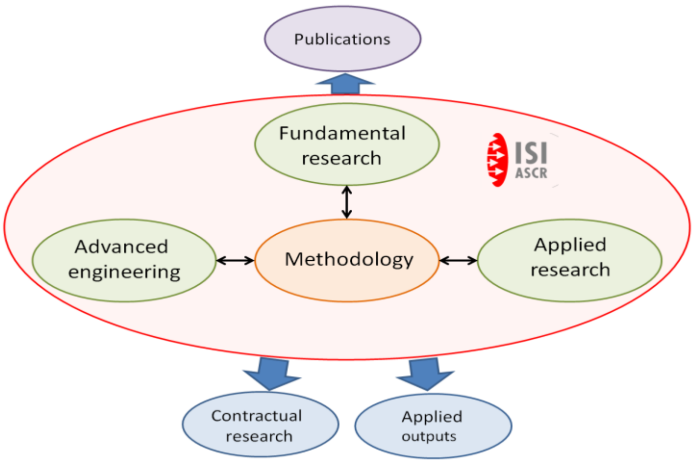
Characteristics of main research directions investigated at the institute and the achievements 2010−2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.

The role of the Institute of Scientific Instruments (ISI) in society is given by its Establishment Deed issued by the Czech Academy of Sciences on 24th May 2006. The ISI is pursuing scientific research in the methodology of examination of physical properties of matter, mainly in the branches of nuclear magnetic resonance, electron microscopy and microanalysis, coherence optics, and acquisition and processing of biosignals, together with research and development of novel special technologies and instrumental elements. The ISI has maintained a tradition in the above mentioned research areas since 1957, when the ISI was established. The ISI contributes to the enhancement of knowledge and learning and to utilization of scientific results in practice.

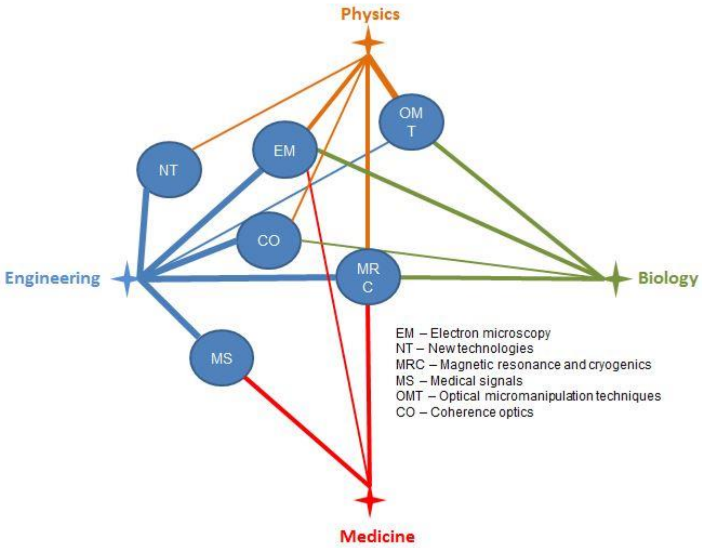
The following chart illustrates how the ISI activities in fundamental research, applied research and advanced engineering are interconnected by methodological activities. Together they participate in the key outputs of ISI in the form of publications, applied output and contractual research for external partners.



The ISI research activities are performed in six research departments that overlap with six teams selected for evaluation. The following table summarizes the teams' numbers, name, main field, and field of science and technology selected for evaluation. This is the original structure of the ISI, which was not formed for the sole purposes of evaluation.

Team		Name of the team	Main Field	Field of Science and Technology (FOS)
1	EM	Electron microscopy	2. Engineering and technology	2.6. Metrology and diagnostic methods
2	NT	New technologies	2. Engineering and technology	2.5. Material engineering, material science and nanotechnology
3	MRC	Magnetic resonance and cryogenics	2 Engineering and technology	2.6. Metrology and diagnostic methods
4	MS	Medical Signals	2. Engineering and technology	2.6. Metrology and diagnostic methods
5	OMT	Optical Micro-manipulation Techniques	1. Natural science	1.3. Physical sciences
6	CO	Coherent Optics	2. Engineering and technology	2.6. Metrology and diagnostic methods

However, each of the teams deals with multidisciplinary topics covering physics, engineering, biology and medicine. Therefore the following chart estimates the rough content of these fields in activities of each team by the distance from the field and thickness of the connecting lines.



1.1. Main achievements of the institute

Scientific achievements are described in detail in texts provided by each team. The quantitative ISI scientific output is summarized in the following table:

Team no.:	1 EM	2 NT	3 MRC	4 MS	5 OMT	6 CO	total
Papers in journals with IF	76	19	52	19	31	25	**222**
Papers in other journals	11	10	8	4	8	12	**53**
Scientific books	0	1	0	0	0	0	**1**
Chapter in scientific books	0	0	0	0	0	0	**0**
Papers at conferences	136	51	87	21	87	170	**552**
Patents	1	0	0	2	0	6	**9**
Applied results	20	67	14	5	28	65	**199**
Number* of Scientists	12,04	7,05	10,48	5,04	9,53	10,00	**54,14**
Number* of other workers in team	6,84	2,23	4,31	2,57	2,99	3,16	**22,09**

**Average number of the load in the evaluated period which is recalculated for full time job.*

Remarkable ISI scientific achievements

2014

Nanotechnology applications of the low energy electron microscopy

Extreme sensitivity of very low energy electron microscopy to ultrafine structure of matter, both in the transmission and reflection modes, enabled us to enter the field of inventing, verification and application of novel approaches to creation of physically or technologically important structures consisting of layers and particles of dimensions in nanometres, as e.g. nanostructured catalyzers or nanocomposite carriers of catalyzers for evolution of hydrogen from water, selective oxidation, etc.

- Zou, X.; Huang, X.; Goswami, A.; Silva, R.; Sathe, B. R.; Mikmeková, E.; Asefa, T. Cobalt-Embedded Nitrogen-Rich Carbon Nanotubes Efficiently Catalyze Hydrogen Evolution Reaction at All pH Values. *Angewandte Chemie*. 2014, **53**(17), 4372-4376. (ASEP ID 436424)
- Das, S.; Goswami, A.; Hesari, M.; Al-Sharab, J. F.; Mikmeková, E.; Maran, F.; Asefa, T. Reductive Deprotection of Monolayer Protected Nanoclusters: An Efficient Route to Supported Ultrasmall Au Nanocatalysts for Selective Oxidation. *Small*. 2014, **10**(8), 1473-1478. (ASEP ID 436427)
- Al-Sharab, J. F.; Mikmeková, E.; Das, S.; Goswami, A.; El-Sheikh, S. M.; Ismail, A. A.; Hesari, M.; Maran, F.; Asefa, T. Low Energy TEM Characterizations of Ordered Mesoporous Silica-Based Nanocomposite Materials for Catalytic Applications. *Microscopy and Microanalysis*. 2014, **20**(3), 1900-1901. (ASEP ID 436806)

Methods for quantitative analysis of MR images of perfusion and MR spectra and for multiparametric segmentation of tumor tissue

Methods for quantitative analysis of MR images of perfusion and MR spectra and for multiparametric segmentation of tumor tissues Methods and software for more realistic and reliable pharmacokinetic modeling of perfusion from MR images, quantitation of metabolite concentrations from MR spektra and for the segmentation of tumors in multiparametric MR images have been developed. Determination and combination of credible quantitative local information about blood microcirculation in tissues, about changes of water relaxation and metabolic anomalies aim to support tumor research, therapy development and clinical diagnostics.

- Bartoš, M.; Jiřík, R.; Kratochvíla, J.; Standara, M.; Starčuk jr., Z.; Torfinn, T. The precision of DCE-MRI using the tissue homogeneity model with continuous formulation of the perfusion parameters. *Magnetic Resonance Imaging*. 2014, **32**(5), 505-513. (ASEP ID 431811)

Light induced sorting and rotation of microobjects

We shaped light beams to spatial patterns similar to „optical sieves" and illuminated microobjects. Force influence of light separated them according to their size, shape or inner structure. The impact of a vortex beam upon a microspheroid led to its trapping and rotation around its axis. As the first we observed mutual synchronization of more rotating spheroids. Understanding this effect reveals motion by flagella and progresses to new optofluidic microtechnologies as light propelled pumps and mixers.

- Arzola, A. V.; Jákl, P.; Chvátal, L.; Zemánek, P. Rotation, oscillation and hydrodynamic synchronization of optically trapped oblate spheroidal microparticles. *Optics Express*. 2014, **22**(13), 16207-1621. (ASEP ID 431695)

Passive localization system for monitoring the distribution of Egyptian fruit bats in the Mediterranean and Egyptian desert

A Localization system for wireless monitoring of the position of Egyptian fruit bats is a unique tool for the community of zoologists who follow the development of the population of these unique mammals. It uses a combination of advanced algorithms of digital signal processing and stochastic analysis of the recorded data for an accurate description of migration, nesting and reproduction of these small vertebrates. The system has now been deployed in the Mediterranean and Egyptian desert.

- Lučan, R. K.; Bartonička, T.; Benda, P.; Bilgin, R.; Jedlička, P.; Nicolaou, H.; Reiter, A.; Shohdi, W. M.; Šálek, M.; Řeřucha, Š.; Uhrin, M.; Abi-Said, M.; Horáček, I. Reproductive seasonality of the Egyptian fruit bat (Rousettus aegyptiacus) at the northern limits of its distribution. *Journal of Mammalogy*. 2014, **95**(5), 1036-1042. (ASEP ID 433567)

2013

Experimental demonstration of an optical "tractor" beam

An optical "tractor" beam transports microparticles over one hundred micrometers against photons flow. The direction of the resulting force depends on the size of a microparticle and thus uneven particles can be separated. More illuminated objects interact via scattered light and self-arrange into optically bound microstructures transported by the beam in different directions. This paves the way to self-assembling and transport of functional microstructures by mere illumination of the suspension.

- Brzobohatý, O.; Karásek, V.; Šiler, M.; Chvátal, L.; Čižmár, T.; Zemánek, P. Experimental demonstration of optical transport, sorting and self-arrangement using a "tractor beam". *Nature Photonics*. 2013, **7**(2), 123-127. (ASEP ID 397687)

On time course of synchronization patterns in the human brain during cognitive tasks

Using intracerebral EEG recordings in a large cohort of human subjects, we investigated the time course of neural cross-talk during a simple cognitive task. Our results show that human brain dynamics undergo a characteristic sequence of synchronization patterns across different frequency bands following a visual oddball stimulus. In particular, an initial global reorganization in the delta and theta bands (2-8 Hz) is followed by gamma (20-95 Hz) and then beta band (12-20 Hz) synchrony.

- Brázdil, M.; Janeček, J.; Klimeš, P.; Mareček, R.; Roman, R.; Jurák, P.; Chládek, J.; Daniel, P.; Rektor, I.; Halámek, J.; Plešinger, Filip; Jirsa, V. On the time course of synchronization patterns of neuronal discharges in the human brain during cognitive tasks. *PLoS ONE*. 2013, **8**(5), e63293:1-9. (ASEP ID 397711)
- Brázdil, M.; Cimbálník, J.; Roman, R.; Stead, M.; Daniel, P.; Halámek, J.; Jurák, P.. Effect Of Cognitive Stimulation On Hippocampal Ripples In Epileptic Patients. *Epilepsia*, 2013, **54**(S3), 268-268. (ASEP ID 423944)

Anomalous heat transport in a two - phase system of cryogenic helium

We investigated heat transfer from colder heated body to warmer cooled body in a liquid-vapor system of cryogenic helium. This anomalous effect was noticed during the study of two-phase convection in a closed vessel. It occurs under non-steady conditions due to evaporation and condensation processes inside the vessel, where temperature inversion originates between the heated cooler bottom and the cooled warmer top. Numerical model affirms that it is not in contradiction with the second law of thermodynamics.

- Urban, P.; Schmoranzer, D.; Hanzelka, P.; Sreenivasan, K. R.; Skrbek, L. Anomalous heat transport and condensation in convection of cryogenic helium. *Proceedings of the National Academy of Sciences of the United States of America*. 2013, **110**(20), 8036-8039. (ASEP ID 397714)

New method for study of live nature in environmental scanning electron microscope

This newly developed method makes electron microscope observation of live mites possible, which the experiments survive without apparent consequences. The procedure was designed on the basis of mathematical-physics simulation which, is on the surface mites or any living tissue formed protective layer of water. The water layer is at a certain moment evaporated, the sample is observed and then the mite is covered with layer of water again.

- Tihlaříková, E.; Neděla, V.; Shiojiri, M. In Situ Study of Live Specimens in an Environmental Scanning Electron Microscope. *Microscopy and Microanalysis*. 2013, **19**(4), 914-918. (ASEP ID 397947)

Properties of absorption cells filled to the saturation point of absorption media

Absorption cells represent a tool for lasers frequency stabilization – light sources for ultraprecise length measurement and evaluating of other physical quantities (acceleration of gravity). Cells prepared by the method of filling to saturation point of absorption media do not need an additional temperature control. Using of borosilicate glass simplifies the cell’s technology. Realization of special multi-pass cell represents the unique tool for gravimeter intended for cosmic research.

- Hrabina, J.; Lazar, J.; Holá, M.; Číp, O. Frequency Noise Properties of Lasers for Interferometry in Nanometrology. *Sensors*. 2013, **13**(2), 2206-2219. (ASEP ID 389837)
- Chiodo, N.; Du Burck, F.; Hrabina, J.; Candela, Y.; Wallerand, J. P.; Acef, O. CW frequency doubling of 1029 nm radiation using single pass bulk and waveguide PPLN crystals. *Optics Communications*. 2013, **311**(15 Jan), 239-244. (ASEP ID 397704)
- Lazar, J.; Holá, M.; Číp, O.; Hrabina, J.; Oulehla, J. Interferometric system with tracking refractometry capability in the measuring axis. *Measurement Science and Technology*. 2013, **24**(6), 067001:1-6. (ASEP ID 399809)

<u>2012</u>

Theoretical and experimental study of near field heat transfer

The near field significantly increases radiative heat transfer by thermal radiation when the distances of radiating surfaces are shorter than the characteristic wavelengths of the spectrum of thermal radiation. With decreasing temperature the wavelengths of thermal radiation become longer and at very low temperatures they reach hundreds of μm. This can be used with advantage in the study of the near field radiative heat transfer.

- Králík, T.; Hanzelka, P.; Zobač, M.; Musilová, V.; Fořt, T.; Horák, M.: Strong Near-Field Enhancement of Radiative Heat Transfer between Metallic Surfaces. *Physical Review Letters*. 2012, **109**(Nov 27), 224302:1-5. (ASEP ID 385282)

Identification of the local crystallinity upon reflectance of very slow electrons

We developed a new method for obtaining the information about local crystallinity of polycrystalline solids in SEM with a high lateral resolution, down to several nm. This result represents an alternative method to EBSD based on the measurement of the reflectance of very slow electrons from the surface. At energies below 30 or 40 eV the incident electrons enter the crystal as so called Bloch electrons existing in the environment of energy bands and gaps, and in dependence of the density of empty electron states in their direction of motion they penetrate the crystal or reflect back. Because the density of electron states and its electron energy dependence is related to the crystal system and its spatial orientation, the relationship between reflectance of very slow electrons and their energy testifies to the local crystallographic information.

- Pokorná, Z.; Mikmeková, Š.; Müllerová, I.; Frank, L.: Characterization of the local crystallinity via reflectance of very slow electrons. *Applied Physics Letters*. 2012, **100**(26), 261602:1-4. (ASEP ID 384097)
- Müllerová, I.; Hovorka, M.; Mika, F.; Mikmeková, E.; Mikmeková, Š.; Pokorná, Z.; Frank, L.: Very low energy scanning electron microscopy in nanotechnology. *International Journal of Nanotechnology*. 2012, **9**(8/9), 695-716. (ASEP ID 375383)
- Frank, L.; Hovorka, M.; Mikmeková, Š.; Mikmeková, E.; Müllerová, I.; Pokorná, Z.: Scanning Electron Microscopy with Samples in an Electric Field. *Materials*. 2012, **5**(12), 2731-2756. (ASEP ID 385193)

Interferometer with compensation of the fluctuations of the refractive index of air

The concept of the proposed interferometric measuring system is based on referencing derived not from the optical frequency and following conversion to wavelength through indirect evaluation of the refractive index of air but from the link (stabilization) of the wavelength to mechanical reference. In this configuration the measuring system can be interpreted as a standing-wave interferometer where within the measuring range there is a fixed “grid” of wavelengths and the positioning system moves within these steps. If the wavelength (or average) wavelength constant within the whole range and the control feedback loop of the laser fast enough, the system can react adequately to the fast fluctuations of the refractive index of air caused by air flow. Experiments show an agreement at least one order better than the fluctuations themselves.

- Lazar, J.; Holá, M.; Číp, O.; Čížek, M.; Hrabina, J.; Buchta, Z.: Displacement interferometry with stabilization of wavelength in air. *Optics Express*. 2012, **20**(25), 27830-27837. (ASEP ID 385285)
- Lazar, J.; Holá, M.; Číp, O.; Čížek, M.; Hrabina, J.; Buchta, Z.: Refractive Index Compensation in Over-Determined Interferometric Systems. *Sensors*. 2012, **12**(10), 14084-14094. (ASEP ID 385287)

Measure of the QT–RR dynamic coupling in patients with long QT syndrome

The patients with long QT syndrome type-1 (LQT-1) have an impaired adaptation of the QT interval to heart rate changes. Yet, the description of the dynamic QT/RR coupling in genotyped LQT-1 has never been thoroughly investigated. We proposed a method to model the dynamic QT/RR coupling by defining a transfer function characterizing the relationship between a QT interval and its previous RR intervals measured from ambulatory Holter recordings. The QT/RR dynamic profiles are significantly different between LQT-1 patients (97) and controls (154). The results provide insights into the types of arrhythmogenic triggers a patient may be prone to and explain the higher prevalence of arrhythmias in LQT subjects even if they have QTc comparable with healthy people. With sudden a increased of heart rate the corresponding shortening of QT does not occur in LQT subjects.

- Halámek, J.; Couderc, J. P.; Jurák, P.; Vondra, V.; Zareba, W.; Viščor, I.; Leinveber, P.: Measure of the QT/RR Dynamic Coupling in Patients with the Long QT 28 Syndrome. *Annals of Noninvasive Electrocardiology*. 2012, **17**(4), 323-330. (ASEP ID 385229)
- Halámek, J. – Jurák, P.: Method of ventricular repolarization analysis. Patent no.: EP2155055. Awarded at: 15-Feb-2012 (ASEP ID 387841) – Patent no.: US8600485. Awarded at: 3-Dec-2013 (ASEP ID 423542)

New methods for magnetic resonance and ultrasound quantitative perfusion measurement

Two new techniques for the quantitative measurements of perfusion by magnetic resonance imaging and ultrasounds have been introduced and represent the next step towards the reliable assessment of perfusion changes, which provide valuable data for preclinical research and clinical diagnostics of conditions associated with vascular changes such as neoplasms, ischemia, neurodegeneration, or blood-brain barrier damage.

- Taxt, T.; Jiřík, R.; Rygh, C. B.; Grüner, R.; Bartoš, M.; Andersen, E.; Curry, F. R.; Reed, R. K.: Single-Channel Blind Estimation of Arterial Input Function and Tissue Impulse Response in DCE-MRI. *IEEE Transactions on Biomedical Engineering*. 2012, **59**(4), 1012-1021. (ASEP ID 385359)

2011

Interferometric system for the calibration of gauge blocks

As a result of a project of applied research in cooperation with the Mesing company, we have developed a system for the calibration of gauge blocks. The measuring method is based on low-coherence interferometry and allows us to measure the length and surface topography of gauge blocks in a non-contact bi-directional way. The system represents a unique solution of the interferometer that is protected by patent.

- Buchta, Z.; Mikel, B.; Lazar, J.; Číp, O.: White-light fringe detection based on a novel light source and colour CCD camera. *Measurement Science and Technology*. 2011, **22**(9), 094031:1-6. (ASEP ID 366037)
- Lazar, J.; Číp, O.: The way of length calibration of the object and the system for the calibration of the length. Patent no.: CZ302948. Awarded at: 14-DEC-2011 (ASEP ID 387845)

MR-based perfusion imaging for biomedical research and cancer diagnostics

Perfusion is a physiological process significant for cancer diagnosis and therapy development because its characteristics get altered by the abnormal angiogenesis accompanying the growth of tumors. For reliable determination of these parameters by data modelling based on MR images, we developed measurement protocols and data analysis methods and applied them to current biomedical research. The methods were tested preclinically in Bergen and clinically at Masaryk Memorial Cancer Institute in Brno.

- Keunen, O.; Johansson, M.; Oudin, A.; Sanzey, M.; Rahim, S. A.; Fack, F.; Thorsen, F.; Taxt, T.; Bartoš, M.; Jiřík, R.; Miletic, H.; Wang, J.; Stieber, D.; Stuhr, L.; Moen, I.; Rygh, C. B.; Bjerkvig, R.; Niclou, S.: Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma. *Proceedings of the National Academy of Sciences of the United States of America*. 2011, **108**(9), 3749-3754. (ASEP ID 368977)
- Jiřík, R.; Standara, M.; Malá, A.; Sedláková, S.; Bartoš, M.; Taxt, T.; Starčuk jr., Z.: Flow phantom for validation of absolute quantification in dynamic contrast-enhanced MRI. *Magnetic Resonance Materials in Physics, Biology and Medicine*. 2011, **24**(S1), 247-248. (ASEP ID 371017)
- Bartoš, M.; Jiřík, R.; Taxt, T.: Precision of DCE-MRI parameter estimates using extended distributed capillary adiabatic tissue homogeneity model. *Magnetic Resonance Materials in Physics, Biology and Medicine*. 2011, **24**(S1), 18. (ASEP ID 371205)

- Taxt, T.; Jiřík, R.; Rygh, C. B.; Gruner, R.; Bartoš, M.; Andersen, E.; Curry, F. R.; Reed, R. K.: Single-Channel Blind Estimation of Arterial Input Function and Tissue Impulse Response in DCE-MRI. *IEEE Transactions on Biomedical Engineering*. 2012, **59**(4), 1012-1021. (ASEP ID 385359)

Calculation and optimization of optical properties of systems with perturbed axial symmetry

We developed a method of description for the misalignment aberrations of electron and ion optical systems. It comes from Sturrock's theory of the system with slightly perturbed axial symmetry, which causes the presence of additional weak multipole fields. We have shown that the combination of the sample tilt with the extraction field attracting the secondary ions to the analyzer causes the presence of strong dipole and quadrupole fields, which deform the primary beam in the direction of the sample tilt, and decrease the final system resolution. For these reasons we proposed a correction method based on the application of two octupole stigmators and the refocusing of the objective lens. The system will be implemented in commercial instruments developed in cooperation with the German company ION-TOF within the FP7 framework program 3DNanoChemiscope.

- Zlámal, J.; Lencová, B.: Development of EOD for the design in electron and ion microscopy. *Nuclear Instruments & Methods in Physics Research Section A*. 2011, **654**(1), 278-282. (ASEP ID 358560)
- Oral, M.; Lencová, B.: Correction of sample tilt in FIB instruments. *Nuclear Instruments & Methods in Physics Research Section A*. 2011, **645**(1), 130-135. (ASEP ID 358590)
- Oral, M.; Radlička, T.; Lencová, B.: Effect of sample tilt on PEEM resolution. *Ulramicroscopy*. 2012, **119**(S1), 45-50. (ASEP ID 358323)

Heat transfer efficiency in a natural turbulent convection at high Rayleigh numbers

The heat transfer efficiency in turbulent thermal convection described via the Nusselt (Nu) versus Rayleigh (Ra) numbers dependence was investigated experimentally by using cryogenic 4He (~5 K) in the Ra range of 106 < Ra < 1013. The aim was to elucidate the existence of transition into the theoretically predicted ultimate Kraichnan regime characterized by the Nu~Ra1/2 scaling law. Based on our experimental results and on detailed analysis of the obtained experimental data, we demonstrated mutual agreement among the world's cryogenic experiments for Ra up to about 1011, while at higher Ra all these sets of data differ considerably. For higher Ra, our data correspond to the simplest physical model of Nu~Ra1/3 and do not indicate any tendency to transition to the ultimate Kraichnan regime.

