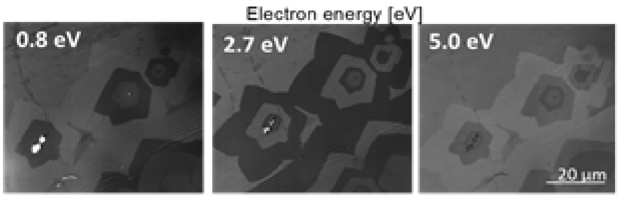
Description of the main research directions investigated by the institute

The main research directions of the ISI are linked with the research directions of individual teams and follow the organizational structure of the ISI.

Electron Microscopy

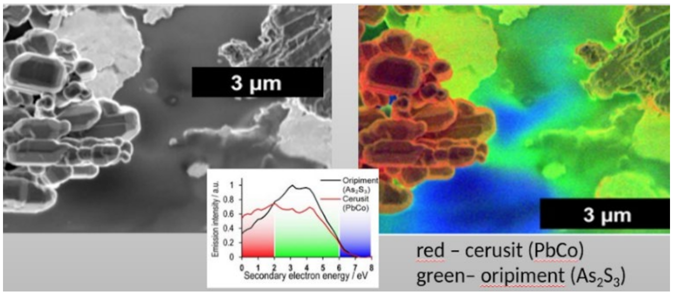
- **Development of cutting-edge methods of scanning electron microscopy (SEM)** that serve as unique tools in both fundamental and applied research in biology, medicine, chemistry, and materials and surface sciences.
- **Leading position** in
  - **Low-energy SEM** - electron microscopy imaging at very low primary beam energies, from units of eV up to 1 keV. This is an extremely surface-sensitive method, which provides crystallographic and surface-potential contrast when combined with ultrahigh-vacuum techniques.
  - **Hyperspectral and quantitative imaging in SEM** - provides additional dimensions to the SEM images. Specifically, hyperspectral imaging utilizes energy filtering to visualize small, nanometer-scale differences in the sample stoichiometry or elemental composition. Quantitative SEM imaging derives quantitative information, such as the local thickness or sample's mass density, from each image pixel. Correlation of various signals then enhances the information content of SEM images acquired from investigated samples.
  - **Cryo-SEM techniques** - allow for high-resolution imaging of biological samples and their inner structure using freeze-fracturing techniques combined with focused ion-beam techniques.
  - **Environmental SEM -** the perfect tool for SEM imaging and analysis of wet biological samples in their native state. The increased gas pressure in the sample's vicinity prevents sample degradation and makes it possible to investigate biological and chemical processes in-situ.
- **Applications of the developed methods,** in collaboration with academic and industrial partners, in
  - advanced characterization of 2D materials,
  - development of new steels,
  - imaging of plant cells, diatoms, and even small live animals in their native state
  - characterization of microbial biofilms,
  - investigation of the structure of hydrogels,
  - characterization of production of biopolymers by bacteria,
  - morphological characterization of ice under dynamically changing conditions.
- **The most remarkable achievements**
  - study of advanced 2-D materials (e.g., graphene) by low-energy electrons[1],



  - application of Super Low-Energy Scanning Electron Microscopy (SLESEM) technique, originally developed at the ISI, to the characterization of advanced steels and other products of JFE Steel Corporation[2],

[1] Frank L, Mikmeková E, Müllerová I, Lejeune M. *Counting graphene layers with very slow electrons*. Applied Physics Letters, 106, 013117 (2015).

- a new in situ method that uses low-energy electrons to clean the sample surface from hydrocarbon contamination to provide undistorted SEM imaging[3],
- a unique instrument combining ultra-high vacuum (UHV) scanning low energy electron microscope (SLEEM) imaging and time of flight (ToF) spectroscopy for analysis of 2D materials for the next generation of electronic devices,
- demonstration of a unique detection system for ESEM appropriate for observation of highly sensitive samples, with the net electron dose about a hundred times lower comparing to the commercial detectors[4],
- filtering of the secondary electrons (hyperspectral imaging in SEM) was applied to medieval paintings to distinguish pigments cerussite and orpiment that are difficult to discern by standard methods, e.g., by energy-dispersive X-ray spectroscopy[5],



