Triplet

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A special edition devoted to the 'Molecular Biotechnology for Health' project

A few words of introduction on the 'Molecular Biotechnology for Health' Project

The project entitled 'Molecular Biotechnology for Health' that is being realised at the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology (POIG.02.01.00-12-064/08) is financed by the European Union funds from the European Regional Development Fund, the Innovative Economy Operational Programme for 2007-2013. The allocated grant is 28,197,842.44 PLN. Project realisation was commenced in September 2008 and is planned to run until March 2012.

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Workshops on the subject of new research methods 'Molecular Biotechnology for Health', Kraków, 4-5 October 2010

On the 4th and 5th of October 2010 there were organised workshops devoted to the latest research methods with the application of the equipment purchased within the framework of the project 'Molecular Biotechnology for Health'. The more than 90 people from the whole of Poland (students and scientists) who took part in the workshops had the possibility to listen to a series of lectures on the functioning and utilization in scientific research of devices such as: FCS/FLIM and STED confocal microscopes, a mass spectrometer, an instrument for real-time analysis of macromolecular interactions , a laser microdissection system, a magnetic cell sorter; these were conducted by, among others: Prof. Alicja Józkowicz, Prof. Jerzy Dobrucki, Prof. Halina Gabryś and Dr. A. Katarzyna Banaś, Prof. Andrzej Kozik, Dr. S. Kędracka-Krok (who presented one of the devices for proteomic analysis purchased from the 'Małopolska Biotechnology Centre' project), Dr. Maria Rąpała-Kozik, Dr. Agnieszka Łoboda, Dr. Joanna Cichy, Dr. Krzysztof Pyrć, Prof. Zbigniew Madeja and Dr. Justyna Drukała. General information on the subject of the project itself, together with its realisation, was presented in an

introductory address given by the head of the project Prof. Józef Dulak. The interest generated by the theoretical part of proceedings exceeded the wildest expectations of the organisers. The lecture halls were full to overflowing.

The practical workshop part of the two-day event also generated a lot of interest, during which the participants were able to personally experience how the instruments purchased within the framework of the project function. Activities were conducted in small groups, enabling one not only to see the equipment but also to conduct simple experiments. The enthusiasm aroused in the participants has resulted in their declaration of attendance at the forthcoming session planned for spring 2011.

Joanna Uchto

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INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

The Molecular Biotechnology for Health project (POIG.02.01.00-12-064/08) is financed by the European Union from the European Regional Development Fund within the framework of the Innovative Economy Operational Programme for 2007-2013.

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND

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The undertaking is an investment project. Its direct aim is the improvement of the research infrastructure at the faculty including the purchase of the highest quality of equipment allowing for the implementation of modern research methods within the field of molecular biology, biochemistry, biotechnology and biophysics, as well as the construction and equipping of an animal facility. The project comprises seven tasks coordinated by the employees of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology. Within the framework of these tasks new laboratories are to be established:

- The Animal Facility (coordinator: Prof. Alicja Józkowicz),
- The Laboratory of Imaging Cytometry (coordinator: Prof. Jerzy Dobrucki),
- The Laboratory of Proteomics and Transcriptomics (coordinator: Prof. Andrzej Kozik),
- The Laboratory of Cell and Tissue Engineering (coordinator: Dr. Justyna Drukała),
- The Laboratory of Viral Molecular Diagnostics (coordinator: Dr. Krzysztof Pyrć),
- The Laboratory of Plant Biotechnology (coordinators: Prof. Halina Gabryś, Dr. Leszek Fiedor),
- The Biological Sample Storage System (coordinator: Dr. Justyna Drukała).

Development of the research infrastructure will result in the realisation (indirectly) of research goals within the project, which are:

- The development and application of new animal disease models and the assessment of experimental therapies and treatments;
- The elaboration of new methods of cell structures analysis and the interaction of studied substances with cells;
- The development of a complex system of gene expression analysis at the transcriptome and proteome level;
- The development of skin tissue cultures with the aim of its application in clinical conditions;
- The elaboration and development of methods allowing for the identification and quantification of human and animal viruses in relation to their application in clinical and research conditions;
- The identification, study and elaboration of plant metabolite derivatives as well as photosynthetic dyes derivatives for use as potential anti-tumour drugs.

The purchased equipment will be used by the scientific employees, PhD and undergraduate students of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology as well as by other faculties of universities and research centres from all over Poland. Due to the new equipment it will be possible to increase the cooperation of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology with scientific centres abroad. In addition, the equipment will be made available for the needs of hospitals as well as projects realised jointly with entities from the commercial sector (including enterprises from the fields of pharmacy, biotechnology and medicine) interested in conducting research projects through a utilisation of instruments developed by the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology.