- Urban, P.; Musilová, V.; Skrbek, L.: Efficiency of Heat Transfer in Turbulent Rayleigh-Benard Convection. *Physical Review Letters*. 2011, **107**(1), 014302:1-4. (ASEP ID 368244)

2010

Scanning transmission microscopy of free standing thin films with very slow electrons

In the scanning electron microscope equipped with the cathode lens, penetration of electrons through free-standing thin films was verified in dependence on the landing energy of electrons, which led to development of the transmission mode at extremely low energies. Using very slow electrons the transmissivity and imaging of graphene were studied at a high lateral resolution of about 10 nm. Image contrast in transmitted electrons of the individual graphene layers has proven itself incomparably higher than the contrast in reflected electrons available so far. The transmissivity of graphene was found reaching its maximum at 5 eV.

- Müllerová, I.; Hovorka, M.; Hanzlíková, R.; Frank, L. Very low energy scanning electron microscopy of free-standing ultrathin films. *Material Transactions*. 2010, **51**(2), 265-270. (ASEP ID 340747)
- Müllerová, I.; Hovorka, M.; Frank, L. Advances in low energy scanning elektron microscopy. In: Proceedings of the 17th IFSM International Microscopy Congress. Rio de Janeiro: Sociedade Brasileira de Microscopia e Microanilise, 2010, pp. 256-257. (ASEP ID 352422)
- Frank, L.; Hovorka, M.; Konvalina, I.; Mikmeková, Š.; Müllerová, I. Very low energy scanning electron microscopy. *Nuclear Instruments & Methods in Physics Research Section A*. 2011, **645**(1), 46-54. (ASEP ID 358595)

Self-organization of microparticles caused by optical interaction between them

Optical micromanipulation techniques utilize light beams with inhomogeneous spatial distribution of optical intensity. In such a beam microparticles or nanoparticles are dragged by optical forces towards the locations of higher optical intensity and they are confined there in the so called optical trap. In the case of more simultaneously illuminated electrically neutral particles, the light scattered by other particles leads to additional force interactions. This long-time neglected phenomenon causes that the final force acting on the particles is also affected by the spatial arrangement of all the particles. Even if the incident light itself does not form the optical traps, the illuminated particles - under proper conditions - create optical traps for themselves and spatially localize in them. This optical binding leads to self-arrangement of particles through light and forms so called optically bound matter. The spatial distribution of the particles sensitively reflects the number and the properties of the particles, surrounding medium and light beam. In our research, we focused on the behavior of polystyrene particles illuminated by two and more laser beams. We created optically bound colloidal structure 100 micrometers long, for the first time we demonstrated behaviour of more microparticles illuminated by counter-propagating optical vortices, and we succeeded in an online tuning of the distances between individual microparticles by changes of the parameters of illuminating beams. These results pave the way towards the self-organization of microparticles into colloidal or photonic crystals just by pure light illumination of the suspension.

- Dholakia, K.; Zemánek, P. Colloquium: Gripped by light: Optical binding. *Reviews of Modern Physics*. 2010, **82**(2), 1767-1791. (ASEP ID 350855)
- Brzobohatý, O.; Čižmár, T.; Karásek, V.; Šiler, M.; Dholakia, K.; Zemánek, P. Experimental and theoretical determination of optical binding forces. *Optics Express*. **18**(24), 25389-25402. (ASEP ID 397687)
- Čižmár, T.; Brzobohatý, O.; Dholakia, K.; Zemánek, P. The holographic optical micro-manipulation system based on counter-propagating beams. *Laser Physics Letters*. 2011, **8**(1), 50-56. (ASEP ID 350886)

Minimization of QT hysteresis by a transfer function description of the QT/RR coupling

In cardiology, QT hysteresis is a well-known property of the RR and QT interval dynamics, which limits the possibilities of analyzing their coupling. No method has been available so far that would explain the hysteresis and eliminate it from the description. Only QT-RR clouds were analyzed, with the aim to extract certain hysteresis parameters usable for diagnostics. We demonstrated that QT hysteresis can be explained on the basis of a general QT/RR coupling model and can be described by a transfer function. In exercise tests of healthy subjects, hypertensive patients and patients with paced rhythm, we were able to virtually eliminate it from the data. Modelling classical QT hysteresis is perfect; in some subjects, QT irregularities remain, such as QT drift or QT changes that precede the RR changes. The elimination of the classical hysteresis from the data opens the way to the analysis of QT irregularity and QT nonlinearity. The QT/RR transfer function parameters, which describe the properties of the classical QT hysteresis, are much more appropriate for diagnostics than parameters derived from the QT-RR clouds.

- Halámek, J.; Jurák, P.; Bunch, T. J.; Lipoldová, J.; Novák, M.; Vondra, V.; Leinveber, P.; Plachý, M.; Kára, T.; Villa, M.; Fráňa, P.; Souček, M.; Somers, V. K.; Asirvatham, S. J. Use of a novel transfer function to reduce repolarization interval hysteresis. *Journal of Interventional Cardiac Electrophysiology*. 2010, **29**(1), 23-32. (ASEP ID 350809)

Applied output have the form done by the governmental Methodology of Evaluation of the Results of Research Organizations and Results of Finished Programs (see www.vyzkum.cz), recently including contractal research. Due to their long-term history in development of scientific instruments, The ISI is active in producing this output as table above demonstrates. Let us list the most important of them:

Filed patents:

- Interferometric assembly for differential measurement of distance. CZ304317, 2014 (ASEP ID 438921)

- Apparatus for laser beam welding and method for controlling quality of weld. CZ303797, 2013 (ASEP ID 423595)
- Method and apparatus for detection of interference phase of two interfering laser beams. CZ304138, 2013 (ASEP ID 423601)
- Method of ventricular repolarization analysis EP2155055, 2012, US8600485, 2013 (ASEP ID 387841, 0423542)
- Ionisation Detector for Environmental Scanning Microscope. EP2195822, 2011 (ASEP ID 372211)
- Interferometric system with compensation of the refractive index fluctuation of the ambiance. CZ302520, 2011 (ASEP ID 372210)
- Method for calibration of the object length and equipment for performing the method. CZ302948, 2011 (ASEP ID 387845)
- Method for detecting dimensional and shape deviations of mechanical components and equipment for implementing this method. CZ303909, 2013 (ASEP ID 423599)

Filed utility samples:

- Controlled breathing apparatus. CZ27503, 2014 (ASEP ID 438929)
- Multichannel whole-body impedance monitor. CZ27564, 2014 (ASEP ID 438930)
- Device for stabilization of laser optical frequency. CZ27304, 2014 (ASEP ID 438926)
- Module for stabilization of laser optical frequency and stabilized laser and module assembly. CZ27406, 2014 (ASEP ID 438927)
- System for sorting of live cells of photoautotrophic microorganisms. CZ2864, 2013 (ASEP ID 423529)
- Device for attachment of radiating source to microscope objective. CZ21642, 2011 (ASEP ID 372212)
- System for monitoring animals with transmitters. CZ23077, 2011 (ASEP ID 387848)

The most important functional samples and prototypes (out of 83)

- Electron gun EG-60/2 V.1.3. 2014 (ASEP ID 437482)
- Absorption cells for the fundamental metrology and spectroscopy. 2014 (ASEP ID 437573)
- Precise high-voltage power supply with integrated low-voltage DC floating power supply. 2013 (ASEP ID 423130)
- The prototype of the solar cell with sputtered Al2O3 layer on the backside or Si3N4 layer on the foreside. 2013 (ASEP ID 426800)
- Functional samples of electrochemical sensors with deposited B-DLC layer. 2013 (ASEP ID 423126)
- Precise reference divider for voltage up to 120 kV. 2013 (ASEP ID 423129)
- Positioning system with high dynamic range driven by a piezoelectric motor. 2012 (ASEP ID 387397)
- New sputtering equipment for depositing B-DLC layers. 2012 (ASEP ID 387557)
- Detector of transmitted very low energy electrons for the ultra-high vacuum scanning low energy electron microscope. 2012 (ASEP ID 379939)
- Fibre optics spectrometer with fibre Bragg grating. 2011 (ASEP ID 375778)
- System for contactless gauge blocks diagnostics. 2011 (ASEP ID 375766)
- Impact tester for measurement in controlled atmosphere. 2010 (ASEP ID 356020)
- Device for calibration of different types of low temperature sensors for temperature measurement. 2010 (ASEP ID 356101)
- Apparatus for study of microscale radiative heat transfer at low temperatures. 2010 (ASEP ID 355914)
- Advanced acquisition system for electrophysiology. 2009 (ASEP ID 336422)

Software

- Software for interference phase detection for two interfering laser beams. 2014 (ASEP ID 435099)
- Virtual scanner NMRScopeB 2.0. 2014 (ASEP ID 437477)

The most important contractual research is given in Appendix 3.2 (out of 92)

- Electron Beam Welder MEBW-60/2. 2014 (ASEP ID 437361)
- Development of welding and soldering technology for testing expansion turbines. 2014 (ASEP ID 438047)
- Research and development of permanent joints for X-ray sources. 2014 (ASEP ID 438050)
- Concept and pilot practical evaluation of system for rifle-barrels straightness measurement. 2014 (ASEP ID 426263)
- Relief structures based on diffractive optics. 2014 (ASEP ID 437369)
- Nanolithography system based on two-photon photopolymerization. 2014 (ASEP ID 437396)
- Development and validation of the methodology of physical realization of optical interference coatings for interferometry. 2014 (ASEP ID 426276)
- System for precise generating and motoring the 3D magnetic field. 2013 (ASEP ID 426256)
- Analysis of the microstructure and chemical composition of the synthetic diamond powders. 2013 (ASEP ID 426228)
- System for measuring angles on ELI manipulator. 2013 (ASEP ID 426230)
- Experimental evaluation of thermal radiative properties of device surfaces designed for space operation. 2013 (ASEP ID 426241)
- A module to measure optical frequency spectrum in the range of wavelengths 1300 – 1600 nm. 2013 (ASEP ID 426262)
- Scintillation detector of backscattered electrons for SEM. 2012 (ASEP ID 426274)
- Research and development of permanent joints of metal materials for vacuum technology by electron beam welding and vacuum brazing. 2012 (ASEP ID 426245)
- Data processing in cardiology. 2012 (ASEP ID 426289)
- Investigation of optical frequency references for lasers stabilization in visible spectral range. 2012 (ASEP ID 426237)
- Absorption cell for laser interferometry. 2012 (ASEP ID 426300)
- Optical tweezers. 2012 (ASEP ID 426248)

Appreciation of scientific results of the ISI researchers by external community

- Zemánek et al.: Price of Werner von Siemens - category: “The most important result of the fundamental research”, for *Demonstration of the optical tractor beams*; awarded by Siemens s.r.o., Czech Republic (2014).
- Brzobohatý O.: Prize of Otto Wichterle for *The best young scientists* (up to 35) of the Czech Academy of Sciences (2014).
- Hrabina J.: Prize of Otto Wichterle for *The best young scientists* (up to 35) of the Czech Academy of Sciences (2014).
- Číp O.: Annual price for the best innovative product or process in the field of the automatiosation for *Automatic contactless calibration of gague blocks*; awarded by Electrotechnical society of the Czech Republic (2014).
- Delong A.: Honourable Doctor Honorius Cause (dr.h.c.), for *Whole life contribution to the electron microscopy*; awarded by the Scientific boar of Technical university, Brno (2014).
- Delong A.: price of the South-Moravian Region for *An entire life’s work in technical science, which remarkably contributed to the good name of the district* (2014).
- Dupák L.: Academic Achievement Award for *The progress in field of electron beam technologies*; awarded by CEEC and FNTS Bulgaria (2014).
- Horáček M.: Main award in Micrographs competition of the International Microscopical Society at IMC 2014 for *Micrograph Raindrop: computer generated diffractive optical performance of fractal originated by e-bem writer* (2014).
- Plešinger F.: Award of Computing in Cardiology-Challenge 2014 for *Development of Robust Detection of Hearth Beats in Multimodal Data* (2014).
- Müllerová I.: Czech Head for *Long and systematic research in electron microscopy*; awarded by the Board of government of the Czech Republic (2014).

- Mikmeková E.: China government scholarship awarded for *Achievements of the scientific results in field of carbon layers*; awarded by Ministry of Education, China (2013).
- Pokorná Z.: Award of the Czechoslovak Microscopical Society for *The best Ph.D. dissertation with the use of microscopical techniques* (2013).
- Mikmeková Š.: *Bursary for the support of the research in the field of Electron microscopy*; awarded by FEI Czech Republic s.r.o. (2013).
- Buchta Z. et al.: Gold Medal at the International Engineering Fair (MSV) for *Automatic contact less calibration of gauge blocks* (2012).
- Číp O. et al.: Siemens Excellence Award (prize for the most important result in development and innovation) for *Automatic contact less calibration of gauge blocks* (2012).
- Müllerová I.: Award of Czechoslovak microscopical society for *The whole life contribution to the electron microscopy* (2012).
- Brzobohatý O.: Award for young sciences of the Czech and Slovak Society for Photonics (2011).
- Frank L.: *Testimonial to contribution to mutual collaboration with Graduate school of Science and Engineering*. Awarded by Dean of faculty of the University of Toyama, Japan (2010).

1.3. Main changes since the last evaluation

Here we briefly summarize the main changes since the last evaluation:

- The organizational structure of the institute was changed from three departments to six and the number of research groups increased to 14 since 2012. Dr. Ilona Müllerová was elected at the new ISI direction since 1st June 2012.
- The ISI ran infrastructure project ALISI which financed the construction of new laboratories and their equipment to pursue new topics. Each ISI team participated in this project.

1.4. Support of the research

Researches are supported by several departments: The Human Resources and Payroll departments (1.5), Economic administration (10.6), Centre of Information Technologies (3.25), the Library (3), Technical department (28), Project Management (5).

Research Report of the team in the period 2010–2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.

Scientific team	Electron microscopy

The research conducted by the electron microscopy department in the given period was concerned with the discovery, development and demonstration of new methods to be implemented in electron microscopes and with the design and application of innovative electron optical elements to extend the capabilities of the commercial instrumentation available in the Laboratories of Electron Microscopy (LEM). The research activities of the team cover scanning electron microscopy (SEM) methods in both their traditional and non-traditional versions such as low-energy and very-low-energy SEM, environmental SEM, ultrahigh-vacuum SEM, cryo-SEM, scanning spectro-microscopy and micro-spectroscopy, quantitative microscopy and in-situ SEM, along with scanning transmission electron microscopy (STEM) at all energies of electrons. The emphasis is placed on the utilization of complete information hidden not only in the total fluxes of the signal species, but also in their energy and directional distributions acquired by means of multiple channel detectors and analyzers of electron backscatter diffraction patterns and energies of emitted Auger electrons, characteristic X-ray radiation quanta and light photons. The general aim was to extend the performance of the available electron beam devices and to pave the way for new microscopic methods in as many branches of materials and biomedicine applications as possible.

Development of methods is enabled by a deep understanding of the field of electron microscopy, electron optics and complementary instrumentation. Having detailed information about electron optical instruments, our insight into the results provided by commercial devices is much deeper than the standard user can ever hope to obtain. Progress in some fields of low-energy SEM, environmental SEM and quantitative microscopy cannot be accomplished using standard instruments, for which reason special electron optical systems were built or extended with innovative optical elements, detectors or sample manipulation assemblies. This would not be possible without a broad background in electron optical design and simulation. Knowledge regarding interaction of the electron beam with the sample is also of vital importance. Our know-how is often expressed in cooperation with scientific and industrial partners in the field of microscopy and microanalysis and in the field of the design of electron optics. Demonstration of the added value of new microscopic methods requires their demonstration in state-of-the-art tasks and samples that we usually share with pioneers in various branches of examination of both living and inanimate matter.

Electron optical design and instrumentation

Electron optical calculations are necessary for any system modification and for correct interpretation of the results. This includes accurate field calculation and calculation of the optical properties of the system and signal electron trajectories. The optical properties of a standard system can be calculated using EOD (Electron Optical Design – commercial program), but knowledge of high-order aberration (correctors) or a large number of electron trajectories is required for more complicated systems containing a general 3D element and analysis of Coulomb interactions, so a more general approach must be used. For these reasons we are developing a set of programs and procedures that utilizes new approaches and procedures for solving these problems.

We implemented a Differential Algebra (DA) method for calculation of the optical properties of a general electron optical system with a straight optical axis. We also developed a new set of methods for calculation of the high-order derivatives of the axial fields and multipole coefficients [ASEP ID 350850] which is necessary for application of the DA method in electron optics. The method was implemented in Fortran and an efficient interface with MATLAB was built which allows its application in the design of the spherical aberration corrector which we are developing with FEI within the project E450EDL. We have recently been working on the implementation of the DA method in EOD.

We devised an aberration analysis method based on real ray tracing and subsequent regression analysis. The method can be used universally with any optical system with arbitrary symmetry, mechanical imperfections and misalignments. It presents an alternative to aberration integrals and the DA method. It has been applied to various problems, such as a LEEM system with a slight sample tilt [ASEP ID 385323], the ion-optical column of a time-of-flight analyzer with a 45-degree tilt [ASEP ID 358590], and parasitic aberrations of a TEM objective lens and their corrections [ASEP ID 421614].

The program for the calculation of the Coulomb interactions in charge-particle beams was generalized for particles with different masses. It was used to determine the optical properties of a bismuth liquid metal ion source [ASEP ID 358593] which was solved as a part of the 3DNanoChemiscope project.

Calculation of signal electron trajectories is necessary for the successful design of detectors and for a deeper insight into the results provided by the electron microscope. These calculations determine which electrons from the emission spectra are detected under a given system configuration. A general tracing routine must be used as the trajectories of the signal electrons can be complicated. Moreover, the detector itself perturbates the axial symmetry of the system. While this is not an issue for the primary beam, the signal electrons are strongly influenced by the general 3D field, for which reason we often use the 3D simulation program SIMION for the calculation of fields and electron trajectories. Sometimes we use a combination of SIMION, EOD and MATLAB.

Monte-Carlo (MC) simulation of the interaction of the electron beam with the sample provides necessary input for understanding the information provided by the instrument. We mostly use third-party software, such as Casino, Penelope and GEANT, particularly its extension to SEM provided to us by FEI. We are able to study the emission of the signal electrons which can then be traced in the electromagnetic field, and the detected particles can be studied primarily on the basis of energy, emission angle and escape depth. We also use MC for the simulation of the X-ray signal used in EDX analysis. The software package MONCA has been developed and is being further improved for the purpose of quantitative imaging in the transmission mode. It gives the exact mass-thickness relationship for specific STEM detector geometry.

The program EOD has been extended with a Monte Carlo module to include the collision phenomena of electrons with the gases in the specimen chamber of the microscope [ASEP ID 358591] in order to understand the signal generation in a gaseous environment. We use the data on cross-sections from the NIST database for ionization for this purpose. Dissociation, vibration, rotation and momentum-transfer cross-sections for water molecules are included in the simulation algorithm. The algorithm covers the effect of elastic collisions and ionization. These effects are vital for obtaining correct results for the signal amplification. We do not include any effects related to generated ions or the secondaries arising from the impacts of BSEs on the microscope parts. The computation starts with a set of electrons with a given initial position, energy and angle. New “environmental” electrons (electrons appearing after ionization collision) are then added to the set. In our experience, it is necessary to trace at least 10,000 electrons for each selected energy and gas pressure if we are to achieve an error of the calculated results of less than 10 %.

Development of detection systems

The information content of signals released by electron impact includes the number of emitted species, the distribution of their directions of emission and the distribution of their emission energies. Traditionally, single-channel detectors have been used in the SEM that acquired only the total fluxes of signal species, merely separated into their slower part (considered as secondary electrons) and their faster part, known as backscattered electrons. The conventional set-up of the detectors of a STEM is composed of multiple coaxial rings of detecting surfaces capable of sorting the transmitted electrons according to the polar angle of their exit from the bottom surface of the sample – most often three segments have been used for the bright field, dark field and high-angle annular dark-field signals. Obviously, these set-ups are far from being capable of completely utilizing the information available.

The output recorded from a SEM standard electron detector is an image that is directly proportional to the signal; usually it is just a qualitative image. We are adding a value to such typical imaging using exact knowledge of the electron scattering of the studied sample as well as precise calibration of the whole detection system. Presently, the main aim of using this method is in the transmission mode of the SEM (also known as low-voltage STEM) where we use the Monte Carlo simulation of electron-matter interactions with the atoms of the specimen that carry information about the number of atoms involved in scattering, hence it allows for quantitative studies of the mass thickness of the specimen. It is then also possible to obtain the total mass of the nanoparticles that would be independent of their shape (this is usual in many analytical techniques) as well as the mass distribution within the specimen. The detection system can be either a commercial STEM detector designed for qualitative imaging or a detector based directly on single-electron counting that is, however, not commercially available for such purposes. We are going in both directions: we use a commercial STEM detector (version 3, FEI) in the SEM Magellan 400L (FEI) and are developing an annular-dark-field detector for the SEM JSM-6700F (JEOL) that should be capable of counting/detecting single electrons that hit the scintillator. In both cases, the exact detection system has to be known, namely the collection angles that are estimated theoretically and proved experimentally. At present, the apparatus is based on the commercial system and is used for the quantitative measurement of mass loss of embedded media ultrathin sections on its aging and various means of protection and imaging conditions (some results have already been presented, e.g. [ASEP ID 436622].

The detection system of the scanning low-energy electron microscope (SLEEM) is composed of five detectors: a spectrometer of energies of the Auger electrons (product from Omicron), a spectrometer of secondary ions (product from Balzers), and three scintillator-based detectors of electrons that have been developed entirely by the team and manufactured at the institute. One detector is situated co-axially just above the sample and also serves as the anode of the cathode lens. The second detector of a narrow acceptance angle is situated to the side of the specimen stage and is movable in two directions in an azimuthal plane passing the optical axis. Its purpose is to analyze the angular distribution of the backscattered electrons (BSE) and to optimize the BSE imaging mode from the point of view of acquisition of crystallographic contrast – this detector was built and installed during the monitored period. The third detector is a retractable, 3D adjustable detector of electrons transmitted through the sample and accelerated in the electric field generated by the sample bias [ASEP ID 379939]. This detector is based on a single-crystal YAG scintillator side-attached to a light-guide made of quartz. It is retractable up to 100 mm and finely adjustable in all three axes from the outside.

The detection systems for Environmental SEM work on the principle of scintillation single crystals and ionisation of gas molecules by signal electrons. Two new detection systems were developed and patented in the evaluated period. The first of these, an ionization detector of secondary electrons (ISEDS) with an electrostatic separator [ASEP ID 372211], uses the suitably situated electrostatic fields of the separators placed above and around the sample for deflecting SEs in the direction towards a positively-biased detection ring electrode located in the field around the specimen. This configuration allows filtering of the high-energy BSEs and detection of the strongly amplified signal of SEs with high signal-to-noise ratio at a small working distance. The original design of the second detection system, a new scintillation secondary electron detector for Environmental SEM [ASEP ID 350812], is based on the emplacement of the single crystal scintillator within a scintillator chamber separated from the specimen chamber by two apertures with small holes in the centre. Biased apertures create an electrostatic lens for the transmission of the signal electrons from the specimen chamber towards the biased scintillator of the detector. This is the world's first detector that allows continual detection of secondary electrons in a pressure range from 0.001 Pa to 1,000 Pa. Both detectors have been experimentally tested.

In the research area of scintillation electron detectors, the experimental device for the study of cathodoluminesce of solids has been significantly improved and expanded, and new methods have been introduced. New materials for electron detectors and/or screens in electron beam devices have been studied with the use of such tools. An automation system for cathodoluminescence (CL) experiments has been developed using the LabVIEW graphical programming environment [ASEP ID 368131] within the framework of improvement of experimental equipment and new method progress. A new specimen chamber with a temperature-controlled specimen holder has been developed and a new method for CL characterization of scintillators applied [ASEP ID 430353]. The new specimen holder enables cooling down to 100 K and heating up to 500 K which allows measurement of CL properties depending on specimen temperature or on a variety of excitation conditions such as excitation energy, electron current (dose) and excitation duration. An original new method for the quality assessment of the scintillation detector, based on mathematical transformation of the time-decomposed image using the detective quantum efficiency (DQE) and modulation transfer function (MTF), has also been developed [ASEP ID 430357]. In addition, the measurement and calculation of the DQE as a function of the spatial frequency for the SEM detector has been described. In this technique, the time-modulated e-beam is used to create a well-defined input signal for the detector.