- extension of quantitative imaging in SEM (i.e., some physical quantity other than intensity is associated to the pixel at the detector) to commercial detectors to enhance SEM characterization methods[6],
- investigation of hydrogels, biofilms, and biopolymer granules inside cells by combined methods of Cryo-SEM and Raman microspectroscopy[7],
- development of an original method for imaging frozen aqueous solutions or even naked ice crystallization in ESEM.[8]

New Technologies

- **Enhancement of technological processes and methods** to satisfy the demands of the ISI teams and external academic and industrial partners.
- **Mastered technologies**
  - **Electron-beam lithography** - uses two electron-beam writers to manufacture large-size, microstructured diffractive optical elements for laser beam shaping, sub-micron diffractive holographic structures for industrial holography applications, and thin-film metallic and dielectric structures on silicon substrates for biosensors and conductive chemical sensors.

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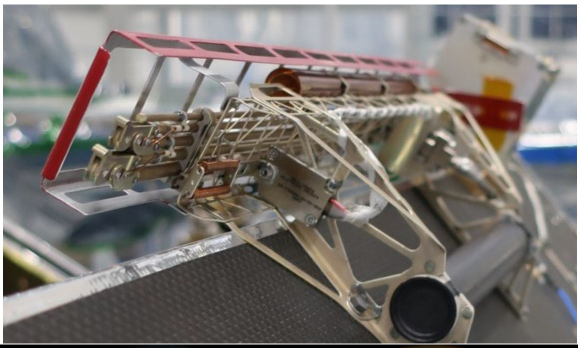
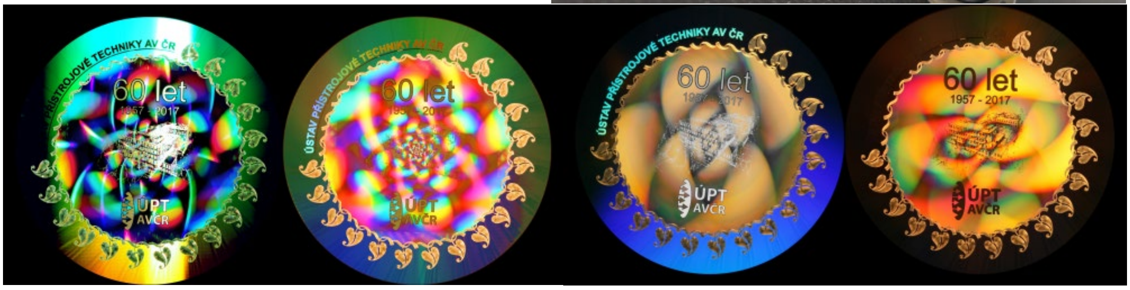
[2] Aoyama T, Mikmeková Š, Hibino H, Okuda K. *Visualization of three different phases in multiphase steel by scanning electron microscopy at 1 eV landing energy*. Ultramicroscopy, 204, 1–5 (2019).
[3] Frank L, Mikmeková E, Lejeune M. *Treatment of surfaces with low-energy electrons.* Applied Surface Science, 407, 105–108 (2017).
[4] Neděla V, Tihlaříková E, Runštuk J, Hudec J. *High-efficiency detector of secondary and backscattered electrons for low-dose imaging in the ESEM.* Ultramicroscopy, 184, 1–11 (2018).
[5] Abrams KJ, Dapor M, Stehling N, Azzolini M, Kyle SJ, Schäfer J, Quade A, Mika F, Krátký S, Pokorná Z, Konvalina I, Mehta D, Black K, Rodenburg C. *Making Sense of Complex Carbon and Metal/Carbon Systems by Secondary Electron Hyperspectral Imaging.* Advanced Science, 6, 1900719 (2019).
[6] Knötigová PT, at al. *Application of Advanced Microscopic Methods to Study the Interaction of Carboxylated Fluorescent Nanodiamonds with Membrane Structures in THP-1 Cells: Activation of Inflammasome NLRP3 as the Result of Lysosome Destabilization.* Molecular Pharmaceutics, 16, 3441–3451 (2019).
[7] Hrubanová K, Krzyžánek V, Nebesářová J, Růžička F, Pilát Z, Samek O. *Monitoring Candida parapsilosis and Staphylococcus epidermidis Biofilms by a Combination of Scanning Electron Microscopy and Raman Spectroscopy.* Sensors, 18, 4089 (2018).
[8] Yang X, Neděla V, Runštuk J, Ondrušková G, Krausko J, Vetráková Ľ, Heger D. *Evaporating brine from frost flowers with electron microscopy and implications for atmospheric chemistry and sea-salt aerosol formation.* Atmospheric Chemistry and Physics, 17, 6291–6303 (2017).

