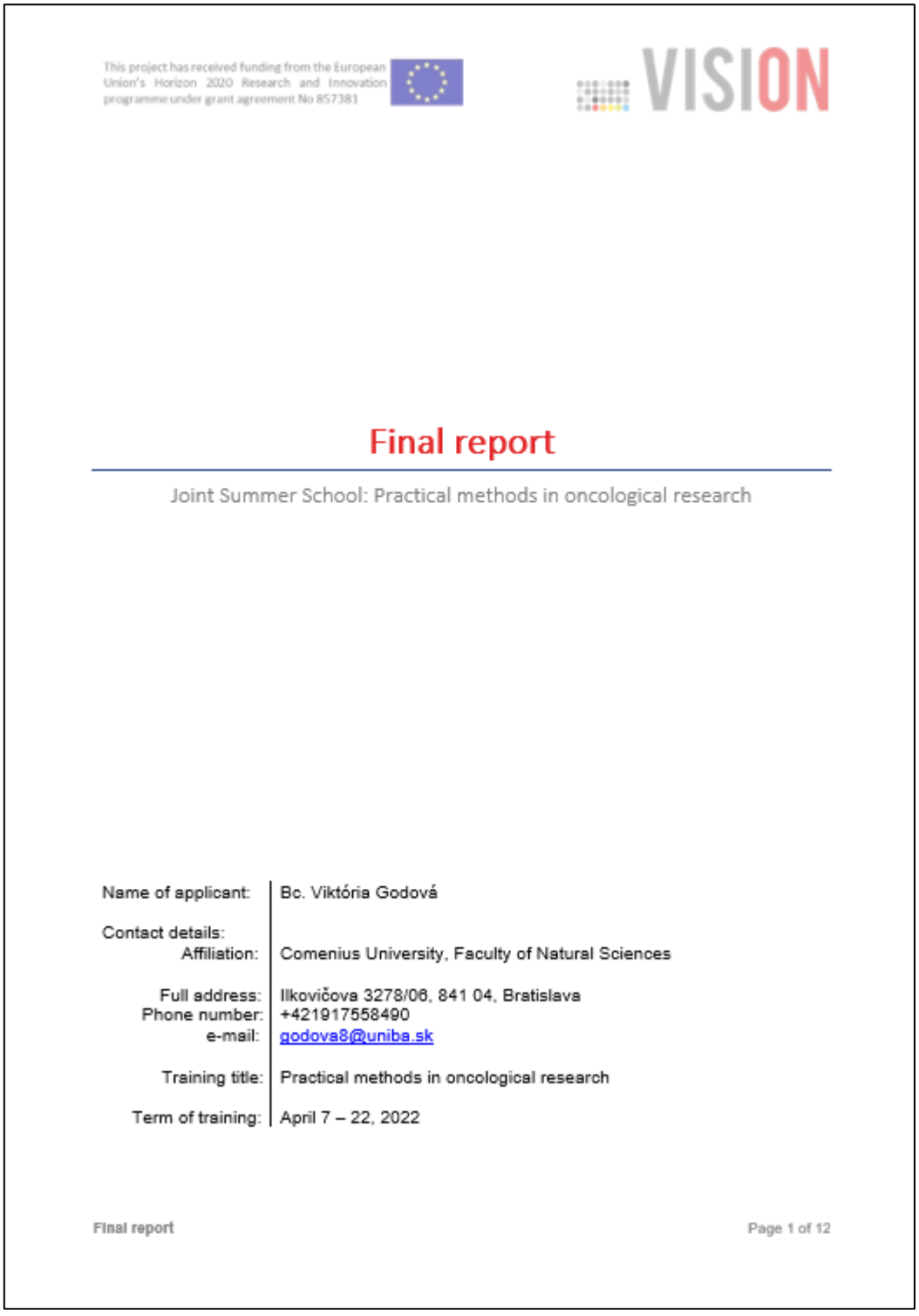
a gallery of cell images (fluorescently-stained nucleoids) provided by the system after analysis. The degree of DNA damage is examined by the software (Metafer 3.6, MetaSystems GmbH, Altlussheim, Germany). The detailed procedure of the comet assay is available for the students on the VISION webpage in educational videos prepared within the hCOMET project (COST Action).

The gallery of cell images after sample analysis

At the end of the Practical methods in oncological research, each undergraduate student attending this course received a Certificate of Attendance.

The undergraduate students who attended both theoretical and practical parts of the Joint summer school

In addition, each student prepared and submitted a final report. All reports are stored on the intranet of the VISION website, together with the list of participants and the agenda.



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VISION

Final report

Joint Summer School: Practical methods in oncological research

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Training title:	Practical methods in oncological research
Term of training:	April 7 – 22, 2022

Final report

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Final report template

2 Deviation from the workplan

The event Joint summer school took place according to the schedule updated in the amendment to the Grant Agreement of the VISION project. Unfortunately, the online lectures originally planned for Session IV were not held due to the severe persistent health problems of the main speaker.

3 Conclusion

The event Joint summer school had a great positive response. Due to the uncertain COVID-19 epidemic situation, interesting theoretical lectures prepared by the VISION partners took place online. Personal contact and interaction between the lecturer and the students always have their advantages. Although we initially considered the distance form to be a limitation/disadvantage, it turned out to be an advantage. Besides undergraduate students, Ph.D. students and early-stage researchers from Slovakia and abroad could attend the lectures. In total, 47 participants registered for the theoretical part.

To maximize the benefit of this event for undergraduate students, BMC SAV also prepared eight tutorial videos. Besides those closely related to the methods that students could train on-site during the practical part, and which were already mentioned above, there are videos of additional three methods generally used in research - how to measure the oxidative stress, protein analysis, and pyrosequencing. Together with the detailed protocols, all videos are available on the VISION website, free to download.

Video: Determination of oxidative stress

Video: Analysis of DNA methylation by pyrosequencing

Video: Protein analysis, Western blot