Up until November 2010 almost 60% of the allocated grant had been utilised. The majority of equipment for the Laboratory of Imaging Cytometry, the Laboratory of Proteomics and Transcriptomics, the Laboratory of Viral Molecular Diagnostics had been purchased. Moreover, the Laboratory of Cell and Tissue Engineering as well as the cell bank had been equipped. Within the framework of project realisation is the construction of an animal facility (Task 1). In September an agreement was signed for Contract management (including the fulfilling of the role of investment supervisor) with the Raciborskie Przedsiębiorstwo Inwestycyjne, while in November with Przedsiębiorstwo Budowlane Kompleks for the realisation of the construction-installation work.

Various other projects are being conducted at the Faculty with funds from the Innovative Economy Operational Programme, which are complimentary with the 'Molecular Biotechnology for Health' project. These include: the structural project "Malopolska Centre of Biotechnology" (activity 2.1), a strategic research project entitled: 'Innovative methods in the application of stem cells in medicine' (activity 1.1.2), three TEAM research projects financed by the Foundation for Polish Science as well as the LIDER project financed by the National Centre for Research and Development. Scientific teams from the Faculty also participate in the realisation of the 'Jagiellonian Centre for Experimental Therapeutics' investment project (activity 2.2) and the strategic research project connected with it, 'Vascular Endothelium in Civilisation Diseases' (activity 1.1.2).

The Animal Facility (Task no. 1)

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

- Department of Medical Biotechnology,
- investment plenipotentiary for the Dean, Dr. Ryszard J. Gurbiel

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

The construction of the Animal Facility was commenced in December 2010 and its completion is planned for mid 2011. The purchase of equipment and fit-

tings for the Animal Facility is already underway – in December a modern ultrasonograph for animal testing was installed.

Forthcoming plans connected with the project realisation

In 2010 a programme for the construction of the Animal Facility was devised and drawn up by the architectural company D44 Architektura. On its basis the Architecture Department of Krakow City Council issued building permission on the 12th of April 2010. This enabled work on the executive design to come to an end and tenders to be invited for the supervision and realisation of construction work. Construction supervision will lie with Raciborskie Przedsiębiorstwo Inwestycyjne sp. z o.o, while the building of the Animal Facility will be undertaken by Przedsiębiorstwo Budowlane KOMPLEKS. In December construction work commenced.

Prof. Alicja Józkowicz

The Jagiellonian University's storage space in which is to be located the new Animal Facility

The Laboratory of Imaging Cytometry (Task no. 2)

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

Research task 1: Investigating the involvement of heterochromatin proteins 1 (HP1) in chromatin repair – Cell Biophysics Laboratory;

Research task 2: Investigating the influence of drugs, which exhibit DNA affinity, on the structure and function of chromatin – Cell Biophysics Laboratory;

Research task 3: Testing applicability of the fluorescent probe Col-F in the visualisation of extracellular matrix proteins in vivo – Cell Biophysics Laboratory.

Other research groups, which currently use the two Leica microscopes purchased within the framework of the Molecular Biotechnology for Health grant are: the Department of Physical Biochemistry, the Department of Plant Physiology and Biochemistry, the Department of Medical Biotechnology, the Department of Plant Biotechnology.

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

- A Leica SP5 confocal microscope with STED attachment,
- A Leica SMD confocal microscope with FLIM and FCS modules

Description of the selected instruments

The Leica STED and Leica SMD microscopes are used for, among other applications, typical observations and measurements of subcellular structures (three dimensional images) and metabolic processes (imaging of living cells in time, 3D, 4D, etc.) advanced, high-resolution structural research (the STED technique), advanced research into mobility, dynamic exchange and diffusion of fluorescently labelled proteins (FRAP, FLIP, FCS techniques) and interactions between various proteins in situ, in living cells (FRET technique, which employs fluorescence lifetimes of the acceptor and the donor).

Forthcoming plans connected with the project realisation

- Research into interactions of heterochromatin proteins 1 (HP1) with PCNA in the process of the detection and repair of chromatin damage;
- Research into the influence of adriamycin, daunomycin and dyes exhibiting affinity to DNA, on the structure and functions of chromatin, as well as investigating the binding of these drugs to double-stranded RNA;
- Investigating the mechanism of the binding of the fluorescent probe Col-F to collagens and elastin in live animal tissues.

Prof. Jerzy Dobrucki

The Laboratory of Proteomics and Transcriptomics (Task no. 3)

Magnetic cell sorter

A Laser Microdissection System

An instrument for real-time analysis of macromolecular interactions

A mass spectrometer

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

- Department of Analytical Biochemistry,
- Department of Medical Biotechnology,
- Department of Immunology,
- Department of Physical Biochemistry

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

- A Laser Microdissection System,
- An instrument for real-time analysis of macromolecular interactions based on the measurements of changes in surface plasmon resonance (SPR) – model BIACORE 3000 (HVD Holding AG),
- A mass spectrometer with an ESI ion source and an ion trap analyzer – model HCTultra (Bruker),
- A magnetic cell sorter autoMACS pro, Miltenyi Biotec,
- A plate reader,
- A shaker for the breeding of insect cells Unimax 1010/Incubator 1000, Heidolph,
- A shaker for bacteria breeding, Innova 43, New Brunswick Scientific,
- An ultra centrifuge Sorvall WX Ultra Series, Thermo Scientific,
- A high-speed centrifuge, Thermo Scientific.