- **Thin-layer deposition and characterization** – focuses on a wide variety of coatings made of Al, Si, Mo, Ti, Ni, Ag, C, ITO, Nb, W, TiN Si_3N_4, SiO_2 or their combinations deposited by magnetron sputtering. It prepares multi-layered systems for X-ray optics with individual double-layers of nanometer thickness, total thickness of tenths of micrometers and accuracy of tenths of nanometers. The only laboratory in the Czech Republic where the hard, wear-resistant coatings can be characterized by an originally developed impact dynamic tester.
- **Electron-beam technologies** - deal with applications of intense focused electron beams, vacuum brazing and glass-sealed vacuum feedthrough technology. They manufacture vacuum tight joints of metal parts made of steel, stainless steel, copper alloys, titanium alloys, zirconium alloys, high-temperature resistant super-alloys such as Inconel, and weld / solder various combinations of these materials. They developed a new method of joining metals with brittle non-metal materials by brazing using ductile active brazing solders and of electron beam welding of metal materials with different physical properties.
- design and manufacturing of special on-demand instruments.

- **The most remarkable achievements**
  - the number of industrial and academic partners exceeded 75,
  - the total revenue was around 880.000 Euro during the last five years,
  - construction of a precise 100 kV power supply for the field emission gun and a reference divider for TESCAN company,
  - design and manufacturing of a spring-powered self-erecting rod antenna for the detector of electric field for ExoMars mission coordinated by the European Space Agency and the Russian space agency,



  - fabrication of diffractive optically variable image devices (DOVID) [9,10],



  - manufacturing of multilayer mirrors for the X-ray and extreme ultraviolet spectral domains with reproducibility of the bilayer thickness better than 0.1 nm - unique position in the Czech Republic and in the EU,
  - various metal joints of the highest quality for more than 50 industrial partners from the automotive, nuclear research, heavy machinery, and aviation sectors.

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[9] Horáček M, Matějka M, Krátký S.Meluzín P, Kolařík V. *Diffractive Optically Variable Image Device* (2018). Czech utility model no. 30627.

[10] Horáček M, Kolařík V. *An optically variable imaging device and the method of its preparation* (2018). Czech patent no. 306956.

Magnetic Resonance

- **Development and application of techniques for quantitative nuclear magnetic resonance (NMR, MR) imaging and spectroscopy** for biomedical R&D, involving
  - research into the mechanisms adversely affecting the accuracy, precision or speed of MR measurements,
  - design of pulse sequences for specific applications,
  - development of advanced data models and efficient analysis techniques,
  - utilization of the state-of-the-art equipment (9.4T small animal MR scanner and a supporting animal facility),
  - access of external researchers to a wide range of established and in-house developed MR techniques available at the MR open access facility, which is a part of the **Czech-BioImaging and Euro-BioImaging infrastructures**.
- **Leading position in NMR physics, physiology modelling and computer simulations**, which is employed in
  - perfusion measurements for quantitative pharmacokinetics,
  - spectroscopy and spectroscopic imaging for metabolomics,
  - advanced diffusometry,
  - application-driven development of specialized MR imaging,
  - utilization of animal models (mouse, rat, rabbit) to support research of external partners in (patho)physiology, testing of therapies or development of theranostic approaches for precision medicine,
  - complex support for project preparation, experiment design, evaluation and interpretation for biomedical research partners.
- **The most remarkable achievements**
  - while dealing with advanced pharmacokinetics models of perfusion measured by MR, we developed procedures suppressing sensitivity to noise and image artifacts, which allows 3D data acquisition[11],
  - for the perfusion analysis, software PerfLab has been developed, including a web-interfaced version providing its tools to the public,
  - the above perfusion techniques represented the key element involved in the study of pathophysiology of schizophrenia, whose findings support the hypothesis that in schizophrenia, functional changes precede structural ones[12]
  - J. Starčuková coordinates the development of jMRUI software [13] for quantitative analysis of MR spectroscopic signals by decomposition of the signal into single-metabolite components[14]. jMRUI has currently about 5000 licensees from all over the world.
  - in vitro and in vivo tests of experimental contrast agents and potential drug carriers based on graphene,[15]