Decay kinetics of new YAG:Ce single crystal scintillators for electron detectors in S(T)EM have been studied with the intention of eliminating their undesirable afterglow in the context of new materials for scintillators and CL screens [ASEP ID 366953]. The CL decay characteristics of YAG:Ce single crystals of different Ce concentrations, cleaned and annealed under specific conditions, were measured in dependence on excitation pulse duration and specimen temperature. A kinetic model of the cathodoluminescence of the YAG:Ce single crystals was created to interpret the presented results. The effects of quenching impurities and defect centres in YAG:Ce were specified. Some possibilities of decay time and afterglow reductions of the mentioned scintillators were proposed. Upgrade options of a scintillation electron detector for SEM were evaluated focusing on seeking and studying new inorganic materials and finding new technologies for their preparation [ASEP ID 434107]. We also investigated the usability of scintillators for low-energy electrons without post-acceleration, including the effect of the scintillator surface and its conductive cover. It was concluded that commonly used scintillators strongly lose their light yield with the decrease of the incident electron energy. Moreover, a thinner conductive layer on the scintillator surface has to be used to allow low-energy electrons to pass through. The CL study of scintillators based on epitaxial layers was also included. In particular, single crystal layers with partial substitution of Gd and Ga for (Y,Lu) and Al ions, respectively, were investigated as prospective scintillation materials. The detrimental effect of shallow traps was significantly reduced in these epitaxial layers. In addition to scintillation properties, the spatial resolution of CL screens was also studied using theoretical simulation as well as an experimental method [ASEP ID 430366].

Cooperation has been established with FEI (Brno, Eindhoven, Hillsboro) regarding detection principles and particular detectors, primarily within the project of the Centre of Competence: Electron microscopy (2012-2019) supported by the Technology Agency of the Czech Republic. Several new detectors are being prepared within this project for commercial microscopes offered by FEI. Another collaboration on unique sophisticated electron optical instruments and detectors is being implemented with Delong Instruments in Brno.

Microscopic and microanalytical methods

One of the main activities of the team is the development of methods for microscopy and microanalysis of various types of samples, including samples that are difficult to image by standard methods of electron microscopy. These include non-conductive samples such as biomedical samples, samples sensitive to irradiation, wet samples, etc. Two alternative methods are available for the imaging of non-conductive samples, namely non-charging electron microscopy and environmental SEM. We have developed an efficient algorithm for the imaging of sensitive samples for adjusting the current and energy of the primary beam to prevent sample degradation. We apply two complementary methods for wet and biological samples that degrade very quickly in the high vacuum of the standard microscope: cryo–SEM and environmental SEM. The latter of these is the subject of continual development and improvement.

Low–energy SEM and STEM and electron spectroscopy

Traditional electron microscopy worked with electron energies in hundreds of keV in transmission modes (TEM and STEM) and tens of keV in SEMs. The principle of the cathode lens, i.e. biasing the sample under examination to a high negative potential, that was developed by the team in the 1990s enabled a further decrease in the energy of the electrons landing on the sample. FEI has marketed a series of instruments with electron energies as low as 50 eV in collaboration with the team. However, the team maintains its worldwide edge in low-energy electron microscopy and presents both SEM and STEM imaging and measurement down to fractions of eV.

The motivation for lowering the energy of electrons in microscopes follows from the physical laws governing the scattering of electrons in solid samples. Firstly, the rates of all scattering mechanisms grow with decreasing energy, generally as a square, so image contrasts are significantly enhanced, particularly for targets composed of light elements and for weakly manifested phenomena such as internal strains. Secondly, the scattering of incident electrons on atom cores that dominates at high energies is combined with scattering on target electrons and on the complete inner potential distribution so new contrasts emerge and some standard contrasts, such as the crystallographic contrast, are advantageously modified. The third factor appears in the range of units and tens of eV, and consists of the electron wavelength approaching the interatomic distances so wave-optical contrasts originating in electron diffraction and interference can be observed. Finally, at ultralow energies the incident electrons convert themselves into Bloch waves in the sample so their interaction directly responds to the energy band structure of the sample, thereby providing extremely valuable information about crystals.

Reduction of the charging of non-conductive specimens can be optimized if the critical electron energy is used for observation. At this energy the total electron yield is equal to one. When measuring the time development of the image signal from a pixel since its first illumination, the critical energy is then the energy for which the integrated signal change is a minimum. This procedure is implemented in the control software of a computer-controlled SEM in such a way that for a choice of the landing electron energies an assembly of the signal vs. time curves is registered in a lattice of separated pixels and statistically processed. This method reveals the real nanostructure and microstructure of the studied sample without coating artefacts. Recently we have focused on:

- Natural photonic crystals with interesting optical properties present in butterfly wings [ASEP ID 366080, 0385186]
- Imaging of doped structures to reveal the distribution of dopants in semiconductor devices to improve the control of the manufacturing process [ASEP ID 340745]
- Observation of metal nanoparticles in nonconductive materials at an appropriate electron landing energy and low electron beam current has enabled the study of samples without the commonly used metal covering of the surfaces which can lead to a loss of detail or even to the destruction of sensitive samples [ASEP ID 436424, 436427].
- The team has also been cooperating on imaging and sample preparation methodology at room temperature; an immunogold labelling of proteins on natural rubber particle surfaces from Taraxacum brevicorniculatum has been developed for imaging in SEM [ASEP ID 385325], as well as characterization of polycaprolactone film biodeterioration [ASEP ID 436617]. The team has also been involved in structure characterization using a TEM, specifically sieve element occlusion [ASEP ID 385330] and artificial forisomes [ASEP ID 385352].

Several important achievements have been made in low-energy microscopy during the reported period. As regards the SEM, i.e. experiments with imaging by slow electrons reflected from surfaces and measurement of their signals, we might mention the study of identification of the local surface crystallinity on reflection of electrons below 30 or 40 eV. Close above the vacuum level of energy, the energy band structure of a crystalline target already differs from the parabolic bands of (quasi) free electrons, being influenced by the crystal potential. For this reason, the unoccupied electron states above the vacuum level acquire dispersions that are characteristic to the crystal system and its spatial orientation. The reflectance of such slow electrons should, therefore, be inversely proportional to the local density of states coupled to the incident electron wave. This method has been proven on aluminium and copper single crystals and polycrystals [ASEP ID 384097] and would seem to be extremely promising as a possible alternative to the traditional EBSD method as it offers higher lateral resolution and faster data acquisition.

Another important result concerns microscopic imaging of doped areas in semiconductors. It is crucial for the development and diagnostics of nanometer range structured semiconductor devices to have a microscopic method capable of providing reliably quantifiable data showing the local density of dopants. This team began working on this problem in the 1990s and has published a series of papers demonstrating the usefulness of low-energy microscopy in measuring dopant density at a high lateral resolution. We have prepared two series of planar patterned semiconductor structures with four different densities of dopants on a substrate of the opposite type of doping in cooperation with a colleague from the Faculty of Science at Masaryk University and using the faculty's equipment. Both p-type patterns on an n-type substrate and n-type patterns on a p-type substrate have been prepared. The densities of dopants were measured at the ON Semiconductor Czech Republic factory. The samples were carefully cleaned before examination and their crucial feature was that patterns with different dopant densities were treated in an identical way so as to ensure the greatest possible reproducibility of data. One result achieved during the reported period concerns n-type patterns on a p-type substrate examined using threshold and core-level photoemission imaging and measurement in a photoemission electron microscope at the WERA beamline of the ANKA synchrotron in Karlsruhe where we were provided with beamline time free of charge, and also using our own SLEEM. The published results [ASEP ID 340745] unambiguously reveal both PEEM and SLEEM image contrasts monotonically dependent on the doping level and therefore available for reliable measurement of the local density of dopants.

The third result to be mentioned again concerns the acquisition of grain contrast by means of slow electrons, but here a real sample was studied, namely a sample as difficult to crystallographic analysis as an ultrafine grained (UFG) metal subjected to severe plastic deformation using the ECAP method. The UFG copper sample was cleaned in-situ with an ion beam and comparison of micrographs taken on as-inserted and cleaned areas demonstrated the essential influence of this treatment step on the crystallographic contrast in the images. The grain contrast was acquired and measured through the full energy scale from units of eV to units of keV and, when compared with EBSD maps, the ability of these contrasts to identify the grain orientation was demonstrated for the case in which reference data is available for interpretation. The lateral resolution of SLEEM imaging and recognition of grains proved higher than that of the EBSD [ASEP ID 34746]. This paper is co-authored by two colleagues from Brno University of Technology who prepared the UFG Cu sample.

The reported period has, to a large extent, been devoted to the development of low-energy and ultra-low-energy STEM. As mentioned above, the UHV SLEEM operated by the team was equipped with a detector for transmitted electrons in the reported period. Pilot experiments confirmed the possibility of operating the scanning transmission microscope down to units of eV when employing the biased sample principle. The transmissivity of freestanding Au foil around 3 nm thick proved sufficient for imaging down to 10 eV. The first experiments have also been performed with two graphene-like samples, i.e. few-layer CVD graphene provided by various suppliers. The contrast of mutually overlapping flakes of graphene was found to increase steeply with decreasing energy of electrons down to 5 eV, and the contribution of the secondary electrons released close to the bottom surface of the sample and accelerated towards the detector in the sample bias field was also identified and was verified by its suppression by means of the blind central spot (the bore) of the detector.

The global uniqueness of performing transmission microscopy at such low energies led us to publishing two papers in the reported period [ASEP ID 340747, 383741].

From the very beginning of experimentation with the ultralow energy STEM, 2D crystals, and graphene as the most accessible of them, were identified as promising objects for demonstration of the capabilities of the new method. Our next step was to use Raman microspectroscopy for counting the layers of graphene on a commercial CVD sample with the aim of comparing the abilities of this way of counting layers with the possibilities of STEM. Measurements of Raman spectra were made in cooperation with the university in Amiens. Examination of the graphene flakes identified by Raman as single-layer graphene (1LG) revealed an assembly of very tiny flakes and empty holes, unsuitable for measurements of graphene transmissivity. The first rough measurements of electron transmission through graphene provided a local maximum near 5 eV [ASEP ID 395127]. Comparison micrographs were produced by M. Unčovský on FEI premises using the first commercial microscope equipped with a cathode lens as a result of cooperation between FEI and the team.

Armed with previous experience, we embarked on the task of accurately measuring graphene transmissivity across the full energy scale down to 1 eV for 1 to 7 graphene layers. CVD graphene samples of acceptable quality were purchased from a US supplier and the number of layers was again verified with Raman spectra in Amiens. This time, we were able to obtain reliable and correct data regarding the transmissivity which was found at units of eV much lower than originally expected. Moreover, we have also established a side effect consisting of the removal of adsorbed contaminants including hydrocarbon molecules, the source of disastrous carbonaceous contamination, by means of prolonged bombardment with electrons below 50 eV. We consider this result crucial for the methodology of electron microscopy of surfaces as it paves the way to the performance of certain surface studies even outside an ultrahigh vacuum. This result was obtained in the reported period, but the paper [ASEP ID 443322, L. Frank et al., Appl. Phys. Lett. 106, 2015, 13117] did not appear until January 2015.

We ascribe an importance similar to the previous item to pilot experiments with the application of low-energy STEM in biomedicine, namely to the imaging of ultrathin tissue sections. We imaged sections less than 10 nm in thickness received from colleagues at the Biology Centre of the Academy in České Budějovice at various energies in UHV and standard vacuum microscopes. We established the contrast of images growing steeply with the decreasing energy of the electrons. We have established that at hundreds of eV the contrast of samples free of any heavy metals is not only higher, but also reveals all structure details, in contrast to stained samples in which merely details are highlighted where the staining agent has been adsorbed. Moreover, at low electron energies we have found a phenomenon of partial depolymerization of the resin in which the tissue is embedded and evaporation of monomers at a greatly enhanced rate, making the sample significantly more transparent for slow electrons. Due of this effect, we were able to acquire micrographs down to tens of eV, although the best performance was found between 500 and 1,000 eV. This work was conducted in the evaluated period, though the paper appeared in January 2015 [ASEP ID 436878, L. Frank et al., Ultramicroscopy 148, 2015, 146].

Finally, we might mention our priorities in introducing low-energy and very-low-energy electron microscopy techniques that are internationally acknowledged, for which reason we are often asked to submit review papers written either with an emphasis on the electron optical aspects of the method or on pilot applications, mostly in the materials science. Two large reviews were published in the reported period [ASEP ID 358595, 385193].

Cryo-SEM

Cryo-SEM prevents sample degradation by very fast freezing (a kind of physical fixation) and imaging the sample at a very low temperature. The resolution obtained can be extremely high, though its limit depends not only on the electron optical properties of the microscope, but primarily by the frozen sample that is usually extremely beam sensitive. 3D analysis and quantitative microscopy can also be used. A multifunctional external cryo-high-vacuum chamber equipped with sputtering and coating devices and a freeze-fracturing system (ACE600, Leica Microsystems) was purchased in the year 2013 for cryo-SEM. The Magellan 400 SEM (FEI) was equipped with a cryo-stage. The ACE600 is compatible with the cryo-high-vacuum-shuttle VCT100 (Leica Microsystems) such as the Magellan SEM that allows the transfer of the sample between ACE600 and SEM without leaving the high vacuum and very low temperatures.

The applicability of the cryo-SEM method is shown in the imaging of highly hydrated samples such as microbial biofilms – specifically in the visualization and characterization of yeast culture structure and extracellular matrix [ASEP ID 436621,384149, 421774, 441095]. The idea of this research was to find the optimal imaging method and to select a sample preparation protocol that allows a result approaching the native state. It can thereby help describe in detail the structure of yeast biofilm, whose presence deteriorates the healing of infection in patients, and to quantify its amount under varying environmental conditions, thereby proving helpful in finding more effective treatment. Several methods of sample preparation for SEM and cryo-SEM were performed and compared. Cryo-SEM is an excellent technique for imaging liquid and semi-liquid materials in which nanometric details are to be studied. In addition, it is the only means of sample preparation that does not result in artefacts affected by chemical preparation techniques. Various cryo-fixation protocols were tested (plunging into liquid nitrogen and liquid ethane, high-pressure freezing (HPF). The amount of biofilm was investigated using a freeze-fracturing technique (physically breaking apart (fracturing) a rapidly frozen biological sample) where structural detail exposed by the fracture plane is then visualized. An optional step, involving vacuum sublimation of ice, may be carried out after fracturing. Freeze-fracture is unique among electron microscopic techniques in providing planar views of the internal organization of membranes or biofilms. Deep etching of rapidly frozen samples permits visualization of the surface structure of cells and their components. Images provided by freeze-fracture and related techniques have profoundly shaped our understanding of functional morphology. The samples investigated were cultures of the yeast *Candida parapsilosis* and *Candida albicans, Staphylococcus epidermidis* bacteria and various mixtures of them that were cultivated on different substrates depending on the freezing technique. Our results show that fixation by freezing with plunging caused the growth of ice crystals (a sponge-like structure). These crystals are responsible for biofilm destruction. On the other hand, fixation by HPF caused a relatively smooth structure without any ice crystals. We conclude that fixation by HPF for one-day cultures is more appropriate under the selected conditions than fixation with the use of plunging, and that cryo-SEM can be an extremely effective imaging method for investigating biofilm structure.

Cryo-SEM is going to be improved in various directions and extended in the transmission mode for both standard imaging and qualitative imaging. The first experiments in cryo-sample preparation for STEM imaging have already been performed for polymeric nanoparticles in aqueous dispersions of copolymers [ASEP ID 429252].

Environmental SEM

The Environmental SEM provides slightly reduced resolution, but samples can be imaged in a physical condition close to their native state. In the Environmental SEM, the samples can be observed in a wide range of pressure conditions, from a vacuum (comparable to the SEM) to high pressures of various gases (over a thousand Pa) in the specimen chamber. In high-pressure conditions, electrically non-conductive wet samples can be observed without a conductive coating, and their natural fully hydrated surface structure is preserved. The pressure of water vapour in the specimen chamber of the Environmental SEM, along with the specimen temperature, plays a crucial role in obtaining and maintaining the state of thermodynamic equilibrium between the environment of the specimen chamber and the sample itself. At a thermodynamic equilibrium of 100 % relative humidity, the dependence of saturated water vapour pressure on specimen temperature must be monitored. A small change of the sample temperature at a stable water vapour pressure can be used for study of the transition between hydration and dehydration phenomena. Other interesting and extremely useful “in situ” dynamical experiments, such as the study of sample solidification, dissolution, chemical reactions, etc., can also be realized.

The team is equipped with an Environmental SEM – ESEM AQUASEM-II. It was designed and realized by our team. During the evaluated period, the Peltier-cooled specimen holder and a hydration system for precise control of water vapour flow into the chamber were developed, designed and implemented. Moreover, a new hydration system has been specially developed to maintain the precisely controlled wet environment in the specimen chamber of the microscope. The hole in the BSE detector simultaneously acts as a pressure-limiting aperture that restricts the gas flow and allows the length of the primary electron trajectories in the gas to be minimized. The microscope can also work in high and low-vacuum modes. In a low vacuum, the secondary electron signal is detected by an electrode deposited on the input surface of the single crystal detector. Additionally, our ESEM AQUASEM II is equipped with the ISEDS described above and a scintillation detector of secondary electrons. An integral part of all types of detectors working on the electron-gas ionization principle is a very fast and low-noise signal amplifier.

Our research focuses on the invention of new methodology and instrumentation for the study of a wide range of samples above and beyond the capabilities of commercial instruments. The possibility of precisely controlling thermodynamic conditions in the vicinity of the sample and the function of our prototype detector and ionisation detectors with a specially shaped detection electrode deposited on the scintillator surface have been presented in our papers [ASEP ID 333986]. The morphological changes of susceptible biological samples were studied and evaluated under dynamically changing conditions between the liquid and gas state of water leading to controlled dehydration of the sample. The results of these and many other various experiments allow us to improve our microscope, detectors and methods.

A new methodology based on an unconventional initiation procedure of ESEM AQUASEM II chamber pumping, free of purge-flood cycles (generally used in commercial microscopes) and on the ability of advanced control of thermo-dynamical processes close to the sample has been published. The gradual and gentle change of the working environment from air to water vapour enables the study of not just living samples in dynamical in situ experiments and their manifestation of life (sample walking), but also experimentally simulated physiological reactions. Moreover, Monte

Carlo simulations of primary electron beam energy losses in a water layer on the sample surface have been studied, and the influence of water thickness on radiation, temperature and chemical damage to the sample was consequently considered [ASEP ID 397947].

The application possibilities of our microscope for the study of chemical reactions in dynamically changing conditions were also published in the Langmuir journal [ASEP ID 431976]. We used ESEM AQUASEM II for the study of uranyl ion-containing brine layers on ice surfaces at environmentally relevant temperatures (above 250 K) and pressures (below 700 Pa). A uranyl salt indicator was used to obtain high-contrast Environmental SEM images in the electron backscattering mode to resolve small-scale features such as grain boundaries filled with brine, and at the same time to acquire information about uranyl ion chemical speciation in a frozen solution using fluorescence spectroscopy.

Research Report of the team in the period 2010–2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.
Scientific team	New technologies

The Team of New Technologies (abbr. NT) consists of three research groups: the Thin Layers group, the Electron Beam Technology group and the Electron Lithography group. Technologies brought in practice by these groups have a long tradition in the ISI: the electron beam processing technologies began in 1963, the electron lithography technologies in 1978 and the deposition of thin coatings starts in 1987. This long term experience in the narrow field permits both excellent mastery of the mentioned techniques and, afterwards, required the design of our own instruments: an electron–beam lithography system in 1982, micro electron–beam machine MEBW 60/2 in 2007, and a sputtering system in 2012. During the evaluated period our team took part in a number of funded projects and industrial research. We have also supported other teams at the Institute of Scientific Instruments with technologies we have mastered.

Multilayer systems with low interface roughness and high repeatability of bilayer thickness

<u>Creating and characterization of nanostructures with soft x–ray lasers</u>

Project supported by AVČR (code KAN300100702) from 2007-01-01 to 2011-12-31, investigator: Institute of Physics, ASCR, co–investigator: ISI (J. Sobota).

Project objectives: The goal of this fundamental research project was to use state–of–the–art high repetition rate sources of coherent soft X–ray radiation (10–50 nm) to generate and probe nanometric structures on solid surfaces. This qualitatively new topic was aimed at studies of interaction of high–intensity soft X–ray radiation with matter where conventional XUV/X–ray sources are not feasible.

NT role: We carried out the optimization of deposition parameters and subsequent deposition of advanced multilayer Sc/Si, Mo/Si a C/Si XUV mirrors with low interface roughness not surpassing 0.3 nm. Tolerance of the bi–layer thickness of a multilayer system has to be better than 0.2 nm. We have also tested temperature stability of these multilayer systems.

Grant agency assessment of the results: Experimental stations were implemented to study the interaction of intense X–rays with matter. Amongst the achievements are ablation nanostructuring, ablation at intensities 10^{17} Wcm^{-2}, a new type of X–ray wavefront sensor, and advanced XUV multilayers.

More at http://www.isvav.cz/projectDetail.do?rowId=KAN300100702

<u>Nanometrology using scanning probe microscopy methods</u>

Supported by AVČR (code KAN311610701) from 2007-01-01 to 2011-12-31, investigator: Czech Metrology Institute, co–investigator: ISI (J. Lazar).

Project objectives: The project was focused on the development of scanning probe microscopy methods focused on the following fields: 1) development of very precise interferometric measurement methods in nanoscale, 2) traceability of the nanostructures dimensions and forms measurements, 3) quantitative measurements of mechanical properties of thin films in combination of micro–indentation, nanoindentation and scanning probe microscopy, 4) theoretical analysis of the probe–sample interaction effects in scanning probe microscopy and nanoindentation measurements.

NT role: The New Technology team was part co–investigator's team. We prepared a wide range of accurate Mo/Si, Sc/Si, Ni/C and C/Si multilayer samples having both different material compositions and different periodicities to perform SThM conductivity mode calibration, resolution estimation and uncertainty components evaluation. In the course of this project we adapted a plasma etcher, available in ISI, and managed the technique of cross section preparation of nanometer size multilayers.

Grant agency assessment of the results: Set of tools for traceability in small dimensional, force and local mechanical properties measurements was developed, including metrological SPM with six–axis interferometric system, nanoindentation reference samples and calibration procedures.

More at http://www.isvav.cz/projectDetail.do?rowId=KAN311610701

Self–organized growth and structure transitions of nanocrystals

Supported by GAČR (code GAP204/11/0785) from 2011–01-01 to 2013-12-13, investigator: Charles University Prague.

Project objectives: In this project we studied the self–organization of two types of technologically relevant nanoparticles (Ge and Fe_2O_3/TiO_2) in an amorphous matrix (mainly SiO_2) using X–ray scattering methods (X–ray diffraction, small–angle scattering) and other experimental methods (EXAFS, Mössbauer spectroscopy, magnetometry, AFM, TEM), both ex/situ and in situ during magnetron deposition.

NT role: We contributed, as a contracted partner, to solve this project by preparing precise structures – cobalt or germanium nanocrystals embedded in amorphous silicon oxide layer (see paper ASEP #0399613).

GACR assessment of the results: V. The team reached a number of valuable results in growing and in the characterization of nanocrystalline structures of Ge/SiO_2, Fe_2O_3/TiO_2/SiO_2. The results were published in twelve papers in international journals with impact factor.

More at http://www.isvav.cz/projectDetail.do?rowId=GAP204%2F11%2F0785

Impact testing of the coating substrate system

Preparation and modification of DLC coatings for machinery application

Supported by MPO (code: 2A–1TP1/031), from 2006-11-15 to 2010-12-31, investigator: HVM Plasma Ltd, co–investigator: ISI (J. Sobota).

Project objectives: This project was aimed at the research of modified DLC coatings and the technology of the coatings' characterization. It was targeted mostly at the tribological parameters improvement, testing the coatings interaction with their environment, cost reduction of lubrication and restriction of the detrimental environmental effect of lubricants. Another aim of the project was to match the measuring procedures to testing standardization.

NT role: We tested five variations of DLC coatings using an impact tester developed at the Institute of Scientific Instruments. We revealed that humidity could significantly influence not only the tribological behavior of the carbon–based coatings but also unexpectedly alter their impact resistance. By increasing the relative humidity the value of dynamic impact resistance increases more than three times. Results were published in Diamond and Related Materials (see ASEP #0366083).

Grant agency assessment of the results: The authors contributed to significant improvement and implementation of measuring methods and innovative deposition techniques of doped DLC coatings. Furthermore, a strong cooperation between industrially oriented HVM plasma and academic institutions was established.