Description of the selected instruments

Laser Microdissection System (LMD 7000, Leica)

The apparatus for the microscopic laser microdissection serves to precisely isolate cell structures from frozen or formalin-fixed, paraffin-embedded tissue sections. The system enables also to separate and transport living cells. This method is particularly designed for the cutting out of small quantities of cells and is characterised by a high level of accuracy. The extracts obtained are free of pollutants enabling further analyses to be conducted on the cut off material such as the isolation of RNA/DNA or proteins. This system constitutes a unique instrument in such fields as molecular biology, pathology, microbiology, cytogenetics as well as reproduction biology.

An instrument for real-time analysis of macromolecular interactions based on the measurements of changes in surface plasmon resonance (SPR) – model BIACORE 3000 (HVD Holding AG)

The biosensor system BIACORE 3000 enables the testing of the interactions between biologically active macromolecules. The analysis involves the measurements of changes in the surface plasmon resonance that occur upon illumination with polarised light of a gold-coated microchip which is in contact with soluble analyte capable of binding to the microchip-immobilized ligand. On the basis of these measurements one may perform a characterization of the analyte-ligand interaction in terms of the power and specificity of these interactions as well as their kinetic and thermodynamic characteristics. All the determinations proceed very quickly which allows for the testing of biological phenomena in real time and is one of the fundamental advantages of applying this method. Thanks to this it is possible to utilize these tests in following the course of physiological processes as well as pathological states. Besides this advantage of the method there is a possibility to test interactions with analytes of a varied molecular mass without the need

for their additional labelling and with a minimal requirement for the quantity and concentration of the analysed sample.

A mass spectrometer with an ESI ion source and an ion trap analyzer – model HCTultra (Bruker)

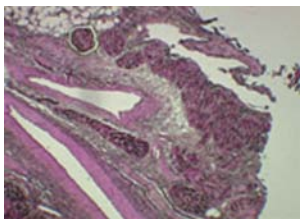
The mass spectrometer with an ion trap as an analyzer and an ion source of ESI type serves in the routine qualitative analyses (identifications) of a wide spectrum of biological compounds on the basis of the precise determination of their weight (mass). In a direct way, one may identify a range of natural compounds of a relatively small particle mass (peptides, lipids etc.). Macromolecules, primarily proteins, are identified through the accurate determination of the masses of their fragments obtained, for example, by means of proteolysis. Because in both application areas the analysed compounds appear in complicated mixtures, the ascertaining of their masses often occurs concurrently with their separation by means of high performance liquid chromatography.

An ultra centrifuge Sorvall WX Ultra Series

Forthcoming plans connected with the project realisation

The Department of Medical Biotechnology

- the isolation of stem cells and the defining of their niches within the brain/bone marrow etc.,



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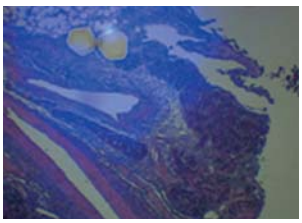

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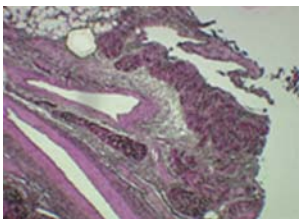


After cutting out

Fig. 1 An example of the application of the LMD 7000 system for the extracting of tissue fragments from a paraffin preparation of a pig's heart, dyed with hematoxylin and eosin stain.

- the designation of the phenotype of transplanted stem cells in the area of the heart following a heart attack in pigs;
- The testing of various kits for the isolation of RNA from the extracted material (from extremely small quantities of material) as well as subsequent analyses, for example, RT-PCR from a single cell (AmpliSpeed);
- The further optimization of work methods with live cells.

The Department of Analytical Biochemistry

BIACORE will be used for kinetic studies of the interactions between proteins of the bioactive peptide (kinin) generation in two models:

- the characteristics of the influence of factors released by defence cells in an inflammatory state, on the interactions between kininogen and prekallikrein (including the influence of oxidizing factors),
- the diagnosis of possibilities for the interaction of these proteins with adhesins appearing on the surface of bacterial and yeast pathogens constituting one of the main factors in the virulence of these microorganisms.