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[11] Bartoš M, Rajmic P, Šorel M, Mangová M, Keunen O, Jiřík R. *Spatially regularized estimation of the tissue homogeneity model parameters in DCE-MRI using proximal minimization.* Magnetic Resonance in Medicine, 82, 2257-2272 (2019).

[12] Horská K, Rudá-Kučerová J, Dražanová E, Karpíšek M, Demlová R, Kašpárek T, Kotolová H. *Aripiprazole-induced adverse metabolic alterations in polyI:C neurodevelopmental model of schizophrenia in rats,* Neuropharmacology. 123, 148-158 (2017).

[13] http://www.jmrui.eu/

[14] Starčuk jr Z, Starčuková J. *Quantum-mechanical simulations for in vivo MR spectroscopy: Principles and possibilities demonstrated with the program NMRScopeB,* Analytical Biochemistry, 529, 79-97 (2017).

[15] Tuček J, Sofer Z, Bouša D, Pumera M, Holá K, Malá A, Poláková K, Havrdová M, Čépe K, Tomanec O, Zbořil R. *Air-stable superparamagnetic metal nanoparticles entrapped in graphene oxide matrix.* Nature Communications, 7, 12879:1-11 (2016).

- MR detection of water diffusion supported the hypothesis of reduced cytosolic water-molecule mobility in genetically modified animal models of Parkinson's disease[16], which could become an objective early-stage diagnostic marker.
- development of an original MR technique for a clinical human 3T scanner allowing direct imaging of myelin, a semisolid substance playing an important role in some neurodegenerative diseases; the same method has also been applied for MR imaging of tendons.[17]

Cryogenics

- Fundamental research focused on
  - **heat transport** mechanisms by **turbulent flows** and **electromagnetic radiation,**
  - **phase transitions in quantum interacting many-body systems**.
- Applied research activities involved in investigation of
  - radiative properties (emissivity and absorptivity) of materials with various surface finishing used in cryogenic applications,
  - heat transport properties of multilayer insulations
  - R&D of components for electron microscopy operated at cryogenic temperatures.
- **The most remarkable achievements**
  - utilization of a unique experimental system, built at the ISI, to determine the scaling of Reynolds number with Rayleigh number in turbulent fluids[18],
  - disprovement of the statement on the experimental observation of the ultimate state of Oberbeck-Boussinesq Rayleigh-Bénard turbulent convection[19]. Existence of such a state would enable extrapolation of laboratory results to extremely large-scale turbulent natural phenomena, not observable in the laboratory.
  - prediction and experimental demonstration of a strong dependence of the transition of low-temperature superconductors to superconductivity on near-field radiative heat transfer, which could be used to control the heat transfer[20]
  - extension of systematic theoretical approach to excited-state quantum phase transitions to truly many-body quantum systems, scrutinizing the interacting boson model of nuclear collective dynamics as an example[21]; generalization of this theoretical approach to 1D and 2D crystals (lattice systems)[22],
  - utilization of a unique home-built experimental system for the determination of hemispherical emissivity and absorptivity of various materials from room to

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[16] Arab A, Rudá-Kučerová J, Minsterová A, Dražanová E, Szabó N, Starčuk jr Z, Rektorová I, Khairnar A. *Diffusion Kurtosis Imaging Detects Microstructural Changes in a Methamphetamine-Induced Mouse Model of Parkinson's Disease.* Neurotoxicity Research, 36, 724-735 (2019).