More at http://www.isvav.cz/projectDetail.do?rowId=2A-1TP1%2F031

<u>Deposition of thermally stable nanostructured diamond–like coatings in dual frequency capacitive discharges</u>

Supported by GA ČR (code GA202/07/1669) from 2007-01-01 to 2011-12-31, investigator: Masaryk University, Faculty of Science, co–investigator: ISI (J. Sobota).

Project objectives: The aim of the present project was to develop a new deposition system based on the application of dual–frequency capacitive plasma (DFCCP) for preparation of thermally stable nanostructured diamond–like carbon (NDLC) films. Complex diagnostics and computer simulation of the DFCCP was used in order to understand the processes of the film growth. The NDLC films were extensively studied from the point of view of their structure (RBS, ERDA, TOF ERDA, HRTEM, SEM, etc.) as well as properties (micro– and nanoindentation, ellipsometry, spectrophotometry, etc.).

NT role: We tested nanostructured diamond–like coatings deposited using dual–frequency capacitive plasma using an impact tester developed at ISI. Structure of the deposited coatings was optimized using the results of the impact tests.

Grant agency assessment of the results: The project was excellent, both in terms of expertise and in terms of disbursement of funds.

More at http://www.isvav.cz/projectDetail.do?rowId=GA202%2F07%2F1669

Deposition of coatings with defined optical and electric characteristics

<u>Effective coating technology of thin passivation and antireflection layers for the production of crystalline solar cells</u>

Supported by MPO (code FR–TI1/603) from 2009-10-31 to 2013-09-30, investigator: Solartec s. r. o., co-investigator: ISI (J. Sobota).

Project objectives: The aim of the project was the development of a new technology making use of plasmo-chemical reactions to the effective crystalline surface cleaning, hydrogenation, deposition of passivation layers and antireflection coatings for solar cells with efficiency above 17%.

NT role: We carried out plasma etching experiments of solar cells using a mixture of argon and hydrogen to substitute wet etching by hydrofluoric acid. To achieve optimal passivation and optical properties of thin transparent oxide and nitride coatings, we optimized deposition technology of Al_2O_3 sputtered from aluminium target, SiO_2 sputtered using silicon target in argon/oxygen mixture, and SiNx sputtered using silicon target in Ar/N_2 plasma. We carried out a series of depositions combining SiO_2 and SiN_x

coatings to reach the colored effect of photovoltaic cells. We succeeded in increasing solar cell efficiency by more than two percentage points. Moreover we developed colored solar cells without a loss to their efficiency. This gives Solartec Company a significant competitive advantage on the market.

Grant agency assessment of the results: The results of this project are: proven technologies for plasmo–chemical cleaning, sputtering of thin dielectric antireflection and passivation layers on surfaces of crystalline silicon solar cells dedicated for aesthetic application.

More: http://www.isvav.cz/projectDetail.do?rowId=FR-TI1%2F603

The influence of the near field to heat transfer by radiation at low temperatures

Supported by GAČR (code: GA14–07397S, running project), investigator: ISI (Tomáš Králík)

Project objectives: We proposed an experimental study of heat transfer in the near field between plane parallel metallic surfaces at temperatures from 5 to 60 K and at distances from 1 to 200 micrometres including the previously unexplored influence of the transition to the superconducting state. We obtained absolute values of the heat transfer in a range of at least four orders of the magnitude. The data was compared with the existing theory for heat transfer by near field by using results of BCS theory of superconductivity and electrical characteristics of the samples.

NT role: The New Technology team was part of the team of investigators. During the first year of the project we prepared radiofrequency magnetron sputtered tungsten coatings with low roughness on sapphire substrates to measure FF absorptivity. The reverse sides of the samples were provided with thin Al and Cu layers. Al layer reflects radiation while Cu pads served as contacting electrodes for capacitive measurement (see ASEP #30437857, #0368238 and #0385282).

More at http://www.isvav.cz/projectDetail.do?rowId=GA14-07397S

Separation of Nanoparticles by High–Resolution Microthermal Field–Flow Fractionation

Microfluidic separation techniques were massively introduced during the last decade, including Microfluidic Field–Flow Fractionation (FFF). Nowadays, Microthermal FFF is experimentally implemented and used for many practical separations. An important field of Microthermal FFF applications is a very precise characterization of nanoparticles (see ASEP #0431682).

We repeatedly asked for grant support, but we didn’t succeed. In spite of this, we are convinced that this topic is worth investigating and we support it using institutional money.

Electron beam lithography and technology

The main focus of the e–beam lithography group was on the research related to the micro and nanostructuring of planar thin layers using an e–beam writer with both the variable shape beam and the Gaussian beam. Two projects formed the framework for this topic. Among several conference contributions, a monographic publication on the practical issues of the e–beam lithography was prepared (ASEP #0423948).

Optimization of electron–beam production technology

Supported by MPO (code FR–TI1/576), from 2009-07-01 to 2013-06-31, investigator Optaglio (Polívka Leoš), co–investigator ISI (Kolařík Vladimír).

Project objectives are optimization of the production flow in electron–beam lithography and its mastering. It is important improvement of the actual technology.

MPO assessment of the results: The goals and material contents of the project under this subject were fulfilled within the extent of the defined terms.

More at http://www.isvav.cz/projectDetail.do?rowId=FR-TI1%2F576

Advanced Microscopy and Spectroscopy Platform for Research and Development in Nano and Micro Technologies – AMISPEC

Supported by TA ČR (code TE01020233) from 2012-03-01 to 2019-12-31, investigator: BUT FSI (Šikola Tomáš), co–investigator: ISI (Lazar Josef).

Project objectives: The project forms a R&D platform bringing together three companies and two research institutions in the CR which will be aimed at 1) building complex scientific instruments and 2) finding methods and procedures for application in fabrication and characterization of functional nanostructures and semiconductor materials/devices. The second activity will result in technologies and analyses for high–tech applications both for the professional and public community ((bio)sensors, security elements, etc.).

TA ČR assessment of the results: the project is ongoing.

More at http://www.isvav.cz/projectDetail.do?rowId=TE01020233

Diffractive devices and masks

Several applications of the lithography originated structures were investigated. Here we mention two of them: the diffractive concentrator for solar panels, and the phase photolithography mask for fiber Bragg grating sensors.

Photovoltaic panel of a new generation

Supported by MPO (code FR–TI1/574) from 2009-01-01 to 2011-12-31, investigator: Optaglio (Baše Radek), co–investigator: ISI (Horáček Miroslav).

Project objectives: The project was focused on an effective conversion of solar energy to electric energy. Photovoltaic panels were based on an internal diffractive concentrator and on a silicon chip with a reduced area. The roadmap of the project was based on a bottom–up solution of photovoltaic elements, cells and panels.

MPO assessment of the results: The project ran according to the defined goals. Part of the expected results were achieved.

More at http://www.isvav.cz/projectDetail.do?rowId=FR-TI1%2F574

A new method of measurement for the containment construction of the nuclear power station, Temelin to guarantee safety in case of catastrophic accidents.

Supported by MV ČR (code VG20132015124) from 2013-04-01 to 2015-12-31, investigator: ÚJV Řež, a.s. (Vomáčka Petr), co–investigator: ISI (Mikel Břetislav).

Project objectives: This project was oriented to research the optical method of measurements of the consistency of nuclear power plants. The research of the measurement technique was optimized for industrial operation with long–time measurement of the containment profile changes. Signals were processed by advanced digital techniques to maintain high resolution and accuracy for the lengthy time measurements.

MV ČR assessment of the results: the project is ongoing.

More at http://www.isvav.cz/projectDetail.do?rowId=VG20132015124

Electron microscopy related topics

The team members focused on the finalization of the forming apparatus for the electron sources, on the research in the field of carbon nanotubes for electron sources, and on the methodology of assessment for the stability and homogeneity of shaped electron beams. Selected results: Modified knife–edge method for current density distribution measurements in e–beam writers (ASEP #0397634); Coincidental imaging system with electron optics (ASEP #0365927).

<u>Coherent imaging of nanostructures in a low–energy scanning electron microscope with an area detector of electrons.</u>

Supported by GA AV ČR (code IAA100650803) from 2008-01-01 to 2010-12-31, investigator: ISI (Horáček Miroslav).

Project objectives: The contrast mechanisms based on the difference in the distribution of signal electrons recorded for every point on the surface of the bulk sample in very–low energy scanning electron microscope were studied. The signal electrons escaping from the specimen were processed using suitable electrostatic electron optics and imaged on a multichannel planar detector. The method was demonstrated on the observation of diffraction contrast on clean surfaces, on the quantum size contrast on thin films with different thicknesses, and on the contrast on semiconductors with differently doped areas. For the experiments, we used a newly designed optical system with the cathode lens for deceleration of primary beam electrons to a very–low energy, a Wien filter for the deflection of signal electrons away from the optical axis and electrostatic transfer lenses. For the acquisition of signal electrons a thinned back–side directly electron–bombarded CCD was used.

GAAV assessment of the results: The very–low energy SEM was newly designed, the experiments with angular distribution of the signal electrons were done, the new method of the specimen bias control in the cathode lens was implemented, the modified Schottky electron emitters were made.

More at http://www.isvav.cz/projectDetail.do?rowId=IAA100650803

<u>Electron microscopy</u>

Supported by TA ČR (code TE01020118), from 2012-03-01 to 2019-12-31, investigator: FEI Czech Republic, s. r. o. (Unčovský Marek), co–investigator: ISI (Müllerova Ilona).

Project objectives: The project aims at introducing in electron microscopes novel techniques responding to needs of nanotechnologies, development of advanced materials, and the application of knowledge acquired in biology and medicine. This concerns the imaging of crystals including 2D ones, non–conductive surfaces, imaging the wave–optical contrasts, etc. Novel materials, technologies of their processing and novel components of the scanning electron microscopes will be developed, accompanied with adequate methodology.

TA ČR assessment of the results: the project is ongoing.

More at http://www.isvav.cz/projectDetail.do?rowId=TE01020118

Glass sealed feedthroughs

We develop glass sealed vacuum and pressure electrical feedthroughs for vacuum instruments, technology equipment, nuclear research, aviation and other industries. The feedthroughs are matched kovar–glass or compression seal type. We recently created several types with different footprints, conductor counts and sizes, and flange types. For example we are able to produce nine conductor feedthroughs compatible with industry standard D–subminiature connectors, high–frequency impendence matched quad SMB connector or military standard MIL–C–5015 14S–2P connector at ISO–KF flanges (see Fig. 1). Temperature ranges of some types of feedthroughs can extend from -196 °C (liquid nitrogen temperature) to more than 400 °C. The majority of components are developed for general-purpose applications but some are tailored for specific purposes (e.g. nuclear plants or aircraft instrumentation (avionics)).

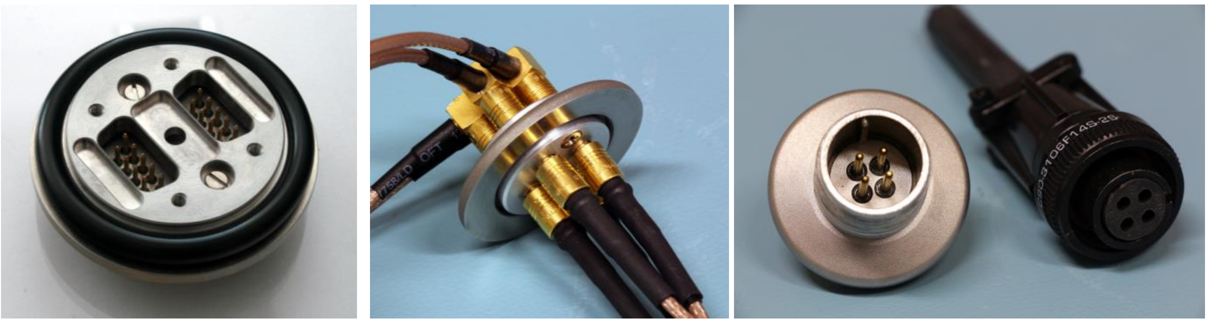


Fig. 1 Glass sealed vacuum feed–through. Two D–subminiature nine–conductor connectors at ISO–KF 40 flange (left), quad SMB at ISO–KF 25 (middle), MIL–C–5015 14S–2P at ISO–KF 25 (right).

Metal joining technologies

Our team is equipped with three electron beam welders (built completely in–house) and two vacuum brazing furnaces. We are able to produce vacuum tight joints of metal parts made of steel, stainless steel, copper alloys, titanium alloys, zirconium alloys, high temperature resistant superalloys like Inconel and many others. We can also weld and/or solder various combinations of these materials.

We provide these technologies for our team but also for colleagues from other ISI teams and external customers. We are able to develop custom joints and, if requested, we also produce a batch for evaluation of a new joint type. Our team cooperates with more than fifty industrial partners from different industries like automotive, nuclear research, heavy machinery, aviation, etc.

Theese are some recent examples of successfully developed joints:

- Electron-beam-welded zirconium-alloy tubes for creeping tests for nuclear research;
- Vacuum brazed stainless steel valve seal for automatic steam condensate drain;
- Inconel superalloy to stainless steel e–beam weld for automotive turbochargers;

- E–beam welded hollow titanium alloy shaft for very high speed turbine helium liquefier;
- Vacuum brazing of thermocouples into steel shaft for experimental rolling mill.

Instrumentation R&D

<u>Electron beam welding machines</u>

The electron beam processing technologies in the Institute of Scientific Instruments began in 1963. Use of the e–beam welding for research activities led to the development and construction of several welding machines in subsequent years. The recent micro–electron–beam machine MEBW–60/2 was finished in 2007 and is now produced by the German company FOCUS GmbH under ISI's licence. Besides the research and development of the welding equipment the welding technology was continuously improved according to ISI and industrial partners' requests. The development of the MEBW continues on both sides.

In 2014, a new version of the electron gun ET–60/2 V1.3, which is the electron generator of the MEBW, was finished and built (see Fig. 2). The recent improvements deal mainly with operator safety and comfort, gun functionality and production optimization. Modifications of the mechanical design were performed by team members, as was the final assembly and testing of the e–guns . For testing, a complete methodology was prepared which comprised the verification of several e–gun parameters like maximum output power, sustainability of high voltage, beam quality, beam deflection system performance and detection of possible X–ray leakage. More information can be found in ASEP #0437482.

Fig. 2 The electron beam welder MEBW–60 at the International Engineering Fair in Brno in 2010 (left). The welding electron guns developed for MEBW–60 welder (right).

<u>New generation of electrochemical sensors and biosensors using thin modified DLC layer</u>

Supported by MPO (code FR–TI1/118) from 2009-05-01 to 2013-04-30, investigator: BVT Technologies, a.s., co–investigator: ISI (V. Neděla).

Project objectives: The project's aim was to develop technology for the production of electrochemical sensors whose working electrode was modified by DLC (diamond–like carbon) layer, which contain fluorine, boron or other elements.

NT role: The New Technology team was an important part of the co–investigator's team. We constructed the sputtering system for deposition of BDLC coatings (see Fig. 3). The sputtering system is equipped by mass flow controllers enabling precise measurement of gas flow, an accurate Baratron vacuum gauge, one magnetron 76 mm in diameter of our own construction, LabView software for deposition process control, turbomolecular and diaphragm pump vacuum system, and a 13.56 MHz RF power supply. The substrate holder can be grounded or DC biased. We deposited a number of modified DLC coatings to discover the proper combination of properties suitable for electrochemical sensors and biosensors.

Grant agency assessment of the results: The project enabled us to expand currently available printing technology of active layer, sintering active layers and inserting active layers of electrochemical sensors, other technologies – sputtering active layers.

More: http://www.isvav.cz/projectDetail.do?rowId=FR-TI1%2F118

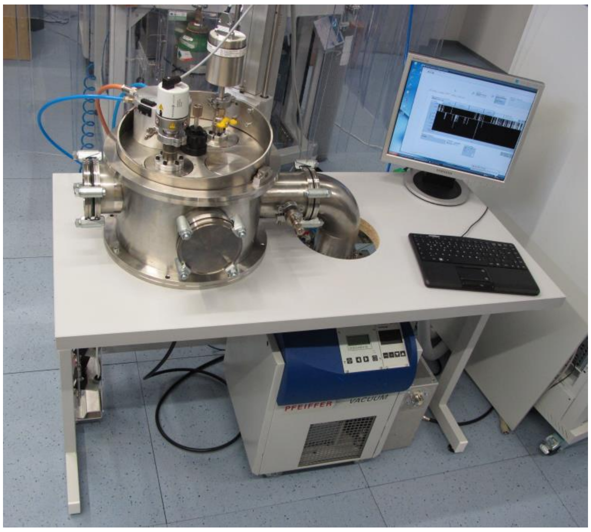


Fig. 3 Sputtering system developed in cooperation with BVT Technologies, a.s. (see project FR–TI1/118).

Special electronics design

XXX

<u>High resolution capacitance meter with remote sensing</u>

For experimental study of the near–field heat transfer (see above *The influence of the near field to heat transfer by radiation at low temperatures*) a special low capacitance measurement device was developed and built. The capacitance meter has to deal with stray capacitances of the interconnecting cables and feedthroughs. For this reason the active shielding technique was applied. The capacitance meter was designed as a stand–alone electronic unit with up to four independent inputs with an input range of 4500 pF. The resolution is about 10 fF. The sampling frequency is adjustable from 1 to 30 readings per second. The sensitivity to ambient temperature changes is less than 20 ppm/K. For direct reading, the measured value is displayed on the built–in LCD or can be transferred to a control system via serial interface (see ASEP #0385282 and #0368238).

Other electronics equipment

Recently we developed several instruments for our internal usage. A brief information list is as follows:

- Accurate multi–channel resistance meter for determination of thermal coefficient and aging of high ohm resistors;
- Thermostatic oven for environmental test of electronics;
- Programmable low noise preamplifier which can be used as an add–on for oscilloscope, frequency analyzer or similar instrument which enhances the particular instrument’s voltage sensitivity by a factor of up to 1000.

Research Report of the team in the period 2010–2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.
Scientific team	Magnetic resonance and cryogenics

For the team of Nuclear Magnetic Resonance and Cryogenics, years 2010-2014 were a period of profound changes, whose explanation may provide useful context. Administratively, the team in the current structure arose in 2013 after the group of Medical Signals had split off from the Department of Magnetic Resonance and Bioinformatics. This was a consequence of long-term divergence of research interests inside what used to be the Department of NMR since 1950s. The process of specialization started in the 1990s, when the department gradually departed from the development of prototypes of commercial NMR systems and the electronics and cryogenics specialists were freed up for research. This history still stands behind the unified coexistence of the NMR and the Cryogenics groups, which now act nearly independently: they share no staff, clients, projects, and publications. The cryogen service provided by the Cryogenics Group to the NMR Group is a rare point of contact. Therefore, the following reports will treat each group distinctly.

Nuclear Magnetic Resonance

During 2010-2014, the NMR Group followed the trend started already in the 1990s, which was research and development of methods for magnetic resonance imaging and spectroscopy for *in vivo* applications in animals and humans. The group entered the evaluated period equipped with a **4.7T/200mm MR scanner** suitable for the measurement of small laboratory animals – mice or rats, but without past animal research background. The age structure was imbalanced, junior researchers were underrepresented. The 4.7T scanner, based on a magnet made by Magnex Scientific Ltd. (UK) in 1990s, had its electronics partially upgraded by the purchase of an MR Solutions Ltd. (UK) console in 2007 and by the acquisition of a new 1000W broadband RF power amplifier in 2010. Before 2010, the scanner was used for imaging of tissues and biological samples from occasional local collaborators, among whom the emerging interest in **testing nanoparticles** (then iron oxides) intended for diagnostics or as therapeutic drug markers could be observed. Another major direction, **MRI measurement and pharmacokinetic modelling of perfusion**, started to be explored thanks to a new team member, Radovan Jirik, whose interest and expertise in this area had roots in his previous work at the University of Bergen (Norway). **Spectroscopy**, the traditional local strength, struggled experimentally with system instability, but thanks to the support by a European FP6 Marie-Curie grant FAST (2007-2010), the NMR Group could join, in 2006, the pan-European activity for the development of spectroscopic quantitation software jMRUI, and particularly its spin system simulation segment. These three areas have remained the dominant focus of the group's research till now, though several new areas started to be explored, such as MR characterization of **diffusion** and **multiparametric / multimodal fusion**.

There were three substantial leaps forward in the evaluated period:

- the acquisition of a modern high-field MR scanner in 2012,

- the establishment of a small modern animal use facility in 2013, and
- radical team rejuvenation.

All these changes could happen only thanks to the substantial support from the **ALISI** grant (MEYS CZ.1.05/2.1.00/01.0017, 2009-2013).

In 2010, the productivity and impact of work with the occasionally unstable MR system was limited, but, fortunately, the preparation of the purchase of a new modern scanner could start. In 2010, a provisional **animal use facility** was established and certified according to the Czech regulations, which allowed the group to start *in vivo* mouse and rat measurements in the 4.7T scanner. In squeezed interim conditions of 2010-2013, the first animal experiments in ISI, mostly related to perfusion and nanoparticle research, were performed. Their global impact was modest but they made it possible to train new staff and start several internal and collaborative projects.

During 2010-2011, the construction of new laboratories and the preparation of a public tender for a new high-field scanner and animal care equipment were under way, taking – unfortunately – a lot of productive capacity. Due to the Public Contracts Law, its controversial interpretation, and the complexity of an experimental NMR scanner, the **9.4T/300mm scanner** (Bruker Biospec with Avance III and high-power gradients of up to 660 mT/m) was installed as late as towards the end of 2012 and applied in research as of 2013. The construction of the **animal facility** required extensive on-the-fly project adjustments in 2010-2012 in order to take into account the growing needs of biological researchers, who got attracted to the arising imaging capacity by pilot studies and intense scientific advertising of the new possibilities among local research biologists. As a result, the facility, capable of accommodating about 200 mice and 100 rats in a virtually barrier-protected environment, was certified in summer 2013. Specific-pathogen-free animals can be accommodated in individually ventilated cages, they are protected by the hygienic loop, air filtration and UV sterilization, air overpressure, and other equipment and procedures. Physiologic monitoring, thermal stabilization, and inhalation anaesthesia with isoflurane have been established as the standard routine for all in vivo MR measurements. In 2014, the license for handling **genetically modified organisms** was obtained for a specific transgenic mice model of Parkinson's disease.

During 2010-2014, the **team was rejuvenated** by the acceptance of several promising pre- and postgraduate students. A newly hired veterinarian-pharmacologist and an animal care specialist started to provide the guarantee of animal service quality, were able to take over standardized measurements, and facilitate interfacing between the mostly technically/physically trained NMR Group members and biological researchers. The direct contact between biologists and physicists proved to efficiently contribute to mutual education and has formed a solid foundation for executing the mission for which the team is best predisposed – to support contemporary evidence-based biological research into pathophysiology and pharmacology by relevant data. Such orientation has not precluded providing support for other scientific branches (e.g. geology – one pilot study in progress since 2014, plant genomics – one pilot study in 2013, …) within limits imposed by the available capacity and equipment. Some further potential application areas and clients were identified but not directly addressed (construction industry – specialized concretes, food industry – product characterization).

Once legally possible, the animal-based research moved fully to the new laboratories in 2013. Numerous pilot studies started and some gradually evolved during 2013-2014 into more serious studies motivated by practical biomedical research interests of **numerous collaborators** from universities (Masaryk University Brno, Palacky University Olomouc, Veterinary and Pharmaceutical University Brno, Mendel University Brno, University of Patras, University of Barcelona) and research institutes (Veterinary Research Institute Brno, Institute of Macromolecular Chemistry Prague, Institute of Analytical Chemistry Brno, Institute of Animal Physiology and Genetics Brno, St. Anne's University Hospital – ICRC Brno). Much of the experimental work was aimed at **Parkinson's disease**, **schizophrenia**, pharmacology in general (**perfusion**), and assessment of prospective **theranostic carriers** with imaging markers represented by gadolinium, or iron or manganese oxides. Other projects (e.g., research of the pathophysiology of subarachnoid haemorrhage and septic encephalopathy, spinal cord regeneration by cell therapy) are still open after some studies and pilot tests were concluded in 2014. In these collaborative pilot studies, a suitable **collaboration modus operandi** has evolved in which the NMR team actively educates the clients, helps them identify experimentally accessible targets, participates in experiment design, sample preparation, optimizes or develops suitable measurement techniques, performs the measurements, evaluates the primary data and assists in result interpretation.