The mass spectrometer will enable the identification of kinin-related peptides generated by yeast proteinases which are expressed in the infections caused by various *Candida* strains. This instrument also serves to identify the substrate specificity of these enzymes in relation to the selected plasma proteins.

Plate reader

Prof. Andrzej Kozik,
Dr. Agnieszka Łoboda

The Laboratory of Cell and Tissue Engineering (Task no. 4)

Laminar cabinet

Low-temperature freezer

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

- Department of Cell Biology

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

- 2 laminar cabinets II class of cleanliness,
- 2 CO_2 incubators for cell culture,
- 2 centrifuges,
- Drier-sterilizer,
- autoclave,
- Phase-contrast inverted microscope,
- Fluorescence microscope,
- Low-temperature freezer.

Description of the selected instruments

Fluorescence microscope

The purchased microscope is a device specially designed for the intravital visualisation of cells with the application of various microscopic optical techniques (epifluorescence, differential interference contrast (DIC), Integrated Modulation Contrast (IMC), phase contrast). The microscopes equipment allows one to observe cells at an assigned temperature and with a determined CO2 concentration. Additionally the highly sensitive cooled CCD camera allows for a limiting to a minimum of the intensity of light arousal. The microscope gives the possibility to register and test many processes occurring within a living cell during the course of experimental work.

Forthcoming plans connected with the project realisation

Work will continue at the Laboratory of Cell and Tissue Engineering within the framework of two currently on-going scientific projects into the clinical application of results obtained. The fluorescence microscope will be utilised in numerous scientific projects both at the Department of Cell Biology as equally at other departments and laboratories of the Faculty of Biochemistry, Biophysics and Biotechnology. As this is a unique piece of equipment it will also be made available to other Polish academic centres (e.g. the University of Wrocław's Institute of Zoology). The microscope is equally an essential instrument for the realisation of other structural projects that the Department of Cell Biology is involved in. Within the framework of the strategic project 'Innovative methods for the use of stem cells in medicine' (activity1.1.2), there is planned in the nearest future a further development of the microscope to allow it to employ FURA, FRET and TIRF techniques.

Dr. Justyna Drukała

Microscope with fluorescence module

Autoclave

CO_2 incubator

The Laboratory of Viral Molecular Diagnostics (Task no. 5)

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

- Department of Microbiology

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

- Flow cytometer (Becton Dickinson; FACSCalibur with sorting and HTS modules),
- Real-time PCR apparatus (Applied Biosystems, 7500fast),
- 4 thermal cyclers (Applied Biosystems, Verity),
- Laminar flow cabinet, biological safety class II (Thermo Scientific, KSP18),
- CO_2 incubator (Heraeus, 150i Duo),
- Inverted microscope with fluorescence module (Leica),
- Laboratory centrifuges (Eppendorf; 5415R and 5804R),
- Electrophoresis unit (power supply unit; 2 containers for electrophoresis) (Biorad),
- Two chambers for work with nucleic acids (UVP, PCR workstation),
- Two cooler-freezer units (Liebherr),
- Thermomixer (Eppendorf; Comfort),
- Magnetic stirrer (IKA; RH Basic 2),
- Personal computer with all-in-one unit.

Chamber for work with nucleic acids

4 thermal cyclers

Description of the selected instruments

Real-time PCR apparatus

The apparatus is used to conduct real time PCR assays in the 96 well plate format. The equipment is supplied with FAM/SYBR Green, VIC/JOE, NED/TAMRA/Cy3, ROX/TEXAS RED, Cy5 filters, which allow simultaneous detection of up to 5 markers. Due to the 'Fast' mode it is possible to conduct a complete real time PCR reaction in a time of <40 minutes, therefore one may significantly increase the quantity of samples analysed. The device's programming allows not only for analysis of the expression level of a given gene in relation to the standard curve, but also microbiological analyses (+/-, relative and absolute), automatic SNP genotyping, population studies and other applications. Further, the apparatus is equipped with a High Resolution Melting Curve (HRM) programme allowing for an analysis of polymorphisms and the identification of DNA fragments based on size, GC content or sequence, on the basis of a DNA denaturation curve without the use of specific probes.

Real-time PCR apparatus

Flow cytometer

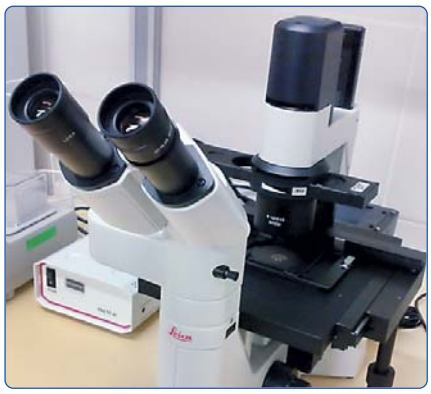
The flow cytometer is equipped with two lasers (488 nm and 635 nm). The apparatus's equipment allows one to simultaneously register up to 6 markers. The HTS module enables this apparatus to be used for the automatic collection of samples from the 96 and 384 well plates. The equipment allows for the rapid analysis of a large quantity of samples, which is something particularly beneficial for screening assays, employed among others in the research for new medicines. Furthermore, the apparatus is equipped with a sorting module, changing the FACS into a simple cell sorter, enabling the preparative approach.

Flow cytometer

Chambers for work with DNA/RNA

The chambers are used for work on RNA and DNA in sterile conditions. Thanks to the use of a four-stage filtration of the air via mechanical filters, carbon filters, a HEPA filter as well as an ozone filter in connection with an UV filter, the equipment warrants ideal conditions for work with nucleic acids. The chamber allows one to

Laboratory centrifuge



Inverted microscope with fluorescence module

obtain repetitive results and to prevent contamination, which is particularly important in the analysis of viral infections.

Cell culture laboratory

The cell culture laboratory has been fully equipped within the framework of the project, allowing for work with class II biological safety pathogens. The equipment of the laboratory comprises: a laminar flow cabinet with HEPA H14 filters and an additional segmented filter (the safety class allowing for work with cytostatic agents), a CO_2 incubator for work with eukaryotic cells as well as viral pathogens, a set of laboratory centrifuges and a bright field / phase contrast / fluorescence microscope.

Forthcoming plans connected with the project realisation

- The continuation of work on viral infections in patients with asthma (identification of the etiologic factor in patients with respiratory tract disease) in cooperation with the Jagiellonian University's Collegium Medicum (Prof. Andrzej Szczeklik);
- The continuation of work on viral infections in lung transplant recipients (identification of the etiologic factor in patients with respiratory tract disease) in cooperation with the Silesian Centre for Heart Disease in Zabrze (Prof. Marian Zembala);
- The implementation of diagnostic methods for viral infections in laboratory animals in connection with the Animal Facility organized within the Faculty of Biochemistry, Biophysics and Biotechnology;
- The continuation of work on ex vivo conductive airway tissue culture generation;
- The continuation of work on the identification of new viruses in samples from patients with illnesses of an unknown etiology.

Dr. Krzysztof Pyrć

Laboratory of Plant Biotechnology (Task No. 6)

Apparatus for isoelectric focusing

A horizontal autoclave

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

- Department of Plant Biotechnology,
- Department of Plant Physiology and Biochemistry

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

Basic equipment:

- Laboratory fittings and furnishings,
- A freezer -20 C,
- Vortex type shakers,
- A MiniSpin Eppendorf bench centrifuge,
- Magnetic stirrers,
- A pH-meter,
- A refrigerated centrifuge MPW 350R,
- Laminar chambers with a horizontal through airflow POLON KL-21,
- A 48-well gradient thermocycler MJ Mini, BioRad,
- Systems for measuring oxygen concentrations,
- Shakers with illuminations for suspension cultures,
- Analytical scales,
- Apparatus for vertical electrophoresis MiniProtean tetra System, BioRad,
- Apparatus for vertical electrophoresis Protean II xi Cell, BioRad,

- Apparatus for isoelectric focusing, Protein IEF Cell, BioRad,
- Apparatus for horizontal electrophoresis Mini Subcell, BioRad,
- Spectrophometer NanoPhotometer, Implen,
- A horizontal autoclave Tuttnauer 5075EL.

Special equipment:

- A flurometer for measurements of in vivo chlorophyll fluorescence equipped with a FluorCam 701 MF imaging unit,
- A potentiostat for anodic stripping voltammetry (ASV),
- A corona charged aerosol detector (CAD)
- A two-beam spectrophotometer UV-VIS V650 JASCO,
- A high resolution two-beam spectrophotometer UV-VIS-NIR Cary 5000, with an enhanced detection range from 175 to 3300 nm,
- A radioactivity detector β-RAM 4 IN/US coupled with a HPLC system, designed for the detection of β radiation and soft γ radiation,
- The upgrading of the Varian GC3800 gas chromatograph with a second detection channel and EI mass detector (Varian 220MS) equipped with a turbomolecular pump,
- A portable Helios Gene Gun System BioRad,
- A system for image documentation with a set of filters, BioSpectrum, UVP.