All these collaborations, performed under economically friendly conditions, established an informal **open-access environment**, in which the ISI's interests were exercised by project selection and economic tools. In general, the **principal aim** was to support applications of MR for research into pathophysiology of important diseases or injuries, and pharmacological development including targeted biomarkers and pharmaceuticals. To fully utilize local research potential, the NMR Group teamed up with the MR team of Masaryk University – CEITEC's core facility MAFIL to form a regional biomedical imaging node, now preapproved for the membership in ESFRI project **Euro-Bioimaging** (negotiations since 2010), and aspiring for funding from the **Czech-Bioimaging** project (submission 2014), which has now entered into the negotiation phase.

The timeline outlined above explains the relatively few **published results** in recent years; most projects were just starting and substantial capacity was bound by the construction. The works started to reach maturity towards the end of 2014, which is reflected by the number of papers submitted and accepted for publication in recent months. Four papers [1-4] have been accepted, three [5-7] have been submitted to international journals, while other two have been submitted to Czech journals [8-9]:

[1] E Carenza, O Jordan, P San Segundo Martinez, **R Jirik**, **Z Starcuk jr.**, G Borchard, A Rosell, and A Roig. *Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells.* Accepted to J. Mater. Chem. B (IF not assigned yet, parent journal: Journal of Materials Chemistry (1991-2012), IF=6.626), 2015. http://pubs.rsc.org/en/content/articlelanding/2015/tb/c4tb01895h#!divAbstract

[2] M Mezl, **R Jirik**, V Harabis, R Kolar, M Standara, K Nylund, O H Gilja, and T Taxt. *Absolute Ultrasound Perfusion Parameter Values of a Tissue Mimicking Phantom Using Bolus Tracking*. Accepted to IEEE Trans. Ultrason. Ferroelectr. Freq. Control (IF=1.503), 2015.

[3] **J Kratochvila**, **R Jirik**, M Bartos, M Standara, **Z Starcuk jr.**, and T Taxt. *Parametric Multi-Channel Blind Deconvolution in Dynamic Contrast-Enhanced MRI*. Accepted to Magnetic Resonance in Medicine (IF=3.398), 2014. In press.

[4] S Schafer, K Nylund, F Svaeik, T Engjomd, M Mezl, **R Jirik**, G Dimcevski, O H Gilja, and K Tonnies. *Semi-automatic motion compensation of contrast-enhanced ultrasound images from abdominal organs for perfusion analysis*. Accepted to Computers in Biology and Medicine (IF=1.439), 2014. In press, available online http://www.sciencedirect.com/science/article/pii/S0010482514002716.
[5] **O Macicek**, **R Jirik**, M Bartos, T Taxt, A Sprlakova-Pukova, M Kerkovsky, **K Bartusek**, and **Z Starcuk jr**. *Interleaved DCE and DSC-MRI for Perfusion Analysis*. Submitted to Magnetic Resonance Imaging (IF=2.022).
[6] G Røsland, E Eskilsson, K Talasila, S Knappskog, O Keunen, A Sottoriva, G Solecki, T Taxt, **R Jirik**, S Fritah, P Harter, R Zehtab, J S Piccirillo, I Spiteri, P Euskirchen, G Graziani, M Lund-Johansen, P Ø Enger, F Winkler, S Niclou, C Watts, R Bjerkvig, and H Miletic. *Distinct evolution and functions of wild-type EGFR and its mutant EGFRvIII in glioblastoma development*. Submitted to Cancer Discov. (IF= 15.929), under second review.
[7] T Taxt, T Pavlin, R K Reed, F-R Curry, E Andersen, and **R Jirik**. *Using Single-Channel Blind Deconvolution to Choose the Most Realistic Pharmacokinetic Model in Dynamic Contrast-Enhanced MR Imaging*. Submitted to Appl. Magn. Reson. (IF=1.152).
[8] **L Grossová**, **E Dražanová**, R Kopáčik, J Bednařík, **R Jiřik**, **Z Starčuk.** *Použití metody ASL pro sledování vývoje septické encefalopatie na myších*. Nové směry v biomedicínském inženýrství. Brno: Společnost elektrotechnického inženýrství, 2014. s. 9-15. ISBN: 978-80-214-5049- 3.
[9] **E Dražanová**, **L Grossová**, J Pistovčáková, A S Khairnar, R Demlová, T Kašpárek, **Z Starčuk jr.** *Využití MRI metody ASL v hodnocení změn mozku u POLY I:C modelu schizofrenie u potkanů*. Psychiatrie, 2015, roč. 1/2015, 1/2015, s. 37. ISSN 1211-7579.

The principal scientific results have been selected and described elsewhere (including the team’s contribution). Here a unifying view will be provided.

MR spectroscopy was an area with limited publication output; as a specific and above-the-standard technique, it was never the highest priority of current collaborators, most capacity was aimed at imaging. Nevertheless, the algorithmic development associated with the above-mentioned FP6 project FAST, was continued until it gained funding again by an FP7 project TRANSACT, executed by a consortium of 10 top European research partners and 4 commercial partners (incl. Siemens and Philips). The ISI’s team was recognized as having a unique combination of theoretical NMR expertise, experimental background and computer programming abilities. By a decision of the jMRUI-developing consortium, our team member (Jana Starcukova) was appointed the **coordinator of jMRUI software development** in 2012. The ISI’s quantum-mechanical simulation of coupled spin systems (module **NMRScopeB**, ASEP 0437477) became the chief tool for the preparation of basis signals for metabolite quantitation, and due to its unique flexibility and ease of use it has a good chance to become popular also for method development and education once properly published. In the capacity of coordinator, our team is responsible for the software presentation at major annual conferences (recently at ISMRM 2010 Stockholm, 2011 Montréal, 2012 Melbourne, 2014 Milan, ESMRMB 2013 Toulouse), on the web (www.jmrui.eu) and for the organization of educational courses for users, whose number now approaches 3000 worldwide. Recent and current work in this area aims to provide enough robustness and quality to metabolite quantitation to make spectroscopy applicable not only in biomedical research, but also in medical diagnostics and pharmaceutical development. Development of new features was, however, suspended in 2014 by the need for detailed revision and bug fixing of other groups’ software functionality.

Perfusion is an important process responsible for the transport of oxygen, nutrients, and signal molecules by the blood through the capillary bed to each somatic cell. Its abnormalities are recognized as important markers of cancer (angiogenesis, necrosis) and its treatment (antiangiogenesis), but perfusion also stands behind all other systemic processes and is important for targeted delivery of imaging markers and therapeutic drugs. Unlike the conventional approach – to identify image intensity abnormalities, our work has been focused on improving quantitative determination of pharmacokinetic model parameters. Several models have been proposed so far, which are – as phenomenological models of the physiology of heterogeneous tissues – only approximative. The model developed in the ISI is believed to provide better physical relevance; it is described by spatial maps of several parameters, whose identification is the subject of the measurements and complex postprocessing. The developers of these algorithms utilized the medical expertise of several collaborators (Univ. of Bergen, Masaryk Memorial Cancer Institute Brno); it started with data acquired externally, but gradually the focus shifted to the measurement of animal models in the 9.4T scanner at ISI. Originally, only dynamic contrast enhanced (DCE) methods based on T_1-weighted MRI were studied; later the scope was extended to contrast-enhanced T_2-contrast based method DSC (dynamic susceptibility-weighted) and the native method of ASL (arterial spin labelling). The published results (ASEP 0368977, 0385359, 0431811) proposed improvements in the model and calculation, tested the confidence areas of the model parameters, and described the results of phantom-based validation and cross-validation by comparing estimates of parameters shared by at least two different methods. Similar models were applied to ultrasound (US) imaging (ASEP 0421676, 0421683, 0421688), thus contributing to multimodal validation / substitution. This work showed that systematic errors existed and further work towards standardization was needed.

Besides relaxometry, perfusion techniques started to be applied for in vivo **testing of prospective carriers** (polymers or nanoparticles – dextran capsules, liposomes) of drugs and imaging markers (based on Gd, Fe or Mn oxides). These experiments, testing specific particles (molecules, nanoparticles) in the blood stream, were preceded by extensive in vitro MRI tests in home-built phantoms. Some work tasks in this area were summarized towards the end of 2014 and condensed in article manuscripts which are already in press or have entered the review process.

Diffusion measurement has gained a specific role in the examination of neural tissues. By applying optimized standard diffusion-weighted MRI methods and public software, we executed a series of measurements as parts of animal model validation in Parkinson's disease and two models of schizophrenia. With the assumption of non-restricted yet anisotropic water molecule mobility, diffusion tensor imaging (DTI) was applied. Finally, it was compared experimentally with the more general technique of diffusion kurtosis imaging (DKI). Unpublished simulation results from the end of 2014 laid the foundation for better understanding of the physical reality probed by these techniques and elucidated some conceptual difficulties to be addressed in future. The practical measurements and animal model preparation were accomplished in the ISI in collaboration with MU CEITEC's teams of neurology and pharmacology (PD, 2 long-term secondments in ISI) and MU University Hospital's department of psychiatry (schizophrenia).

Most of these programs could be classified as **quantitative MRI**, which could be seen as the central component of all our work. To complete the survey, two more techniques have been explored and applied: **MR relaxometry** has been applied for the visualization of chemical reaction in geological gels (2013-2014), and to the study of the properties of specific gels for electrochemistry (2013), and a **3-point Dixon method** for fat/water quantitation was demonstrated to be feasible in the 9.4T scanner in 2014. Avoidance of artefacts and improved accuracy of quantitative images are the common denominator of several other results (ASEP 0350811, 0367300, 0366067, 0385190), which should ultimately lead to meaningful multiparametric **image fusion** for both human diagnostics and biomedical research.

In summary, the period of 2010-2014 could be seen as the period in which the NMR team capitalized on historical spectroscopic success and established an important base for translational research of other, local as well as remote, institutions. The personnel hiring and educating strategy managed to develop a specific bio-physical support unit providing service for biomedical research along with self-propelled refinement of measurement methods, with a specific focus on perfusion and spectroscopy.

Cryogenics

Fundamental research

Natural convection is a complex type of turbulent flow occurring on many length scales across the Universe. At large scales (high Rayleigh numbers), for example, it controls the weather through atmospheric and oceanic flows, terrestrial magnetic field, continental drift, and solar or Jupiter flares. Although the equations describing turbulent flow are known, our ability of prediction, especially for very intense convection, is very limited or even absent. The ideal laterally-infinite **Rayleigh-Bénard convection** (RBC) serves as a model for fundamental studies of these flows. The unsettled issue in study of RBC is the existence of the ultimate scaling regime theoretically predicted by Kraichnan in 1962. The transition to the ultimate regime is commonly believed to lie in the range of Rayleigh numbers $10^{13} < Ra < 10^{14}$. The efficiency of the convective heat transfer, described by the Nusselt number via the $Nu(Ra,Pr)$ dependence, should then reach the scaling law $Nu \sim Ra^{0.5}$.

Several years ago, the discrepancy between various experiments motivated us to build a cryostat with a **helium cryogenic experimental cell** with the height $L = 0.3$ m and diameter $D = 0.3$ m (aspect ratio $G = D/L = 1$) with particular effort to minimize the influence of the cell structure and materials on the observed convection (Grant Agency of the Academy of Sciences of the Czech Republic, Grant No. KJB200650902, “Elucidation of fundamental questions in turbulent convection”). Using our new large cryogenic cylindrical cell (ASEP 350813), arguably the best of its kind in regard to various corrections connected with finite thermal conductivity of sidewalls and plates (ASEP 391089), we have studied RBC at Ra numbers of up to 10^{15}. We have obtained valuable experimental results and knowledge about the convective heat transfer efficiency by RBC and, additionally, about the large-scale circulation (LSC) by detection of temperature fluctuations inside the cell.

As regards the **heat transfer efficiency** study characterised by Nusselt number Nu, our aim was to elucidate the disagreement in the published results on $Nu(Ra)$ scaling between various experiments at Rayleigh numbers Ra of up to 10^{15}. In our work (ASEP 368244) we have shown full agreement among all $G \simeq 1$ cryogenic experiments (Brno, Grenoble and Trieste) by applying suitable sidewall corrections, for Ra of up to about 10^{11}, while at higher Ra all these sets of data differ considerably (Czech Science Foundation, Grant No. P203-12-P897, “Cryogenic Rayleigh-Bénard convection at Rayleigh numbers above 10^{11}”). Our experimental data for $Ra > 10^{12}$ does not meet the Boussinesq approximation due to temperature dependent fluid properties; as we have demonstrated by our data, at *Ra* of 10^{12} up to 10^{15}, the non-Oberbeck-Boussinesq (NOB) effects lead to significant changes in *Nu*(*Ra*) scaling (ASEP 383815, 398058). To eliminate NOB effects we have proposed a new method for data evaluation. In the first step of our new method we show that for $10^{12} < Ra < 10^{15}$, the Nusselt number closely follows $Nu \sim Ra^{1/3}$, which corresponds to theoretical models. In the second step of our analysis we show that evaluation of *Nu*(*Ra*) scaling on the basis of NOB data can lead to incorrect conclusions that the ultimate regime has been reached (ASEP 430597). Observation of transition to the ultimate regime has been claimed several times. Our analysis strongly suggests that such claims are not justified and that the observed transitions are spurious, caused by the NOB properties of the working fluids used in the experiments. We believe that consistent application of our new method of data processing will lead to unification of all contradictory data. Our findings have led to very important physical results which have been published in two papers in the high-impact physical journal Phys. Rev. Lett. (11 citations in total).

The statistical properties of **turbulent Rayleigh-Bénard convection** were investigated experimentally in a cylindrical cell of aspect ratio one. We specifically analyzed the large-scale circulation of RBC based on measurements of temperature fluctuations by small Ge sensors placed inside our cell. The resulting dependencies of Reynolds numbers on Rayleigh number within six orders of magnitude of *Ra* of up to 2.10^{14} were identified and compared to available theoretical models and experimental results for similar geometries. The results have been serially presented at international conferences and published in the respective proceedings.

The experimentally observed effect of the **anomalous heat transfer** against the temperature drop via the two-phase liquid-vapour system of cryogenic helium has been described and published in a prestigious journal (ASEP 397714). The phenomenon was investigated with the use of our Rayleigh-Bénard convection cell specially designed to minimize the influence of its structure on the convective flow studied. The technical properties of our cell were crucial for the observation of this effect.

All theoretical investigations were done in cooperation or consulted with colleagues from Charles University in Prague.

Our next aim was directed towards **thermally excited electromagnetic near field** (NF), which is responsible for van der Waals forces and radiative heat transfer enhanced beyond the validity of Planck's law. In connection with the development of applications of micro- and nanoscale objects, NF interactions have attracted attention of theorists and also experimenters during last two decades. The reach of thermally generated NF is on the scale of micrometres at room temperature and tens of micrometres at 30 K. Relevant experimental results are by far not as numerous as theoretical papers. Under a supported project (Grant Agency of the Academy of Sciences of the Czech Republic, Grant No. IAA10065080) we constructed a precise apparatus (ASEP 368238, 386093) enabling measurement of NF radiative heat transfer between plane parallel surfaces (geometry advantageous for direct comparison with theory but highly difficult for experiment) and compared the results with the theoretical model (ASEP 385282). Owing to the well-defined geometry and material properties of our sample surfaces (produced by magnetron sputtering at our institute) and previous experience with measurement of radiative heat transfer at low temperatures, we obtained experimental data in a wide range of temperatures and distances. The range of transferred heat power was unique, covering four orders of magnitude, starting from far field and exceeding 100 times the black-body limit.

Applied research

Since thermal radiation presents one of the basic modes of heat transfer, comprehensive knowledge of **thermal absorptivity and emissivity** are important for calculations of heat flows in cryogenic devices. Although detection of weak thermal radiation of low-emitting metallic samples at cryogenic temperatures presents a challenging issue, our apparatus allows us to determine both total hemispheric emissivity and absorptivity of highly reflecting surfaces at temperatures ranging from 320 K down to 10 K.

Our group focused on the issue of the **influence of condensed water** on absorptivity properties of metallic surfaces at cryogenic temperatures (ASEP 352298, 351426). We prepared *in situ* very thin layers of condensed water and found that the coating with a thickness of 85 nm increases the absorptivity of a cold aluminium surface almost twice. Thus, surface contamination by volatile deposits can substantially affect thermal radiative properties of the exposed surfaces and increase the heat load of cryogenic devices.

We also evaluated the emissivity and absorptivity of samples with various types of **surface finishing and coatings** in the temperature range of 40 K - 300 K (ASEP 426241, 437370). Our research will enable us to optimize the production and the surface finishing of some parts of a cryostat designed for space operation with respect to its required thermal radiative properties.

In the last work (ASEP 434010) we used our experience, gained over 15 years, with measurement of total hemispherical emissivity and absorptivity of various materials at temperatures ~20 K - 300 K, to assess individual factors influencing their thermal radiative properties. The effects of the material and its purity, finishing and of protective coatings or accidental layers (water, oxidation) on the thermal radiative properties were analyzed. Summing up the results, it can be concluded that chemical treatment (polishing, etching) provides surfaces with the lowest absorptivity and without accidental impurities. In addition, a thin layer of water ice on the surface can have the same or stronger influence on the absorptivity enhancement than the natural oxide layer. Likewise mechanical stress applied on the surface by cutting tools or by abrasion has significant impact on the absorptivity, which could be further reduced by annealing.

Nanotechnology is a hot issue and has become a subject of fundamental research of all world-leading universities and research institutes. The application potential is huge, and hence the need for superior technology, characterization tools and related unique products is growing. Our team is engaged in the AMISPEC (Advanced Microscopy and Spectroscopy Platform for Research and Development in Nano and Microtechnologies) project, which is supported by the Technology Agency of the Czech Republic (Grant No.TE01020233, 2012-2019). This project forms a research and development platform bringing together three Czech companies and two research organizations, all of them are well established on the topic of the proposal. The goal of the project is to build an ultrahigh vacuum scanning electron microscopy (UHV SEM) and a scanning probe microscopy (UHV SPM) modular system (**UHV SEM/SPM**) for *in situ* fabrication and characterization of nanostructures and surface analysis. It will also include complex systems combining more technological and analytical techniques (e.g., SEM/FIB, SEM/SPM, SEM/nanoprobes).

We have developed the low temperature parts of an UHV scanning probe microscope (SPM), working at variable temperatures in the range of 20 K - 700 K. To achieve the required temperature range, a flow cooling system using liquid helium (~5 K) or liquid nitrogen (~77 K) as coolants was designed. A new helium/nitrogen flow cryostat is used for cooling, which allows low-loss transfer of cryoliquids from a Dewar vessel to the SPM system placed in a vacuum chamber. The cryostat consists of an inlet and an outlet, heat exchangers and copper strands (braids) for the thermal connection between the heat exchangers and both the sample holder/SPM and the radiation shield around the SPM. A compact sample holder support with low thermal conductivity and high mechanical stiffness was developed and experimentally tested in the vacuum chamber. The thermal and mechanical criteria are fulfilled by using three sets of silica glass balls as a mounting of the sample holder. Every set consists of four balls placed in vertices of a tetrahedron. Low thermal conductivity of the four-ball supports (FBS) is based on the small contact areas between the glass balls (ASEP 395139). Such a solution has the lowest thermal conductance of all feasible supporting structures. The unique low conductive pad (InBallPad) for thermal insulation of a sample holder of a variable temperature UHV SEM/SPM is characterised by low thermal conductance with heat flow of 140 mW between the top plate at 25 K and the bottom plate at 290 K, and by high mechanical stiffness of 1.0×10^{6} N/m. The dimensions of the sample holder with a total mass of 34 g are small - 30mm diameter and 12mm height.

Important for the team is the membership (one person since 2003) in the **COMPASS** Collaboration at CERN (Project NA58, http://wwwcompass.cern.ch/). This membership brings the team not only additional experience in the field of cryogenics but also in the wide field of materials technology. During the period of 2010-2014 one member of our team worked in the COMPASS Polarized Target (PT) Group with a 10% part-time job. The contribution to the collaborative results corresponds to this percentage value. The PT group is responsible for the development and installation of the polarized target and also for the control of the target during the whole time of the experiment. The low-temperature polarized target (solid ^{6}LiD or NH_3 target cooled by dilution refrigerator, two superconducting magnets for target polarization, NMR system for polarization measurement and very high frequency dynamic polarization system) is the heart of the COMPASS experiment and has major influence on the quality of many results obtained in the field of particle physics. During the evaluated period a set of experiments to study hadron spectroscopy and structure of hadrons was performed and important results were published (e.g. ASEP 351513, 351515, 385194, 390817, 428202, 431897 and others).

In summary, the period of 2010-2014 was very important for the Cryogenics Group for its future scientific progress. We have successfully expanded our scientific interest into two new areas of cryogenics research – cryogenic natural convection and cryogenic near-field effect. These areas will be the most important tasks in our group for several subsequent years.

The Cryogenics group has received substantial support from the Czech Ministry of Education, Youth and Sports via grant ALISI (2009-2013), No. CZ.1.05/2.1.00/01.0017. With this support we could install a new helium liquefier and other parts of a closed helium recovery system in 2013. The vast reconstruction suspended our possibilities to perform cryogenic experiments for more than half a year, but this new installation ensures us long-term independence in experimental cryogenics research, which is a great advantage in view of the permanent liquid helium price growth.

Research Report of the team in the period 2010–2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.

Scientific team	Medical signals

The MEDISIG (Medical Signals) department is the smallest department in ISI at the level of one research team. MEDISIG has dealt with measurement and signal processing in medicine for a long time. Twenty years ago, we began a widespread cooperation with medical workplaces.

Our main goal is to develop new methods and technologies for effective treatment in human medicine. In our opinion, the data acquisition and technologies, which carry new information, is absolutely crucial - thus the proposal of measuring protocols, high-tech technology for measurement coupled with targeted methodology and signal analysis. As an example we can mention the new technology of an ultra-high-frequency ECG. For more than a hundred years, the ECG was measured and interpreted in the same way. The details of an ECG curve are the scope of the extensive, serious and long-term discussions. However, the basic information from which it comes is still the same.

Our team structure includes predominately the engineers (P. Jurak, J. Halamek, V. Vondra, F. Plesinger, I. Viscor, J. Chladek, P. Klimes), who carry out the development of new technologies, provide experiments, design and test new methods and interpret the results. An important part of our team is made up of students in doctoral study programs (both internal and external). We very closely cooperate with the medical workplaces and universities. This cooperation is absolutely crucial to meet our objectives.

The primary goal of our work is, the use of the last achievement of the technology in medicine for basic research and for the clinic. We try to find the optimal targeted link between high level measurements, measurement protocols, methods of analysis and diagnostic and physiological contribution. We maintain a close cooperation with the private company M & I Prague.

MEDISIG team research includes three principal areas:

- blood circulation and hemodynamic control
- heart electrophysiology
- deep brain electrophysiology

In each area we perform both basic and clinical research.

1. Blood circulation and hemodynamic control

The important parameters describing hemodynamics are blood pressure, heart rate, stroke volume (SV) and pulse wave velocity (PWV). While heart rate and blood pressure can be measured easily, measurement of SV is complicated and inaccurate. There are no sufficiently reproducible and noninvasive methods. One important parameter used to analyze vessel stiffness is PWV. The increased speed of pressure wave propagation is closely related to reduced arterial compliance. Using this parameter we can analyze dynamic changes in peripheral resistance and refine SV estimation with the help of heart rate and blood pressure.

A completely new possibility for hemodynamic diagnostics is provided by the newly developed multichannel, whole-body impedance monitor, MPM. The MPM provides the time course of pressure related waves from the whole body at a single moment. Moreover, it is able to take measurements during exercise or various respiratory or body position maneuvers with very good signal quality.

Main team contribution:

Complete technology design and construction, experimental measurement management and data evaluation, analysis and interpretation. Since 2010 we have been constructing an experimental device that is able to measure whole-body high-quality impedance signals simultaneously with 12-lead ECG, blood pressure and heart sounds. Such data had not previously been available. Today we're able to conduct detailed analyzes of mutual coupling. The multichannel whole-body bioimpedance monitor (MPM) uses three independent current sources operating at different frequencies – three current generators with adjustable frequency and amplitude (filed US patent).

During 2013 and 2014 we developed and realized a new MPM monitor designed not only for research and experimental use but also for clinical purposes (www.medisig.com). The MPM monitor includes certification as a medical device. It underwent clinical examination and can be used for diagnostics of the cardiovascular system. From our perspective, this is a totally unique result. Certification was very difficult, and it was necessary to establish the clinical significance of MPM and verify outcomes by other methods. It was especially difficult because it was the first measurements of the pressure wave propagation technique in the whole body (up to 18 locations) simultaneously with different hemodynamic load.