Description of the selected instruments

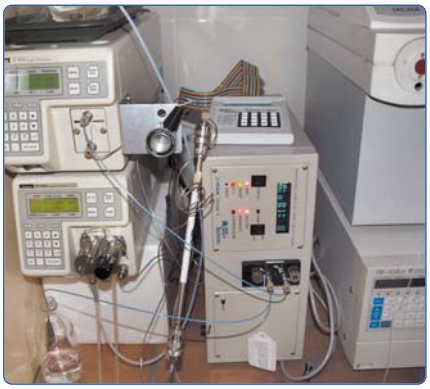
The potentiostat for anodic stripping voltammetry, ASV

Voltammetry is an electrochemical method of analysis based on the measurement of current connected with the course of an electrode reaction. The processes that are the subject of measurement occur on a polarised electrode working on a confined surface. The potential of the working electrode is variable in the course of measurement, in accordance with the program resulting from the technique applied; while the measured current is registered in the form of dependence on the applied voltage, resulting in the curve called a voltammogram. The maximum position on the current-voltage curve characterises qualitatively the analysed substance, for the intensity of the current is proportional to the concentration of the substance. The system for the automatic analyses of voltammetry trace quantities of metals or other substances is composed of a working station (a generator of a linear-variable potential), a measuring vessel equipped with a polarised graphite electrode as well as a system for data acquisition (a PC with software). The equipment allows for identification and precise determination of concentrations of trace quantities (within the range of concentrations below 1 ppm) of heavy metals as well as certain other substances. The precise determination of metal contents in samples of biological origin requires their prior complete mineralization in controlled conditions. The instrument will be used to analyse the collection and accumulation of heavy metals in plant material in order to: 1) evaluate the levels of bioaccumulation of certain pollutants in water plants in relation to the designating of bioindicators as well as the biotechnological methods potentially used in removing environmental contamination brought about by increased concentration of the concentrations of heavy metals (phytoremediation); 2) obtain the characteristics of mechanisms of toleration through an analysis of the differences in the distribution of selected metals in plants derived from populations undergoing selective pressure connected with habitats with various heavy metal content.

Corona charged aerosol detector (CAD)

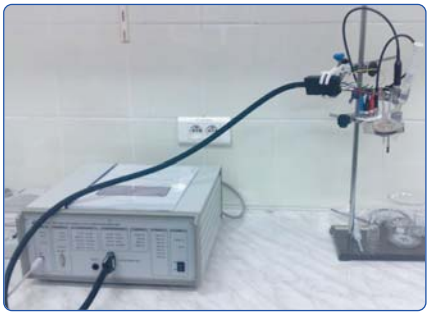
The device is a universal HPLC high sensitivity detector. The specific method of detection allows it to be used for all applications, for the detector's response does not depend on the chemical structure of the separated substances, while the signal, in a very broad range, is linearly proportional to the concentration of the

Gas chromatograph radioactivity detector



A radioactivity detector

Flurometer for measurements of in vivo chlorophyll fluorescence equipped with a FluorCam 701 MF imaging unit



Potentiostat for anodic stripping voltammetry (ASV)

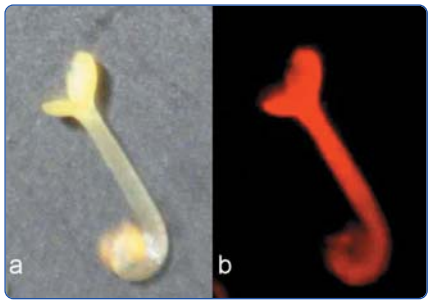
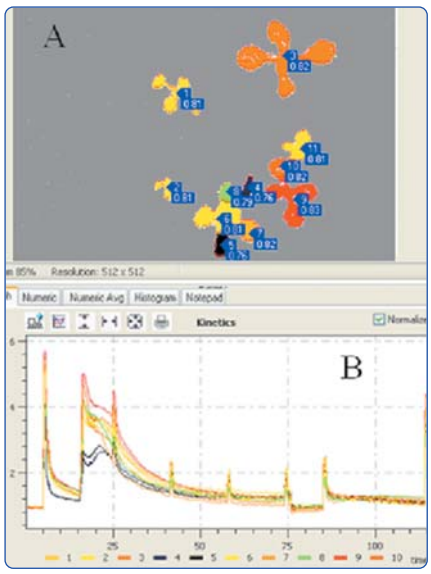


Fig 1. Etiolated (dark-grown) a five-day seedling mutant of the line cop1 Arabidopsis: (a) a photograph in day light; (b) red fluorescence shows the excess accumulation of chlorophyll precursors.



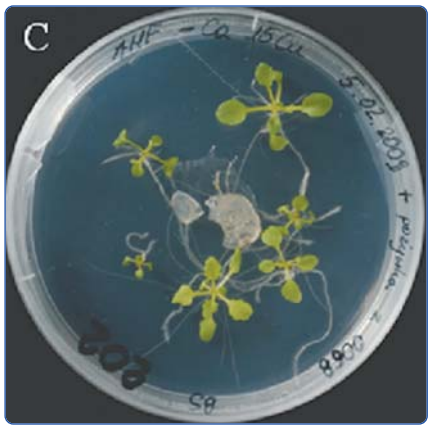


Fig. 2. The spatial distribution of maximum potential photosynthetic efficiency (A) and the kinetics profile of chlorophyll fluorescence (B) for *Arabidopsis arenosa* plants grown in vitro with the presence of Cd ions (C). Data A and B were obtained through the aid of an Open FluorCam (Photon System Instr.) imaging fluorometer.

analysed component in the elute. The elute emerging from the column is subjected to pressure nebulisation (the formation of an aerosol) and dehydration in a nitrogen jet. The stream of particles of the analysed component created in this way subsequently receives an additional electric charge carried by a second stream of gas flowing in the opposite direction additionally charged by a Corona electrode at high voltage. The electric charge delivered by the particles is measured by an electrometer, while the signal is directly proportional to the quantity of the analysed component in the sample. Corona CAD is characterised by high sensitivity, a wide detection range, repetitiveness and ease in service. This detector may also be used for a qualitative and quantitative analysis of substances that do not display (or only weakly) optical activity in the UV/Vis range (lipids, sugars, glycosides, certain alkaloids, etc.), as well as substances that appear in general in small quantities in samples of biological origin (e.g. the precursors of important metabolites, phytohormones).

In recent years there has been observed in Poland a significant interest in the cultivation of plants in artificial conditions, ones requiring additional assistance (e.g. additional lighting, CO2 fertilization etc.). It has become thus necessary to develop technology that allows one to obtain the highest crop without increasing the area under cultivation. This is particularly important in the case of plants grown in closed conditions for research and/or biotechnological aims including biomedical ones. Knowing the molecular mechanisms regulating the biosynthesis of chlorophyll through proteins regulated by photomorphogenesis will constitute an important step towards the above mentioned technologies. At present, through the application of the detector at the Department of Plant Physiology and Biochemistry of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology, a research project is being launched which will be devoted to the role of protein regulators in the photomorphogenesis of plants in the control of chlorophyll biosynthesis.

A fluorometer for the measurement of chlorophyll fluorescence in vivo with a unit for FluorCam 701 MF for e-imaging

Open Fluor Cam from the Czech company Photon Systems Instruments serves in the two-dimensional imaging and analysis of photosynthetic activity. Its operation is based upon a comparison of the efficiency of fluorescence emitted by the photosystems in a photosynthetically inactive and active state – the difference being the energetic efficiency of photosynthesis. The profile of changes in this efficiency provides additional information about the structure and functioning of the photosynthetic apparatus. The measurements, being non-invasive and contactless, may be conducted many times on the same sample, e.g. a group of plants, and the images obtained may be statistically analysed. It is possible to test plants growing in the ground or under in vitro conditions (Fig. 2). This device is used for, among other things, analysis of the microevolutionary adaptation of *Arabidopsis arenosa* in populations exposed to long-term environmental stress.

Helios Gene Gun System BioRad

The gene gun enables the transformation of plants by means of a biolistic method. DNA/RNA-coated balls of gold or tungsten balls are accelerated to great velocities by means of compressed helium and fired into the plant cells. Thanks to this one may supply nucleic acids directly to the cells without the need to damage the cell walls. The biolistic method is widely used in basic research (including to test localisation, the role and interaction between proteins) and applied research (including the introducing of new, beneficial traits to modified species). It constitutes an alternative to the transformation of species, particularly monocotyledonous plants for which other methods of transformation have to date not been developed or are not sufficiently effective. It is also utilised to provide DNA vaccines

to epidermis cells of mice. A particular application of the gene gun has been found in the transformation of chloroplasts. Transgenic plants obtained in this way show a high accumulation of correctly folded and glycosylated heterologous proteins. The earlier used gene guns required the use of vacuum chambers, the dimensions of which seriously limit the selection of material for transformation. The Helios Gene Gun is a portable device which enables its use for in vitro transformations e.g. of leaves on a plant. Within the coming six months there are plans at the Department of Plant Biotechnology to develop protocols of the transient and stable transformation of *Nicotiana benthamiana*, *Arabidopsis thaliana* as well as the water plant Lemna trisulca.

Gene gun

Forthcoming plans connected with the project realisation

Planed purchases:

- An analytical preparatory system of high pressure chromatography equipped with a diode-array type detector (absorption and emission) enabling the registration of absorption and emission spectra in real time during chromatographic separation,
- An NIR detector with a liquid nitrogen cooling system allowing for the detection of emissions in a range from 400 to 1300 nm,
- A time-resolved spectrofluorimetric system for the measurements of absorption and emission spectra in picosecond time domain,
- A two-beam photometer for measuring the motoric activity of chloroplasts.

Employees of the Department of Plant Biotechnology
and the Department of Plant Physiology and Biochemistry

The Biological Sample Storage System (Task no. 7)

Departments and/or Laboratories of the Jagiellonian University's Faculty of Biochemistry, Biophysics and Biotechnology involved in the realisation of the task

- Department of Cell Biology

Equipment purchased within the framework of the Molecular Biotechnology for Health Project

- 2 containers for liquid nitrogen,
- A programmed freezer.