Outcomes: The multichannel whole-body bioimpedance monitor technology certified as medical device, 1 IF article, 1 accepted IF article.

Patent: Device for blood flow property measurement and method of its connection US 20110237966 A1, https://www.google.com/patents/US20110237966

Cooperating institutions: International Clinical Research Center, St. Anne's University Hospital, Brno, M&I Prague company.

Fig. 1 MPM monitor (http://www.medisig.com), Head up tilt table test, medical device certificate

2. Heart electrophysiology

Cardiac electrical activity involves two important phases – depolarization and repolarization. Depolarization is associated with the mechanical action during systole, repolarization with the following relaxation. Malfunction of these two phases can lead to heart failure. Our team focuses on studying the static and dynamic properties of heart depolarization and repolarization.

2.1 Heart repolarization – QT interval

Cardiac repolarization is measured by the QT interval on the surface electrocardiogram (ECG) and has been measured and analyzed for about a century. Its prolongation is associated with a higher risk of cardiac death and arrhythmias. The QT interval is highly dependent on heart rate (RR intervals) and many correction methods have been suggested to compute corrected QT (QTc). The QTc means the QT interval value that corresponds to 60 beats per minute (bpm). QTc is used as the basic parameter in patient diagnosis and drug testing, but its contribution is limited. Limitations may be given by the correction method used and that QTc is a static parameter. Dynamic parameters of QT/RR coupling are mostly neglected, despite the fact that Torsades de Pointes are typically initiated by a variable sudden change in heart rate.

Main team contribution:

Our focus on repolarization analysis began in 2006, when we started to analyze drug tests from the Mayo clinic, NM, USA. The recommended Bazett or Fridericia corrections produced debatable results and on the basis of our ECG measurements with different heart rate excitation, the dynamic model of QT/RR coupling was designed. The model is based on an optimized ARMA model, without any preliminary assumption about dynamic dependency of QT/RR coupling. The optimized QT step responses fully agree with physiological QT step response. All static and dynamic QT properties (QTc, QT memory, QT restitution and time constant of QT adaptation) may be analyzed with this model. Other dynamic QT/RR models were based on mathematical assumption about QT/RR dependency (e.g. exponential weighting), optimized step response is some approximation of physiological QT step response and QT restitution is fully neglected. The model was used in the following studies: QT hysteresis elimination, irregularities of QT/RR coupling, dependency of repolarization parameters on sex and type of heart rate stress, QT parameters in long QT subjects, coupling between QT/RR slope and QTc, linearity/nonlinearity of QT/RR coupling.

Various aspects of QT/RR relationship have been investigated. For an analysis of the whole set of statistical and dynamic parameters of QT and QT/RR relationship, the original and patented method (WO/2008/146168, PCT/IB2008/002198) was developed. The patented description of QT/RR relationship contains five parameters: QTc corrected to the dynamic characteristics of QT and hysteresis; QT/RR gain for slow RR variability ($Gain_S$) - the parameter is equivalent to “QT/RR slope” but does not require a long term stabilized measurement; QT/RR gain for fast variability ($Gain_F$) - the parameter describes immediate change of QT during a change of RR; time constant of QT adaptation to a change of RR (τ) and QT variability independent of RR (QTvar). There is no other analysis that can provide this complete set of parameters.

The most important contribution of our method is the implication of dynamic parameters during analysis of QT/RR relationship. Two parameters ($Gain_F$ and τ) describe newly analyzed physiological characteristics of QT interval.

Testing of the method was performed on selected groups (healthy, hypertonic, metabolic syndrome, patients with pacemakers) and on Holter recordings from LQT THEW database.

We provided complete experimental study management, data recording, processing and analysis. We developed custom software for robust detection of RR and QT intervals during exercise allowing the application of the theory of QT/RR dynamic coupling.

Outcomes: 3 IF articles

US and EU Patent: Method of ventricular repolarization analysis
https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2008146168&recNum=1&tab=PCTDocuments&maxRec=&office=&prevFilter=&sortOption=&queryString=

Cooperating institutions: International Clinical Research Center, St. Anne's University Hospital, Brno, Mayo Clinic, Rochester, MN, USA (S. Asirvatham, V. K. Somers); THEW, University of Rochester, NY, USA, (J. P. Couderc); Department of Medicine, Division of Cardiology, Hôpital du Sacré-Coeur, Montréal, QC, Canada; (Paola A Lanfranchi); University of Milan, Milan, Italy, (N. Montano, E. Tobaldini).

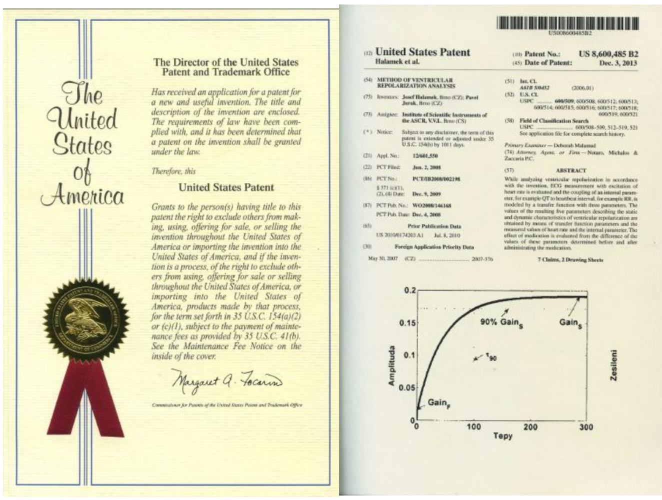


Fig. 2 U.S. Patent – Method of Ventricular Repolarization Analysis

2.2 Heart ventricular depolarization

The myocardial structural changes caused by acute ischemia or cardiomyopathy and conduction system abnormalities affect almost 20% of the adult population. These severe heart diseases reduce cardiac efficiency, worsen cardiac mechanics and often lead to heart failure (HF).

Conventional 12-lead ECG devices create most of the important basic measurements for heart pathology differentiation. Clinical criteria for left ventricular (LV) dyssynchrony quantification are based especially on the QRS complex duration and morphology. Unfortunately, a robust tool for LV dyssynchrony diagnosis, widely acknowledged by cardiology specialists is still missing. The reason may be due to the fact that body surface ECG, acquired and interpreted the same way for more than a hundred years, has nothing new to offer.

Main team contribution:

Entirely new possibilities can be provided by a high dynamic range ultra-high frequency ECG (UHF-ECG 250–2000 Hz) followed by new analytical procedures. Currently, to our knowledge, there are no studies describing the UHF-ECG.

During 2014 we designed methods which were able to precisely identify very weak electrocardiographic potentials, to enable ventricular dyssynchrony quantification and to research the physiological background of UHF information.

The UHF-ECG is based on a top-level acquisition system, with a high dynamic range (24 bit) and high frequency range. Such an acquisition system allows us to analyze weak signals of QRS complex up to 2000 Hz, that are about 100 dB below QRS signals analyzed in commonly used frequency band up to 100 Hz. Signals in higher frequency bands represent completely new information about depolarization. They represent detailed information about action potential distribution and about myocardial homogeneity. UHF-ECG creates a challenge for the application of new signal processing methodologies, hitherto unused in the diagnosis of cardiac diseases and ventricular electro-mechanical research.

Basic hypothesis of UHF-ECG:

- The action potentials of heart ventricles contractive cells during the depolarization phase generate specific energy when sodium channels open. This energy is characterized by a sharp change in voltage and current, and acts as an ultra-high-frequency transmitter. UHF-ECG technology is capable of measuring these very week potentials (tens of nV). The energy of these potentials is more than a million times smaller than the energy of LF ECG. The evolution of these potentials in the chest leads can localize spatial and temporal distribution of the electrical depolarization activation of left and right ventricles.

- UHF-ECG provides more accurate spatial localization of the signal source due to lower tissue impedance at higher frequencies and simultaneously more precise temporal resolution in milliseconds.

- The physiological content of UHF-ECG completely differs from ECG. The UHF-ECG provides unique information about the electrical depolarization temporal-spatial distribution. This information might help to identify ventricular dyssynchrony, structural changes of the myocardium in ventricles, identify patients for CRT and even more CRT settings optimizations.

- While depolarization initiates myocardial cell contraction, the UHF-ECG is the only method that creates a direct link between electrical and mechanical heart activity. Both experimental and clinical outcomes are extremely important. Figure 4 shows a unique comparison of UHF-ECG electrical depolarization map and the left ventricle motion measured by speckle tracking echocardiography.

The team's main contribution covers the complete design and implementation of new technologies. Including: a high dynamic acquisition system, new software for the analysis of UHF-ECG, interpretation of results and diagnostic applications.

Outcomes: UHF-ECG technology, UHF Solver software, 2 national patents pending (2013, 2014), 1 PCT patent pending (2014), one submitted publication.

Cooperating institutions: International Clinical Research Center, St. Anne's University Hospital, Brno, Mayo Clinic, Rochester, MN, USA (S. Asirvatham); M&I

Prague company, Cardion company, IKEM – Institute of Clinical and Experimental Medicine, Prague (D. Wichterle);

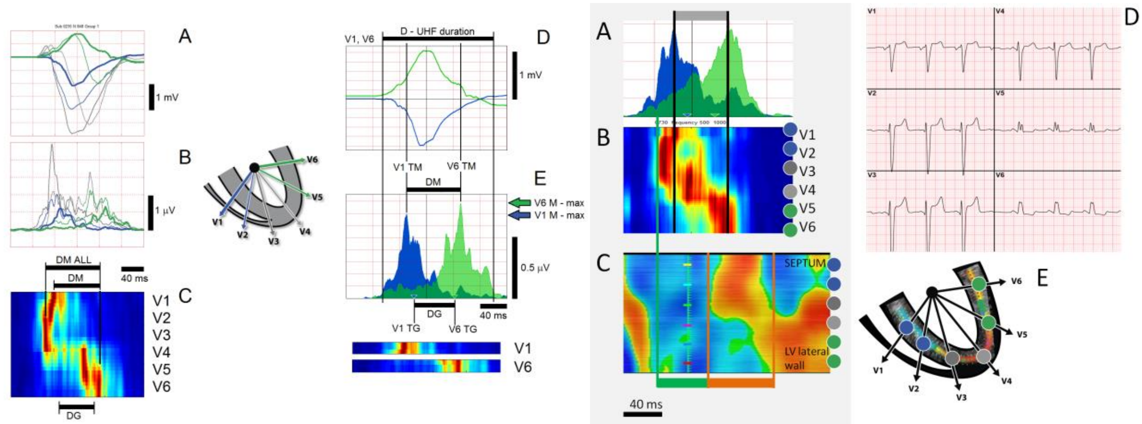


Fig. 3 UHF-ECG ventricular dyssynchrony diagnostics. Electrical maps identify ingoing mechanical contraction of the heart. Patient with left ventricle dyssynchrony.

3. Deep brain electrophysiology

About 30% of all epilepsy cases are pharmacoresistant cases that cannot be pharmacologically controlled and a significant portion of such patients profit from surgical management. Intracerebral or subdural electrodes are implanted within the scope of the epilepsy surgery program in particularly severe cases. These electrodes are usually inserted for a time period of 7–10 days with the objective of precise identification of epileptogenic focus prior to its surgical removal.

The preparatory stage for the surgical treatment of epilepsy allows us to obtain a quantity of interesting complementary physiological data of a high quality. The epilepsy surgery procedure itself is extremely complex, and is presently comprised of synchronized video-EEG monitoring and semi-invasive and invasive EEG (SEEG) examination using sphenoidal, intracerebral or subdural electrodes.

Basic neuroscience research is witnessing exponential growth in the number of groundbreaking findings regarding the activity of the brain and the nervous system. Measurements from depth electrodes represent a rare opportunity to contribute to the latest knowledge. From the medical point of view, research into pharmacoresistant epilepsy is extremely valuable because it allows monitoring of the basic functions and behavior of the brain in its deep structures. Research into the neurosciences does not take place in isolation, but is supported by co-operation with parallel research in technical and technological progress.

It is, therefore, evident that the processing and analysis of intracerebral EEG is strongly connected to technological progress. New findings have been published in just the past few years. Best practices, procedures and methods for the analysis of fast processes in the brain are not yet known or established. It is also not clear how to interpret the results and how to compare individual measurements taken at different workplaces.

The epilepsy and Parkinson’s disease patients measurement with depth brain electrodes include both clinical records and records during various cognitive tasks and stimulations of brain activity. The clinical records cover resting and sleep measurements, and records of epileptic seizures.

Depth electrodes are implanted with the aim of localizing epileptic foci. This means that there are no records of any control group of healthy volunteers. For this reason pathological signals associated with epilepsy are present in both the clinical records and stimulations. While in clinical records this is a carrier of useful information, within the cognitive tasks we are focusing primarily on functional properties of the intact brain structures.

Data obtained from the epilepsy surgery program at the cooperating workplace are used for analysis and method development. A program for the treatment for patients with advanced Parkinson’s disease is running simultaneously with the epilepsy surgery program. It includes the generally successful depth brain stimulation of the subthalamic nucleus. We can record electrical activity directly in the subthalamic nucleus and globus pallidus internus. This activity is connected with cognitive processing.

Main team contribution:

Our main goal in the neurology program is to design and verify methods for identification of epileptic sources within the brain as clinical outcome and the establishment of functional links between brain structures as a basic research outcome. Team contributions can be divided into clinical and basic research outcomes.

Clinical data analysis – pathological High-Frequency-Oscillations (HFOs):

HFOs are observed in some EEG contacts in the frequency bandwidths from 80 Hz to 1,000 Hz. There is a hypothesis that some HFOs most probably have a physiological importance and the others rather reflect intrinsic epileptogenicity of the dysfunctional brain tissue. Determination of the contribution of interictal epileptic oscillations can significantly help localize the epileptogenic zone in patients suffering from intractable epilepsy, and could be helpful as a new diagnostic approach.

HFOs include Ripples (80-250 Hz) and Fast Ripples (FR) (> 250 Hz). Ripples are mostly understood as a reflection of normal brain activity. HFOs above 250 Hz are directly connected with epileptic activity and are labeled High-Frequency Epileptic Oscillations (HFEOs). Three types of areas have been named – the seizure onset zone, which includes epileptic sources, the irritative area where epileptic activity occurs but there is no source, and the remote area without epileptic activity.

The methods of analysis are based in particular on the calculation of the spectral power for the gamma band, ripple and FR frequencies. Time-frequency analysis (TFA) is used for analyzing spectral properties in a frequency band up to 600Hz. Visual record inspection is commonly applied for HFO detection, through successful semiautomatic and automatic HFO detection.

The contributions made by the group are a complete methodology and algorithms for HFOs detection. These are, primarily, identification of the seizure onset zone, irritative zones and remote areas, spikes, ripples and fast-ripple detection, automated detection of HFOs and Welch power spectra analysis. The numerical outputs were statistically evaluated and presented in graphic form.

Experimental data analysis and basic research:

The aim of experimental stimulation is to obtain relevant information on the functional properties of brain areas. In the case of high-frequency records, there are differences in data size, frequency bands and processing requirements. Evoked potentials measured on the scalp are commonly analyzed up to 50 Hz. High-frequency deep brain records allow analysis of signals at frequencies of up to 500 Hz and over. This high sampling frequency, simultaneously combined with improved data quality, enable entirely new research into evoked brain behavior.

In studying the brain activities it is important to know how the brain structures are functionally interconnected and how information is spread. EEG signals are the source data for the evaluation of brain activity. A typical dataset contains many channels covering the entire area of interest in the brain. It is, therefore, possible to explore not only each channel separately, but also dependencies between channels to obtain additional information.

Several methods for the detection of connections and interaction between brain structures are used - correlation function and coherency, the multichannel model, partial directed coherence (PDC), directed transfer function (DTF), and methods based on autoregressive spectral estimation of multivariate signals, etc.

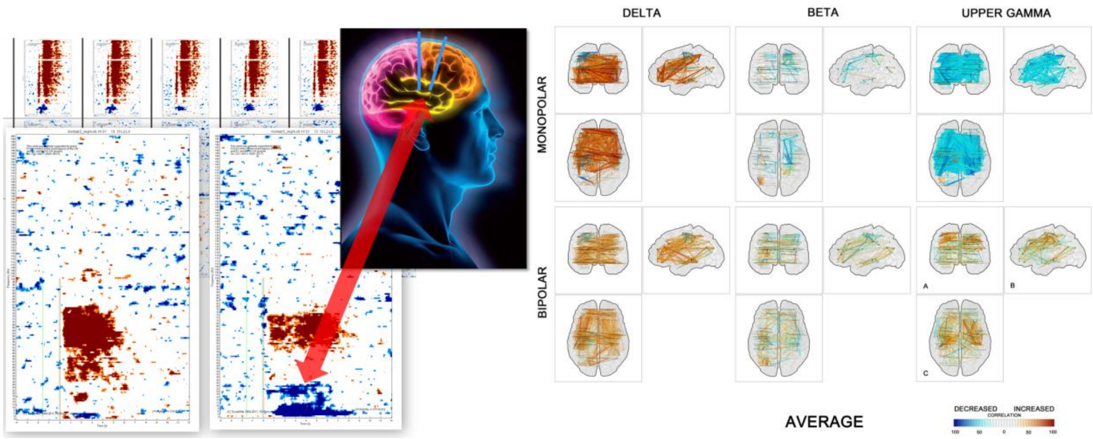


Fig. 4 Deep brain activity research – induced mental activity, structural conectivity.

The team has long focused on intracerebral EEG recording and analysis, including paradigm design and high-quality data acquisition, artefact detection and data scoring, statistical evolution and presentation of the results in graphic form. Additionally, we have also provided a completely new methodology for multidimensional cross-correlation analysis of spatial couplings and two-dimensional interpretation of brain interactions.

Outcomes: 14 IF articles, SignalPlant - system for medical data visualization.

Cooperating institutions: Faculty of Medicine, Masaryk University, St. Anne's University Hospital, Brno, Mayo Clinic, Rochester, MN, USA (G. Worrell)

Medical software team activities

During 2013-2014, three software products were being developed:

PulsWave – Software evaluating pulse wave velocity in blood stream. It is part of the MPM (Multichannel PulsWave monitor) product. The associated citable article was awarded (May, 2014) the „ESGCO 2014 Award for Technology Transfer". PulseWave was certified as a medical device in September 2014.

SignalPlant – Software for bio signal viewing, signing and post processing. It is in frequent use by our department of ISI and collaborating departments of FNUSA-ICRC and CEITEC.

It is unique in that it has an expandable plug-in architecture and one of its plugins (QRS detector for multimodal data) has been awarded fourth place in the „CinC Challenge 2014".

UHF Solver – Biomedical software for ultra-high frequency ECG analysis. Currently, it runs at one of our department servers and fully automatically process data from St. Anne's University Hospital and Institute of Clinical and Experimental Medicine Prague.

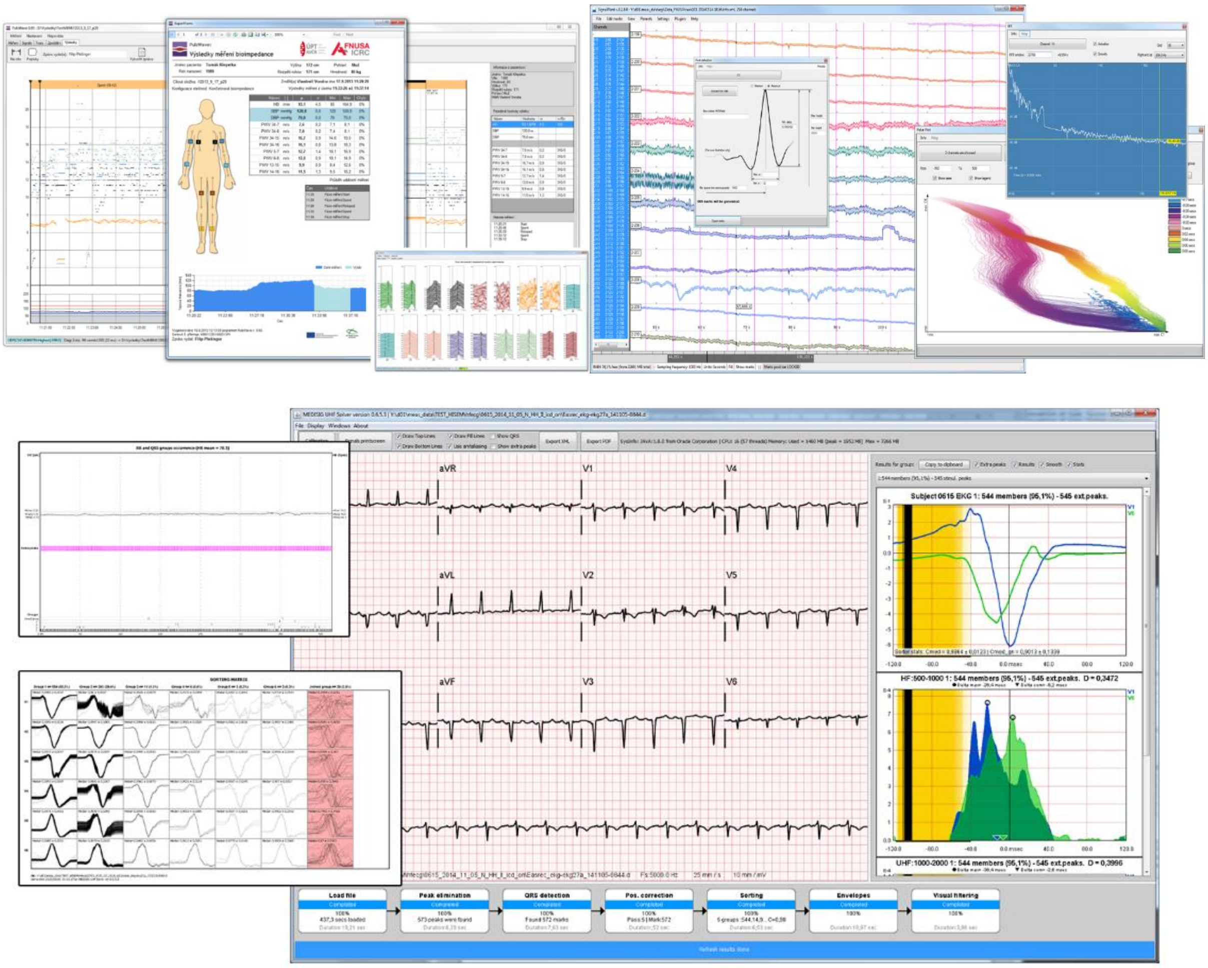


Fig. 5. Top panel: PulsWave, SignalPlant , bottom panel; UHF Solver

Research Report of the team in the period 2010–2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.

Scientific team	Optical micromanipulation techniques

The long term vision of the team is focused on the forefront research of interaction of laser beams with matter at the microscale and nanoscale. During the evaluated period we namely dealt with the transfer of momentum and angular momentum from spatially shaped laser beams upon objects of various properties, shapes and sizes. We further combined focused laser beams with Raman microspectroscopy, fluorescence detection, and microfluidic systems and used them for the determination of properties of trapped microorganisms, emulsion droplets or manufacturing of microstructures by two-photon polymerization. Our long-term experimental experience was employed for custom-made experimental setups for external partners. The following table summarizes our activities.

The full list of our publication outputs is available at the group webpages http://www.isibrno.cz/omitec/ together with more information about the group.

I. Fundamental Research	**II. Applied Research**
1. Theoretical and experimental background	1. Optical diagnostics, trapping and sorting of living micro-organisms
2. Optical binding	2. Photopolymerization
3. Optical trapping of non-spherical particles	3. Droplet microfluidics
4. Optical sorting of particles in shaped optical „potentials	
5. Optical „tractor“ beam	
6. Optical manipulation in air and vacuum	**III. Advanced Engineering**
7. Optically manipulated droplet microlaser	1. Custom made experimental setups for external partners

I. Basic Research

These types of activities are driven by our eagerness to reveal the hidden secrets of nature, understand them and finally employ them in applications useful for mankind. Our activities usually cover theoretical or numerical studies combined with experimental measurement in the laboratory.