Description of the selected instruments

Containers for liquid nitrogen

Cell Bank

The system of the programmed freezing and preservation of cells in liquid nitrogen enables for the safe storage of valuable and unique cell lines which are subsequently used in academic research. The system possesses its own certificates enabling the storage of human cells, to be utilised clinically and reared in the Laboratory of Cell and Tissue Engineering. The workshop, at present in the process of applying for a GLP certificate, is fully equipped to conduct cell freezing and banking. The human cells cultured in vitro in the laboratory are utilised clinically and used in basic research.

Programmed freezer

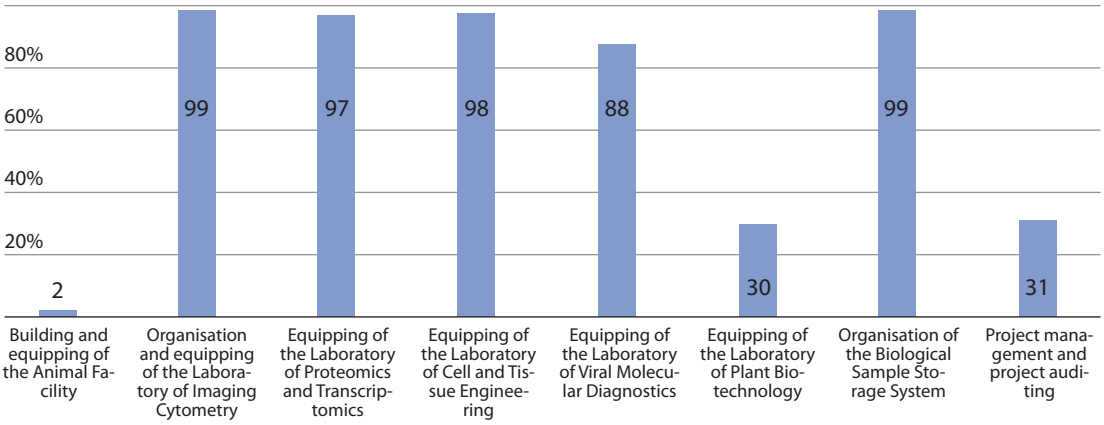
Forthcoming plans connected with the project realisation

Cultured in vitro autologous skin cells, which will be used in burns and trophic wound treatment, will be stored in the cell bank. The Cell Bank will be utilised both at the Laboratory of Cell and Tissue Engineering as equally at other departments and laboratories of the Faculty of Biochemistry, Biophysics and Biotechnology.

Dr. Justyna Drukała

A summing up of project costs

Up until November 2010 16,384,718.66 PLN had been spent within the framework of the project i.e. 58% of the total grant given. The percentage of monies spent in relation to the relevant tasks is presented in the graph.



Task nos.	Task name	Planned sum (PLN)	Amount spent (PLN)
1	Building and equipping of the Animal Facility	6 868 963,00	119 741,06
2	Organisation and equipping of the Laboratory of Imaging Cytometry	7 011 686,00	6 929 753,53
3	Equipping of the Laboratory of Proteomics and Transcriptomics	5 215 030,00	5 036 675,60
4	Equipping of the Laboratory of Cell and Tissue Engineering	864 842,00	846 900,73
5	Equipping of the Laboratory of Viral Molecular Diagnostics	1 009 402,00	889 698,28
6	Equipping of the Laboratory of Plant Biotechnology	4 892 754,00	1 473 524,69
7	Organisation of the Biological Sample Storage System	549 117,00	541 263,95
8	Project management and project auditing	1 786 048,00	547 160,82
	TOTAL	28 197 842,00	16 384 718,66

In the table is presented the degree to which tasks have been realised with regard to the planned and actual expenditure to date.

Editorial Board:
Martyna Elas,
Józef Dulak,
Magdalena Tworzydło

Contact details:
martyna.elas@uj. edu. pl

The editorial board reserves the right to shorten and modify texts. Unaccredited texts are those of the Editorial Board.

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'Molecular Biotechnology for Health' Project Coordination Team:

Prof. Józef Dulak – Project leader
Prof. Alicja Józkowicz – Animal Facility coordinator
Dr. Krzysztof Pyrć – Scientific coordinator
Piotr Widerski MSc – Finance coordinator
Aneta Pazik MA – Administration specialist
Dr. Ryszard Gurbiel – Dean's representative for investments

We invite you to visit our website at: **www.wbbib.uj.edu.pl/bmz**

Contact details: Prof. Józef Dulak
Faculty of Biochemistry, Biophysics and Biotechnology
Jagiellonian University
7 Gronostajowa St., room 3.0.25 (B121)
30-387 Krakow, Poland
tel. (+48) 012 664 63 75
email: jozef.dulak@uj.edu.pl

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