I.1. Theoretical and experimental background

I.1. Theory We have gradually improved our own theoretical codes for the calculation of optical forces and torques acting upon a single particle and even more particles of various properties. Namely we employed coupled dipole approximation (od discrete dipole approximation DDA), Mie theory, finite elements methods (based on COMSOL package) and T-matrix and used them to study behavior of dielectric or metal, spherical[1] or nonspherical[2] (ellipsoidal, spheroidal, decahedral, triangular prism) particles illuminated with laser beams with various spatial distribution of intensity and phase (e.g. vectorial description of aberrated focused laser beam, Gaussian, Bessel[3], Laguerre-Gaussian, evanescent[4], and their interferences under different propagating angles). The acting optical forces and torques are used to solve the equation of motion in an overdamped regime and to obtain trajectories of particles. Recently we added to the code hydrodynamic interactions between particles based on Rotne-Prager approximation to cover all leading physical interactions. Therefore we can simulate even our demanding experimental configurations and these simulations, performed in advance, usually save experimental effort and eliminate blind attempts due to relatively large experimental parametric space.

I.1. Experimental setups. For the purposes of our experimental basic research we built several experimental systems. All of them use PC controls based on LabView software.

a) Holographic dual beam. In cooperation with our former PhD student T. Čižmár we built a unique experimental setup that uses counter-propagating (C-P) beam geometry with the possibility to modify the number of C-P beam pairs and shape the beam profile of each beam using a spatial light modulator (SLM)[5]. Thus we can modify in a contactless way the parameters of the beams, optical trap/s via PC controlled interface while keeping the same particle/s trapped and observe them from from a direction perpendicular to the beam propagation. This system represents the most flexible dual-beam optical trap and was used to obtain the majority of the experimental results mentioned below.

b) Holographic optical tweezers. We built two experimental setups that enable modification of the beam properties of the focused beam. One of them was mainly used to generate vortex beams and to study behavior of spheroidal and spherical particles using fast CCD and a quadrant photodiode. The second one enables easy modification to generate, instead, focused beams, wide interference structures for investigation of stochastic particle dynamics, or particle sorting in shaped optical potentials using a CCD camera.

c) Time-sharing multi-beam optical tweezers. This experimental setup generates one highly stable optical trap with a possibility to generate several time sharing traps

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[1] M. Šiler, L. Chvátal and P. Zemánek: "Metallic nanoparticles in a standing wave: Optical force and heating", *Journal of Quantitative Spectroscopy and Radiative Transfer* **126**, 84-90, 2013 (IF 2.380)

[2] J. Trojek, L. Chvátal and P. Zemánek: "Optical alignment and confinement of an ellipsoidal nanorod in optical tweezers: a theoretical study", *J. Opt. Soc. Am.* A **29**, 1224-1236, 2012 (IF 1.562)
O. Brzobohatý, M. Šiler, J. Trojek, L. Chvátal, V. Karásek, P. Zemánek: "Non-spherical gold nanoparticles trapped in optical tweezers: shape matters", *Optics Express* **23**, 8179-8189, 2015

[3] M. Šiler, P. Jákl, O. Brzobohatý, and P. Zemánek: "Optical forces induced behavior of a particle in a non-diffracting vortex beam", *Optics Express* **20**, 24304-24319, 2012, (IF 3.546)
M. Šiler and P. Zemánek: "Optical forces in a non-diffracting vortex beam" *Journal of Quantitative Spectroscopy and Radiative Transfer* **126**, 78-83, 2013 (IF 2.380)

[4] M. Šiler, and P. Zemánek: "Parametric study of optical forces acting upon nanoparticles in a single, or a standing, evanescent wave", *Journal of Optics* **13**, 044016, 2011, (IF 1.573)

[5] T. Čižmár, O. Brzobohatý, K. Dholakia, and P. Zemánek: "The holographic optical micro-manipulation system based on counter-propagating beams", *Laser Physics Letters* **8**, 50-56, 2011, (IF 9.970)

displaced laterally from the first one. This system uses stable laser, opto-mechanical components, and quadrant photodiodes for the detection of tiny forces (optical binding, thermophoretic, etc.) acting upon the trapped particle serving as the force probe.

d) Optical "tractor" beam. Based on our simulations we designed an experimental system which is able to pull objects against the intuitively expected radiation pressure[6] – similar to the tractor beam seen in the Start Trek series. Besides this phenomenon, we also observed the sorting of particles of different sizes or their self-arrangement into optically bound structures that moved in opposite direction in contrast to individual unbound particles.

e) C-P Evanescent beams. In this setup we used the total internal reflection at the prism surface to create C-P evanescent beams on its other side forming standing wave traps in its intensity nodes or antinodes. Controlled change of the phase in one beam causes motion of the interference fringes above the surface together with confined particles. A fast CCD camera followed their motion, while image processing provided the position of the particles with respect to the trap bottom. Stochastic particles jumps across the potential barrier between neighboring potential wells were also studied.

I.2. Optical binding

Motivation: Would it be feasible to illuminate a stochastic sample of microparticles with a structured laser beam and force them to self-arrange in as a functional microstructure?

a) One dimensional longitudinal binding. Areal illumination of more particles leads to their mutual force interaction via scattered light – so called optical binding[7]. This interaction was usually considered too weak to significantly influence particles behavior. However about 20 years ago it was rediscovered that such a process can create new equilibrium positions for the particles and self-arrange them into a structure kept together by light. While this one-dimensional optically bound structure has been demonstrated in Gaussian beams, we used our holographic dual beam system I.1.a to compare quantitatively the measured distances between particles in C-P incoherent Bessel beams with our theoretical models. The coincidence was excellent for lower distances however slightly deviated for interparticle distance close to 20 micrm due to the real experimental properties of the Bessel beam[8].

In the same geometry but with interfering C-P beams, forming a periodic potential landscape for a single particle, we demonstrated experimentally and confirmed by theoretical simulations that the presence of two particles leads to significantly different behavior as opposed to the case of a single trapped particle. It is caused by an extra tilt of the periodic potential, due to the optical binding between particles, and

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[6] O. Brzobohatý, V. Karásek, M. Šiler, L. Chvátal, T. Čižmár, P. Zemánek: "Experimental demonstration of optical transport, sorting and self-arrangement using a 'tractor beam'", *Nature Photonics* **7**, 123-127, 2013 (IF 27.254)
[7] K. Dholakia, and P. Zemánek: "*Colloquium:* Gripped by light: Optical binding", *Reviews of Modern Physics* **82**, 1767-1791, 2010 (IF 51.695) (review)
[8] O. Brzobohatý, V. Karásek, T. Čižmár, and P. Zemánek: "Dynamic size tuning of multidimensional optically bound matter", *Applied Physics Letters* **99**, 101105, 2011 (IF 3.844)

consequent stochastic jumps between neighboring optical potential wells[9]. Finally the equilibrium distance between two such optically bound particles was almost the same in both cases of interfering and non-interfering beams. Therefore existing potential barriers comparable to the thermal activation energy just slow down the process of formation of optically bound structures but do not significantly change its dimension.

Using the geometry I.1.d, we studied the properties of optical traps[10], measured the first mean passage time of the single particle to the neighboring optical traps using fast CCD camera[11], and then we focused on delivery of more particles confined in the travelling standing wave (periodic potential). We then found that more particles can be delivered about 5x faster than a single particle due to almost equal contribution of optical binding and hydrodynamic effects[12].

b) Two-dimensional optical binding. We also demonstrated for the first time two-dimensional optically bound microstructures with tunable sizes that were formed in C-P incoherent ellipsoidal Gaussian beams of variable beam widths[8]. The interplay of the thermal activation with possible stable configurations of the structure is clearly visible in the experimental data.

c) One dimensional optical binding in the “tractor” beam geometry
In cooperation with the group led by prof. K. Dholakia at the University of St. Andrews, together we built an experiment system combining together geometries of I.1.c and I.1.d. For the first time we determined quantitatively the optical binding force between two and three particles and compared it with the theoretical results based on DDA. This proved we were able to measure forces as low as hundredths of pN[13].

Experimental formation of two and more optically bound particles revealed that such optically bound structures feel much stronger optical force which pulls them against the direction of the final wavevector[6] (e.g. against the intuitively expected direction of the radiation pressure).

We also theoretically studied the optical binding force between two unequal gold nanoparticles and their stable configurations in this geometry. By increasing the difference between particle sizes, they are more displaced from the symmetrical configuration. The larger the distance is between the particles the closer to the beam axis they are[14].

I.3. Optical trapping of non-spherical particles

[9] O. Brzobohatý, V. Karásek, M. Šiler, J. Trojek, and P. Zemánek: "Static and dynamic behavior of two optically bound microparticles in a standing wave", *Optics Express* **19**, 19613-19626, 2010 (IF 3.587)
[10] M. Šiler, and P. Zemánek: "Parametric study of optical forces acting upon nanoparticles in a single, or a standing, evanescent wave", *Journal of Optics* **13**, 044016, 2011, (IF 1.573)
[11] M. Šiler, and P. Zemánek: "Particle jumps between optical traps in a one-dimensional (1D) optical lattice", *New Journal of Physics* **12**, 083001, 2010 (IF 3.849)
[12] M. Šiler, T. Čižmár and P. Zemánek: "Speed enhancement of multi-particle chain in a traveling standing wave", *Applied Physics Letters* **100**, 051103, 2012 (IF 3.844)
[13] O. Brzobohatý, T. Čižmár, V. Karásek, M. Šiler, K. Dholakia, and P. Zemánek: "Experimental and theoretical determination of optical binding forces", *Optics Express* **18**, 25389-25402, 2010, (IF 3.749)
[14] L. Chvátal, O. Brzobohatý, P. Zemánek: "Binding of a pair of Au nanoparticles in a wide Gaussian standing wave", *Optical Review*, **22**, 157–161, 2015

Motivation: Majority of optical trapping experiments and theoretical studies deals with an isotropic object of spherical shape. Its behavior is governed by the optical force acting upon the particle center of mass. Optical manipulation with nonspherical particles however also leads to optical torque that rotates the particle around its axis, and thus modifies the strength of the trapping optical force. Behavior of the nonspherical particles is thus much more complex, rarely described in literature, and offers interesting applications if properly understood.

We mastered the manufacturing of spheroidal polystyrene microparticles from spherical ones and illuminated them with a circularly and linearly polarized Laguerre-Gaussian (vortex) beam (using setup I.1.b). The spheroid was trapped at the beam center and rotated around its axis. We found that the frequency of particle rotation was by an order of magnitude more sensitive to the increase of topological charge than the polarization of the beam. Parallel rotation of two spheroids trapped in two separated optical traps indicated synchronization of their rotation for certain trapping powers due to hydrodynamic coupling via the surrounding medium[15].

Performing experiments with larger gold nanoparticles, we observed their three dimensional trapping even in relatively wide beams, which were obtained by focusing optics with a numerical aperture of 0.37. This behavior was in contrast to theoretical predictions for expected spherical shape of the nanoparticles. Images from the electron microscopy (obtained by our colleagues from the Electron Microscopy group) revealed decahedrons, icosahedrons, hexagonal and triangular prisms, but no spheres in the sample. Finally we obtained encouraging agreement between measured and calculated trap stiffnesses for triangular prism NPs[16]. This supports our conclusion that the 3D trapping of larger Au NPs is caused by their non-sphericity and proper stable orientation in the trap even though they are smaller than the trapping wavelength.

I.4. Optical sorting of particles in shaped optical „potentials“

Motivation: Could laser illumination of heterogeneous suspension of particles lead to spatial separation of its components?

In the past we developed several methods based on travelling or static periodic potentials that enables sorting of spherical particles according to their sizes. We gradually improved sorting methods based on travelling and switching periodic potentials (e.g. I.1.b) and demonstrated several methods on how to spatially separate spherical particles according to their sizes, spherical and spheroidal particles or alga cells of different sizes[17]. This approach is compatible with commercial optical microscopes and samples placed in Petri dishes.

A. V. Arzola, K. Volke-Sepulveda, J. L. Mateos: **"Dynamical analysis of an optical rocking ratchet: Theory and experiment"**, Physical Review E **87**, 062910:1-9, 2013, ABSTRACT

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[15] A. V. Arzola, P. Jákl, L. Chvátal, P. Zemánek: "Rotation, oscillation and hydrodynamic synchronization of optically trapped oblate spheroidal microparticles", *Optics Express* **22**, 16207-1622, 2014 (IF 3.525)

[16] O. Brzobohatý, M. Šiler, J. Trojek, L. Chvátal, V. Karásek, A. Patak, Z. Pokorná, F. Mika, P. Zemánek: "Three-Dimensional Optical Trapping of a Plasmonic Nanoparticle using Low Numerical Aperture Optical Tweezers", *Scientific Reports* **5**, 8106, 2015

[17] P. Jákl, A. V. Arzola, M. Šiler, L. Chvátal, K. Volke-Sepulveda, P. Zemánek: "Optical sorting of nonspherical and living microobjects in moving interference structures", *Optics Express* **22**, 29746-29760, 2014 (IF 3.525)

I.5. Optical „tractor“ beam

Motivation: Could we manufacture functional microstructures with light and sort them automatically away from the individual building components?

Using the setup I.1.d. we demonstrated that it is at least possible with polystyrene spherical particles[6].

I.6. Optical manipulation in air and vacuum

Motivation: The majority of optical trapping experiments were performed in liquid, where the stochastic system is overdamped. What kind of new phenomena can be observed if the damping is low and the particle inertia plays a role? Could we slow down (cool down) the particles center of mass by light similarly as it is done in atom cooling? How low temperature could be reached?

We started with a trapping of aerosol droplets using the setup I.1.a and successfully reached their fusion, rotation in three C-P beams, and confinement even in wide Gaussian beams[18]. In 2014 we started building a new setup for trapping and manipulation in a vacuum.

I.7. Optically manipulated droplet microlaser

Motivation: Would it be possible to manufacture a laser that can be manipulated and tuned by laser beams?

In cooperation with the group led by prof. A. Kiraz from Koc University in Istanbul, we demonstrated for the first time the frequency tuning of a laser light emitted from an emulsion droplet that is optically deformed. Such an emulsion droplet having a diameter of 47 micrometers was optically confined and deformed in two C-P beams and separately optically pumped by another laser beam. The spectral profile of the emitted radiation from whispery gallery modes was repeatedly shifted by the power variation of the deforming beams[19]. We also started experiments with liquid crystal droplets which are birefringent and thus strongly dependent on the polarization of the incident beams.

II. Applied research

Activities in this area cover our research, which is focused on finding methods or techniques to solve a particular practical problem to the level of proof of concept at the running laboratory setup.

II.1. Optical diagnostics, trapping and sorting of living micro-organisms

For these activities we use home-made experimental systems and commercial system Renishaw inVia (for some experiments enhanced with our module described in III.1.b).

II.1.a Algae

Motivation: Algae cells represent a perspective source of lipids either for biofuel or nutrition purposes because they can produce unsaturated fatty acids using

[18] O. Brzobohatý, M. Šiler, J. Ježek, P. Jákl, P. Zemánek: "Optical manipulation of aerosol droplets using a holographic dual and single beam trap", *Optics Letters* **38**, Iss. 22, 4601-4604, 2013,

[19] M. Aas, A. Jonáš, A. Kiraz, O. Brzobohatý, J. Ježek, Z. Pilát, P. Zemánek: Spectral tuning of lasing emission from optofluidic droplet microlasers using optical stretching", *Optics Express* **21**, 21381-21394, 2013, (IF 3.546)

photosynthesis. Deeper understanding of their reaction to different light conditions at the level of the individual cell would be a step forward in their subsequent utilization. However, a major step forward would be represented by a method that provides diagnostics of an individual cell with its subsequent separation for breeding – ***all in a contactless and harmless way for the cell****. In cooperation with Photon Systems Instruments we followed this vision to the experimental system proving the principle.*

We demonstrated that the ratio of intensity of Raman peaks corresponding to cis C=C stretching mode (unsaturated bond) and CH2 scissoring mode (saturated bond) represents a sufficient parameter to determine the level of unsaturation of the investigated lipid droplet inside the cell[20]. As a “side product” of this effort we found a way how to measure the concentration of beta-carotene in lipid bodies inside an individual living cell (using Raman spectroscopy) and we determined the dependence between this concentration, volume of the lipid body, and light intensity during the alga cultivation[21]. Since the algae are extremely sensitive to light intensity, we used the pulse amplitude modulation technique to assess the photodamage of individual alga cells at the wavelengths 735-1064 nm which are frequently used for optical trapping. We found negligible influence of the trapping laser at wavelengths longer than 935 nm[22]. In the next step we built an experimental system that automatically analyses the image of the incoming algae, determines which alga is the closest to the trapping laser beam and navigates the stage to trap it (using 1064 nm), acquires the Raman spectrum which is processed and analyzed online. Based on this analysis, the cell is either delivered by the trapping beam to the collection channel or released from the beam and wasted. This technical procedure is protected by Czech Utility Model No. 25864.

II.1.b. Bacteria

Motivation: A fast and nearly real-time method for identifying the type of bacteria is highly demanded for medical purposes, e.g. for fast targeted treatment by antibiotics. The classical way uses cultivation of the bacterial sample on agar for several days and microscope observation followed by strain determination by an experienced expert. In cooperation with the Institute of Microbiology (providing cell cultivation) from the Faculty of Medicine Masaryk University and the group of Microscopy for Biomedicine, the ISI (providing SEM images of samples) we were looking for a faster method of how to determine different bacterial strains or biofilm positive and negative variants of the same strain. Based on the interest of our medical colleagues we were also looking for a method of faster recognition of how the antibiotic influences the bacterial cell – whether it kills the bacteria (bactericidal) or just suppresses its growth (bacteriostatic).

Based on our previous experience we decided to use Raman microspectroscopy to investigate the bacterial samples. It turned out that individual bacterial strains have

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[20] O. Samek, A. Jonáš, Z. Pilát, P. Zemánek, L. Nedbal, J. Tříska, P. Kotas, and M. Trtílek: "Raman microspectroscopy of individual algal cells: Sensing unsaturation of storage lipids *invivo*", *Sensors* **10**, 8635-8651, 2010 (IF 1.771)
O. Samek, P. Zemánek, A. Jonáš, and H. H. Telle: "Characterization of oil-producing microalgae using Raman spectroscopy", Laser Physics Letters **8**, 701-709, 2011 (IF 9.970
[21] Z. Pilát, S. Bernatová, J. Ježek, M. Šerý, O. Samek, P. Zemánek, L. Nedbal and M. Trtílek: "Raman microspectroscopy of algal lipid bodies: beta-carotene quantification", Journal of Applied Phycology **24**, 541-546, 2012 (IF 2.411)
[22] Z. Pilát, J. Ježek, M. Šerý, M. Trtílek, L. Nedbal, P. Zemánek: "Optical trapping of microalgae at 735-1064 nm: Photodamage assessment", *Journal of Photochemistry and Photobiology B: Biology* **121**, 27-31, 2013 (IF 3.110)

different Raman spectral “fingerprints” that provide their identification after mathematical analyses of the spectra (using principle components). We applied this technique to many bacterial samples placed in a petri dish or trapped in optical tweezers and we succeeded in the determination of biofilm positive and negative strains of *Staphylococcus epidermidis*[23].

We also used Raman microspectroscopy to determine how the antibiotic affects an individual bacterial cell trapped in optical tweezers. It turned out that bactericidal antibiotic fragments the cell DNA and thus the ratio of the Raman peak related to DNA and phenylalanine varies with antibiotic concentration. In contrast, this does not happen for bacteriostatic antibiotics. This is an encouraging result because it proves Raman spectroscopy as the appropriate tool for monitoring biological changes introduced by antibiotics at the level of a single cell[24].

II.1.c. Yeast cells

Motivation: Similarly as in bacteria, i.e. fast and nearly real-time method identifying the type of Candida and its biofilm positive and negative variants.

We used the Renishaw inVia Raman system to analyze different samples of *Candida parapsilosis* using Raman microspectroscopy and we succeeded in determination of their biofilm positive and negative variants using Principle Component Analyses[25].

II.2. Photopolymerization by focused laser beam

Motivation: Due to our skills in shaping of the focused laser beam and fast and precise positioning of the sample, we were interested in mastering two-photon polymerization to manufacture non-spherical microstructures with submicrometer details. These structures can be used for a deeper understanding of the linear and angular photon momentum transfer to micro-objects, or can be used to shape channel walls of the microfluidic chips to reach proper fluid or droplets behavior.

We built our own experimental system that uses a near-infrared laser with femto-second pulses to initiate the polymerization in both epoxy-based monomers and organically modified ceramics. Using a spatial light modulator we optimized the beam to get diffraction limited focus, tune the laser beam power to set the voxel size and/or create multiple focal spots to speed the manufacturing process. We successfully

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[23] O. Samek, J. F. M. Al-Marashi, and H. H. Telle: "The potential of Raman spectroscopy for the identification of biofilm formation by *Staphylococcus epidermidis*", *Laser Physics Letters* **7**, 378-383, 2010 (IF 6.010)
O. Samek, S. Bernatová, J. Ježek, M. Šiler, M. Šerý, V. Krzyžánek, K. Hrubanová, P. Zemánek, M. Holá, B. Růžička: "Identification of individual biofilm-forming bacterial cells using Raman tweezers", *J. Biomed. Opt.* **20**, 051038, 2015

[24] S. Bernatová, O. Samek, Z. Pilát, M. Šerý, J. Ježek, P. Jákl, M. Šiler, V. Krzyzánek, P. Zemánek, V. Holá, M. Dvořáčková, F. Růžička: "Following the mechanisms of bacteriostatic versus bacericidal action using Raman spectroscopy", *Molecules* **18(11)**, 13188-13199, 2013 (IF 2.428)

[25] O. Samek, K. Mlynariková, S. Bernatová, J. Ježek, V. Krzyžánek, M. Šiler, P. Zemánek, F. Růžička, M. Holá, M. Mahelová: "Candida parapsilosis Biofilm Identification by Raman Spectroscopy", *Int. J. Mol. Sci.* **15**, 23924-23935, 2014 (IF 2.339)

created structures with details well bellow 250 nm in the area of 100x100 μm using exposition times in units of microseconds per voxel.

II.3. Droplet microfluidics

Motivation: New biotechnological methods are looking for cheap procedures that save large amounts of expensive chemicals and enable parallel processes with a high level of time control. It seems that microfluidic systems, known as lab-on-a-chip, can offer these possibilities. Their recent generation using emulsion droplets formed from immiscible liquids represents an exciting step forward, provided that such individual droplets can be investigated and navigated in a contactless way.

We gradually mastered the method of soft-litography to produce microfluidic channels in a PDMS matrix bound to a glass surface. Content of such chips can be easily observed by optical microscopy and investigated by laser spectroscopy. We have also mastered the generation of emulsion droplets of water-in-oil, or oil-in-water, detection of their fluorescence, whispering gallery modes, and their navigation in the microfluidic channels using dielectrophoresis. Now we enter the phase of investigation and optimization of biochemical reactions in cooperation with the group led by prof. J. Damborsky from Loschmidt Laboratories from Masaryk University.

III. Advanced Engineering

These activities link together our long-term expertize in the areas of fine mechanics, optics, laser physics, electronics, software system control and building of laboratory opto-mechanical experimental systems. It enables us to design and build semi-laboratory experimental systems for external partners on-demand. A brief example of such systems follows:

III.1.a. Holographic Raman tweezers.

This system combines holographic optical tweezers and Raman microspectroscopy. Holographic optical tweezers use a spatial light modulator to generate several focused trapping beams independently placed in three dimensions and to shape their spatial intensities and phase profiles. Raman microspectroscopy allows detection of Raman spectra from a sample placed at the focus of another (so called Raman) laser. The whole system represents a unique tool to optically trap and manipulate one or several microobjects (including living cells, emulsion droplets, etc.), and to investigate their fluorescence or Raman spectral response. The system has the form of a laboratory setup, open for modifications, placed on the optical table and equipped with home-made software which provides control, detection, acquisition, processing and storage of measured data in a user-friendly manner. In the cooperation with our partner (Institute of Experimental Physics of the Slovak Academy of Sciences in Kosice) we enhanced the system with natural user interface integrating hand tracking, gestures recognition, eye tracking and speech recognition using “Leap motion,” “MyGaze” low-cost sensors and a “Tazti” program[26].

III.1.b. Equipment for connecting laser source to microscope objective.

[26] Z. Tomori, M. Antalík, P. Keša, J. Kaňka, P. Jákl, M. Šerý, S. Bernatová, P. Zemánek: "Holographic Raman tweezers controlled by hand gestures and voice commands", *Optics and Photonics Journal* **3**, 331-336, 2013

This system provides a tool for easy coupling of a laser beam to any commercial microscope. It is placed in between the microscope objective and microscope body so that the inner optical paths of the microscope are untouched. An optical fiber connector is used for laser implementation and a fine screw is used for adjusting the beam focus to the sample plane of the microscope. This result is protected by Czech Utility Model No. 21642.

III.1.c. Optical scalpel

This system is based on a pulsed laser which is coupled with a fiber to a commercial optical microscope using our compact module described in III.1.b. The system is equipped with home-made software to control the parameters of the laser. This system runs at the Institute of Experimental Physics of the Slovak Academy of Sciences in Kosice.

III.1.d. Optical trapping system for manipulation of charged particles in plasma

The system uses dual-beam geometry of two counter-propagating laser beams overlapping in the vacuum chamber where the dusty plasma is formed. This system runs at Christian-Albrechts University in Kiel.

III.1.e. Optical nanolitographic systems.

These systems are based on a focused femtosecond laser that initiates photo-polymerization in its focal spot so that solid structures of dimensions in hundreds of nanometers can be formed byrapid scanning of the spot. These systems run at the Institute of Experimental Physics of the Slovak Academy of Sciences in Kosice and at the ISI.

Research Report of the team in the period 2010–2014

Institute	Institute of Scientific Instruments of the CAS, v. v. i.

Scientific team	Coherence optics

Position of the Department of Coherence optics

The Department of Coherence optics derives its research focus from the history of research and development in the field of gas lasers, lasers with high coherence and laser metrology and technology. The first gas (He-Ne) laser in the Czech Republic (Czechoslovakia) was put into operation here and other highly complex laser systems followed, such as laser interferometers and standards of optical frequencies.

Research in this field stands on the border between physics and technical sciences, it includes fundamental, targeted and applied research as well. Our research is methodologically oriented. The goal of most of our projects is an introduction of a novel method, technique or approach and its experimental verification. This includes design of often highly complex and sophisticated experimental assemblies. In optical metrology it often means pushing the limits of resolution and precision.

The Department of Coherence optics tries to keep an optimum balance between fundamental and applied research. This seems to be the key challenge in the environment of dominating project funding. The boundary between fundamental and applied research is quite blurred but the projects funding our research effort are specific. With a growing shift towards preference of applied research (often due to political reasons) it is becoming more difficult to maintain the necessary key portion of fundamental research. This is a crucial investment into the future of the Department filling the pool of knowledge and expertise, which can later be tapped in application-oriented projects.

The Department of Coherence optics is overwhelmingly project funded. Nearly the whole staff and all consumables are financed from running projects. This is the main limiting factor of any strategic thinking, decision-making and management and also a great burden of administrating the whole project portfolio. Not to speak about the need to prepare new project proposals under the conditions of quite low success-rates in project calls. A handful of researchers who perform their own scientific direction annually have to come up with a host of new ideas of project goals, to write proposals and only a few of them materialize in funded projects. This results in a great deal of wasted manpower in completing the failed proposals and it also introduces a random factor guiding our research orientation, which ultimately remains partially out of our control.

The position of the Department of Coherence optics is unique in some aspects. We are a research-oriented institute of non-university research. The same way we are not a metrology institute. We closely cooperate with the Czech Metrology Institute and we are also involved in education within a framework of commonly accredited study programs of undergraduate as well as postgraduate courses. This gives us a chance to unify research topics that are usually followed by different groups or departments at national metrology institutes and it allows students from technical subjects as well as physics to get involved. Thus our team is built as a team combining specialists from various fields, including optics, solid-state physics, spectroscopy, physics of thin films, electronics, information technologies, computing, precision mechanics, vacuum technology, material sciences, laser power technology, etc. Thanks to this, our research is always that of a team effort between specialists, compared to that of separate individual projects of Ph.D. students at universities.

Fundamental research in our case means predominantly research in fundamental metrology. However, when we speak of the technical sciences it is sometimes difficult to speak about "fundamental science" at all. We would prefer to say targeted research. It means that the research has its practical goal that can be foreseen (mostly in some new and more precise measurement technique) but its transfer into practice is quite distant and unsure. In our case this includes our involvement in the research of laser optical frequency synthesis, optical clocks, the transfer of highly stable optical frequencies over fiber networks and some selected topics of laser interferometry.

Our involvement in applied research is motivated by the tradition of the Institute of Scientific Instruments, which has always tried to promote technology transfer and applications of its research in practice. Besides this, there is a significant shift in the research policy of the Czech Republic and of project funding which promotes applied research. Part of our applied research motivation lies in the need to ensure the funding of our group. We have been involved in a large number of applied research projects with partnering companies. A specific situation of our post-transformation economy resulted in a great dominance of foreign ownership of the Czech industry, where the foreign management tend to restrict local research and cooperation with local research institutions. Many mostly small and medium enterprises operate on the basis of contract production, and they show little or no interest in support of research cooperation. Only a handful of companies are really innovative and promote research partnerships. In such an environment the applied research projects, we were involved in, were in nearly all cases motivated from our side, when we were looking for a partner. It seems that only in the last few years there’s been a change in the tide, as more and more managers see that the partnership in research is the only way how to survive in this hypercompetitive market environment.

The Department of Coherence optics has built a portfolio of partnering companies that are interested in our research and see us as trustworthy partners. Thanks to their understanding we are able to compose our applied research projects with a portion of targeted research as well, which helps us to benefit from these projects by strengthening our knowledge portfolio. Our position as an institute within the framework of the Academy of Sciences puts us in a position where the contractual, "demand driven" research should not be our primary goal. The cooperation with industries should be predominantly "idea driven," which we try to fulfil in all of our projects.

Key research topics and results of the period

Optical frequency synthesis

The Department of Coherence optics operates several femtosecond pulsed optical frequency synthesisers (optical frequency combs) ranging from the near-infrared to visible spectral ranges. These systems are referenced at the radiofrequency end to an in-house Hydrogen active maser standard. The lasers serve as references for calibration of lasers, stabilized laser systems and for spectroscopy applications.

We started a new research topic involving the optical frequency synthesis. The synthesizers allow direct "precision transformation" from the optical to radiofrequency frequency ranges thus linking the dimensional and time metrology. We tried to extend the immense potential of frequency precision of discrete frequency components of the comb of optical frequencies referenced to the rf standard into nanometrology – ultraprecise measurement of dimensions in the nanoscale. We introduced a technique of locking of a mechanical standard – an etalon to a highly stable optical frequency comb component by optical filtering of selected frequency components by a fibre Bragg grating. Length tuning of the cavity locked to the discrete comb components results in nanometrology displacement reference. Fine-tuning of a locked cavity following the tuning of repetition frequency through introducing a synthesized rf offset to the H-maser produces a true ultraprecise nanopositioning.

Laser cooling of trapped ions

Our Department of Coherence optics became involved in a project of so called Centre of Excellence on the investigation of the unexplored area between quantum and classical physics of mechanical oscillators, approaching it from both sides. It is a multidisciplinary project unifying fundamental quantum physics and laser technology and bringing together teams from the Department of Optical Micromanipulation Techniques, the Department of Coherence optics from the ISI, and a research team from Palacký University. Our department is involved in the investigation of quantum mechanics with trapped ions, especially Calcium ions that may lead to the development of optical ultrastable laser oscillators.

The project combines fundamental research in the field of quantum physics of light and matter interaction with fundamental metrology. The complex experimental instrumentation, which is still being built, will serve both purposes. The project has been running for a little more than one year and its results will be presented in the near future. The project takes advantage of the long-term experimental experience and expertise of our department. Thanks to its interdisciplinary approach it will unavoidably lead to fundamental discoveries and technological advancements with broad applicability in both nanotechnology and quantum physics. We have prepared the vacuum setup with Paul trap and Ca atom oven. The work on control of linewidth and absolute optical frequency stabilization of all lasers needed for cooling of Ca ion to ground state is running.

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Interferometry techniques

Dimensional metrology is predominantly a domain of interferometry techniques. The precision and stability of a laser standard has to be transferred into a real measurement via interferometer. We invested significant effort into interferometry where some of our projects and research topics can be considered fitting within a fundamental metrology and some of them an applied research or industrial metrology.

Low-coherence and laser interferometry

We introduced a novel method of contactless short gauge blocks calibration and diagnostics within a collaborative project with an innovative company known as MESING, Czech Republic. The patented principle of double-ended interferometer combines coherent and white light.

The optical setup combines a Michelson interferometer and a Dowell interferometer placed in the reference arm of the Michelson interferometer. The research of the method was fully exploited in a design of a unique measuring instrument. The fully automated computer controlled system is able to perform serial calibration of 126 pcs. of gauge blocks in one measuring cycle with the help of automatic feeder. The standard deviation from a traditional multicolor absolute interferometer with a reference plane is in the order of tens of nanometers. The key added value here is in the quality repeatability of measurements thanks to the concept of the combination the measuring of a double-ended interferometer with an automatic feeder, where personal assistance is no needed during the measuring process.

This complex automated system was awarded a Gold medal at the International Engineering Fair in Brno, 2012, as the most innovative instrument and received Werner von Siemens Excellence Award 2012 for the most important innovation.

Interferometry with compensation of the varying refractive index of air

Within the framework of our involvement in high-resolution and high-precision interferometry techniques in optical metrology and nanometrology, we focused on a key problem of any interferometry on air: the fluctuations of the refractive index of air, which produces an optical length noise. The instability of the refractive index of air is a key limiting factor in any interferometric dimensional measurement and represents a dominating source of uncertainty when evaluated indirectly from the physical parameters of the atmosphere.

We proposed a principle which is based on referencing the wavelength of the coherent laser source in atmospheric conditions instead of traditional stabilization of the optical frequency and indirect evaluation of the refractive index of air – in fact, stabilization of the air wavelength over the beam path of the measuring range. The reference length is derived from a mechanical frame made from a material with a very low thermal coefficient. The technique was verified in a setup with a tuneable laser with optical frequency tracking the varying optical length. Another approach used was a technique which allowed us to track the variations of the refractive index of air on-line directly in the line of the measuring beam and to compensate for the fluctuations.

Optical setup for testing of this concept is a novel configuration of single-axis differential interferometer where the key advantage is in identical beam paths of the position measuring interferometers and the interferometer used for the wavelength stabilization or tracking refractometer. This concept allows simultaneous measurement of the carriage position from both sides together with monitoring of the overall range – optical length. This technique can find its way into high-precision positioning systems in nanometrology.

The method of stabilization of wavelength within the measuring range has been tested also in a cavity-based setup where the displacement was measured with a transparent photodetector detecting the standing-wave.

Interferometry with passive Fabry-Perot cavity

Direct transfer of displacement on the nanometer level into frequency is a measuring technique where the length-tuneable passive Fabry-Perot cavity represents a displacement standard referenced to an optical cavity. The setup we proposed and tested is used for calibration of interferometers at the scale below one interference fringe. The cyclic error, which is a result of nonlinearity of the fringe subdivision, can be evaluated by high-linearity positioning where the transfer length-to-frequency plays a role, especially when the referencing is done to the optical frequency synthesizer.

The initial and complex measuring technique works as a generator of precise length displacement. The passive optical cavity is integrated in the measuring part of Michelson interferometer and shares a common geometrical length. The length of the cavity is locked through one selected eigen-mode to a highly coherent tuneable laser diode. The optical frequency of the diode is controlled with help of a phase lock loop to an H-maser stabilized optical frequency comb at the same time. Then the beatnote of the laser diode and optical frequency comb controls the length of the passive cavity through a piezoelectric transducer. We demonstrated the generation of an etalon of a length in range of up to 1000 nm with picometer resolution. The measuring setup is able to evaluate scale non-linearity of a homodyne Michelson interferometer.

The other experimental setup has been developed for characterization of the coefficient of thermal expansion (CTE) of low-expansion materials. We verified the method with a 160 cm long rod of Zerodur ceramics. The rod was manufactured so as to be equipped with cavity mirrors attached via optical contact. The cavity was illuminated with a tenable laser diode. This diode was locked to a selected mode of the passive cavity. While the length of the cavity changed thanks to a temperature sweep of the low-expansion ceramics, the optical frequency of the locked laser detuned too. We observed a beatnote signal between the optical frequency comb and this locked laser diode. On basis of the beatnote signal we were able to calculate the length of the cavity and CTE as well. The resolution of the method is in picometer range and seems very promising for testing of some low CTE materials.

Digital derivative detection technique of the interference signal

We proposed a technique of the evaluation of the phase of the interference signal in displacement interferometry that is able to overcome the complexity of optics in homodyne detection as well as the need for a two-frequency laser source in heterodyne interferometry. The detection of the direction of motion – transfer of the signal to rotating phase representation is done by a digital derivative technique generating the additional cosine part of the signal through synchronous detection from the frequency modulated laser source. The technique brings a significant simplification of the detection chain to interferometry and has great potential for applications in industrial interferometry. The key advantage of the technique can be used in a case of interferometry with coherent semiconductor lasers allowing fast modulation via injection current. Extension of the technique is able to tackle residual amplitude modulation problem as well as nonlinearity correction.

SIB 60 - EMRP Long distance surveying with femtosecond lasers

Due to the involvement of our department in activities of the European metrology research program (EMRP), we are a partner in the project for absolute distance measurements of long distances with laser interferometers (SIB60 – Surveying). We did an experimental setup for the filtering of selected components of optical the frequency comb. The idea stems from multi-wavelength interferometry where the optical frequency comb produces a wide spectrum of narrow-linewidth components. The problem is that when those components are detected with a VIPA spectrometer, because of the small frequency difference between components some of these cannot be resolved well. Our filtering setup consists of a passive optical cavity equipped with a piezoelectric transducer for cavity length control. The cavity free spectral range is then tuned to an integer multiple of the frequency comb repetition rate. Then only some optical components are transmitted through the passive optical cavity. We proofed the principle and filtered spacing up to 1.5 GHz of optical frequency comb components has been demonstrated. Within this project we work in cooperation with the national metrology institute VSL Delft and Technical University of Delft, Netherland. We have also done verification measurements of our design of the passive cavity setup with control electronics on the optical frequency comb and VIPA spectrometer in the TU Delft.

Coordinate interferometric measurement

Coordinate, multi-axis measurement is a great field of applications of interferometric measuring methods and in combination with positioning represents a complex system that is a core technology in nanometrology, coordinate measuring machines, lithography, etc. We participate(d) in several projects oriented towards the development of various coordinate measuring systems.

Nanometrology standard for Czech Metrology Institute

The concept of instrumentation for nanometrology represents a combination of a high resolution microscopy technique with a measuring system capable of delivering the resolution and precision needed on the nanoscale with traceability to the primary length standard. The measurement of the sample is done by a coordinate length interferometer linked to the positioning system.

We developed and designed a nanometrology measuring setup which is a part of the national standard for nanometrology operated by the Czech Metrology Institute (CMI) in Brno, Czech Republic. The system employs a full six-axis interferometric position measurement of the sample holder consisting of six independent interferometers. This small range system is based on a commercial nanopositioning stage driven by piezoelectric transducers with the range (200 × 200 × 10) μm. The novel approach of our design is represented by a specially designed interferometer units with homodyne detection and thermal expansion compensation. The units with a flat mirror configuration show a very high sensitivity to the tilt of the target mirror. Together with an accurate alignment of the mirrors of the sample holder, this enables the alignment of the orthogonality of the measuring beams with a precision at the level of a few tens of arc seconds by angular adjustment of the measuring axis of each unit. This allows achievement of maximum contrast of the interference signal.

Micro-positioning systems with high dynamic range

Thanks to our involvement in the development of a nanometrology short-range positioning system we tried to extend the measuring range and to introduce positioning systems combined with high-precision measurement of the displacement for medium-range coordinate dimensional metrology.

We proposed two designs of positioning stages. The first one relying mainly on ball bearing straight guideways and a DC motor with a precise optical encoder serving as an actuator. This complex system achieves a quality performance with respect to repeatability and range of positioning along the X-Y axes. But thanks to the guideways with ball bearings, the Z-axis position stability of the stage is limited due to random displacements caused by the roughness of guiding rods and balls in the bearings.

The other concept uses a principle with flexible joints. This design is free from the unwanted random displacement along the Z-axis. If there are some displacements on the Z-axis, they are repeatable and predictable. The long range stage with a minimum of unwanted displacements along the Z-axis is in combination with flexible joints and piezoelectric motors where continuous movement can be provided without steps.

Interferometry systems for dimensional measurement in nanometrology

Development of interferometer systems for dimensional metrology and nanometrology is a result of a common collaborative project of applied research of our department and the company Meopta-optika. The main aim of the project is to arrive at the design of a complex interferometric measuring system in the form of a prototype serving as a master for further production. The concept is a modular family of components configurable for various arrangements primarily for multi-axis coordinate measurements in nanotechnology and surface inspection. Within the project we developed a compact, solid-state frequency stabilized laser referenced to iodine transitions and technology of iodine cells of simplified design for laser frequency stabilization. The company Meopta-optika contributed with the development of a new glass polishing technology and the manufacturing of the interferometer optics purely out of fused silica joined through optical contacting which signifies a great improvement in thermal stability and overall precision.

Within this project we proposed measuring setups for single-axis measurement through reflection from a corner-cube retroreflector, a configuration suitable for multi-axis coordinate systems measuring a displacement of a flat mirror and a novel concept of a differential interferometer with unified coaxial measuring and reference axes especially suitable for coordinate measuring systems where the preserving of Abbe principle is crucial.

Coordinate interferometry for e-beam lithography

Our experience in coordinate interferometric measuring systems resulted in our participation in a large collaborative project aimed at the development of an e-beam lithograph in a partnership with the company Tescan in Brno. High-precision positioning is a key technology in exposition of wafers and structures in the vacuum chamber. We proposed a combination of differential interferometers compensating for deformations of the vacuum chamber and laser sources and interferometer optics operating in the near-infrared region due to collision with acceptance spectral bands of highly sensitive detectors in the chamber. The project includes the design of the IR stabilized laser, optics, signal processing electronics and development of a complex instrumentation. The project is currently running and will continue of the next few years.

IND 58 6DoF Metrology for movement and positioning in six degrees of freedom

Within a framework of coordinate interferometric measurement research we participate in the EU funded collaborative project involving several European metrology and research laboratories. The main objective and focus of this part of the JRP is research in the field of nanometrology, namely techniques of topography measurement in the nanoscale for the purposes of fundamental and applied metrology. The research itself concentrates on the development of interferometric techniques for large scale measurement and positioning in all six degrees of freedom.

The tasks for our department are focused on the development of stabilized semiconductor laser sources suitable for interferometry. Laser sources stabilized on external references such as spectroscopy in molecular iodine or stabilization of pairs of laser diodes to a constant beat frequency needed for heterodyne detection schemes. The project is currently running and will continue throughout this year.

Optical fiber sensors for strain detection

Within our department we developed a technology for the manufacturing of phase-masks for fiber Bragg gratings (FBG) and a technology for manufacturing FBGs themselves acting as highly selective optical filters. We also developed the theoretical background and method for calculation of FBG spectral profiles. The technique was explored in a project for monitoring the deformations of shape of a containment building in a nuclear power plant in Temelín via a set of FBG strain sensors.

A super-luminescent LED as a broadband light source illuminates the set of sensors. An optical circulator transmits the light to the sensor network and receives the reflected part of the LED spectrum from each FBG filter at the same time. The first real operational test of the pilot FBG sensors was been done in cooperation with the company UJV Řež, CR and an installation on the containment is underway.

Technology of absorption cells for metrology of optical frequencies

The technology of absorption cells has been developed at the ISI for a long time; it follows the tradition of research in the field of highly coherent and stable lasers for metrology and interferometry. The Department of Coherence Optics established itself as a dominant and much respected supplier of the best quality absorption cells in the metrology community. In some aspects they are not only the dominant laboratory, but also the only one able to manufacture, prepare and fill especially custom designed cells for highly demanding applications including space research, optical clocks and national standards of optical frequencies. Our absorption cells – optical frequency references are in use at many world metrology laboratories.

The cells are made by welding high-quality fused silica even with large diameter optical quality windows. We also developed a technology of deposition of AR coatings on outer as well as inner surfaces of the cell windows. Thanks to our in-house technology of deposition of optical coatings we are able to optimize the AR coatings according to any specification. We mastered, and further perfected, methods of evaluation of the cell quality. This includes a measurement of the Stern-Vollmer coefficient, as it relates to the level of induced fluorescence, direct measurement of the absolute frequency shift by beat-frequency comparison with an optical frequency synthesizer, and also a technique of high-resolution line-profile scanning and linewidth measurement of the selected hyperfine subdoppler spectral components.

Technology of laser welding and adaptive control of the welding process

Thanks to a long-term experience of members of the Department of Coherence Optics with power laser industrial technologies we are involved in research in the field of laser welding. We investigated the physical phenomena associated with the welding process especially the behaviour of the plasma plume above the weld. We discovered that the properties of the frequency spectrum of radiation of the plasma plume generated by the laser welding process depend on the weld depth. The maximum depth generates a broader spectrum containing more frequency components and comes closer to the white noise spectrum. This has been exploited for generating a control value for a servo control loop.

Together with the novel concept of making use of adaptive mirrors, we put together a control algorithm adjusting on-line the position of the focal point and control of the welding process to achieve a high-quality weld with constant parameters. The principle has been tested on a host of samples and evaluated by metallography techniques.

Design and technology of optical coatings

An important part of the technology background of the Department of Coherence Optics is a laboratory for deposition of optical coatings well equipped with a state-of-the-art deposition apparatus with ion support and instrumentation necessary for measurement of the spectral properties of coatings. The technology again draws on a vast pool of knowledge and expertise in this field accumulated over decades with an older piece of instrumentation, now obsolete. Our ability to design and manufacture special coatings even on special substrates helped us to get involved in power laser technologies, deposition of low-loss mirrors and AR coatings for active optical components for pulsed lasers. We participated in project HiPER, European High Power laser Energy Research facility, dedicated to demonstrating laser driven fusion as a future energy source, with coatings for the power optical amplifiers. We also developed a methodology to measure the laser induced damage threshold (LIDT) of optical coatings under vacuum and cryogenic conditions.

Special electronics for laser control and signal processing

The staff of the Department of Coherence Optics consists of a mixed group of physicists and engineers as well. There has always been a significant effort invested in good engineering of the experimental apparatuses that have been built either for demonstration of a novel principle or for applied research projects. Several interesting and innovative designs in electronics should also be mentioned here as achievements in the field of technical science.

The technique of two-stage control of repetition and offset frequency of femtosecond comb has been developed with our staff. This technique employs the frequency lock loop and phase lock loop for both quantities at the same time. When the sensitive phase lock loop is dropped out thanks to some acoustic ripple signals the stage with frequency lock loop guards the comb frequency within a limited interval and phase lock loop is switched on again. The complex electronics were completely designed and manufactured within our team. In operation are two femtosecond combs, which we employed in our experiments.

We also exploited the detection technique for precise measurement of noise properties of single frequency lasers, which we stabilize to atomic reference based on absorption cells, high finesse passive cavities and a beatnote with an optical frequency comb. The degree of supressed frequency noise can be measured with an unbalanced Michelson interferometer with a frequency shift of the optical frequency of the laser in one arm of the interferometer. This heterodyne mixing is done with the help of an accousto-optic modulator and a fast photodetector in the output of the interferometer. In our setup, we have used a long spool of optical fiber for the unbalancing of the Michelson interferometer. The whole setup is based on fiber optic components so it is very robust. We have developed this detection technique for 1550 nm range and for visible parts of the spectrum.

Correlation identifier of transmitters for tracking of small vertebrates

Slightly apart from the laser, metrology and optoelectronic direction of the department may be an automated system for UHF radio tracking of animals. It may be considered a novel, state-of-the-art result of methodological research in engineering science for fundamental zoological research.

Radiotracking is important. It’s often the only possible method to explore specific habits and the behavior of animals. We developed a system in cooperation with leading

Czech zoologists where a substantial qualitative and quantitative improvement in spatio-temporal behavioural data collection was desired.

The components included lightweight automated tracking stations easily transportable and deployable in the area of interest. The station consists of an antenna array with omnidirectional coverage, digitally controlled custom radio receiver, and a single-chip based computer system. The stations read the signals from transmitters, typically over a distance of several km (depending on the transmitter), and record their position. The primary position data from multiple stations and manual tracking are later combined to obtain more precise positional information. The resulting performance of the system in terms of bearing estimation accuracy has been vastly field tested under harsh conditions in Turkey, Cyprus and Egypt on fruit bats.