[17] Latta P, Starčuk jr Z, Gruwel MLH, Lattova B, Lattova P, Štourač P, Tománek B. *Influence of k-space trajectory corrections on proton density mapping with ultrashort echo time imaging: Application for imaging of short T2 components in white matter.* Magnetic Resonance Imaging, 51, 87-95 (2018).

[18] Musilová V, Králík T, La Mantia M, Macek M. Urban P, Skrbek L. *Reynolds number scaling in cryogenic turbulent Rayleigh-Benard convection in a cylindrical aspect ratio one cell.* Journal of Fluid Mechanics, 832, 721-744 (2017).

[19] Skrbek L, Urban P. *Has the ultimate state of turbulent thermal convection been observed?* Journal of Fluid Mechanics, 785, 270-282 (2015).
Urban P, Hanzelka P, Králík T, Macek M, Musilová V, Skrbek L. *Elusive transition to the ultimate regime of turbulent Rayleigh-Benard convection.* Physical Review E, 99, 011101 (2019).

[20] Musilová V, Králík T, Fořt T. Macek M. *Strong suppression of near-field radiative heat transfer by superconductivity in NbN*. Physical Review B, 99, 024511 (2019).

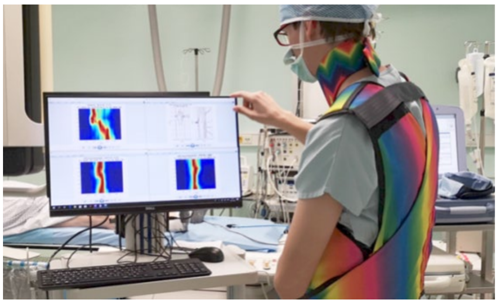
[21] Macek M, Stránský P, Leviatan A, Cejnar P. *Excited-state quantum phase transitions in systems with two degrees of freedom, III, Interacting boson systems.* Physical Review C, 99, 064323 (2019).

[22] Dietz B, Iachello F, Macek M. *Algebraic Theory of Crystal Vibrations: Localization Properties of Wave Functions in Two-Dimensional Lattices.* Crystals, 7, 246 (2017).

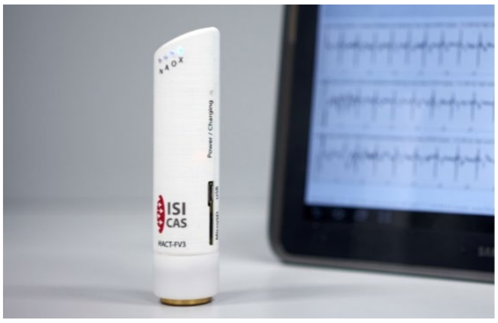
cryogenic temperatures and determination of an optimal surface treatment providing the lowest emissivity and absorptivity[23],
  - assessment of radiative properties of metallic and polyimide foils contained inside multi-layer insulation and of gold coatings used for components of the third generation of meteorological satellites of RUAG Space Company and Frentech Aerospace company,
  - development of low-temperature parts of an UHV SEM/SPM modular complex system serving for in situ fabrication and characterization of nanostructures under UHV conditions in a wide temperature range of 20 K-700 K.

Medical Signals

- Research and development of **new methods** based on cutting-edge technologies with **high potential for effective treatment in human cardiology and neurology**.
- **Introduced breakthrough technologies**
  - worldwide new field of cardiac electrophysiology - **ultra-high-frequency electrocardiography (100-1000 Hz)** - which measures the temporal and spatial distribution of myocardial cell depolarization,
  - acquisition, by standard clinical deep brain electrodes, and processing **of very and ultra-fast oscillations (up to 2000 Hz) in the human brain** serving as markers of areas of epileptic activity[24],
  - **ventricular dyssynchrony imaging (VDI) technology** that is easy to implement into clinical care through standard 12(14)-lead ECG setup and is currently tested in 6 hospitals to monitor in real-time ventricular dyssynchrony, e.g., during implantation and positioning of pacing electrodes and to optimize the settings of cardiac resynchronization therapy (CRT) device[25],



  - **fast, handheld arrhythmia detector with on-chip artificial intelligence**, which is able to determine, after a 15s long measurement, if ECG contains atrial fibrillation, other arrhythmias, or regular sinusoidal rhythm,



  - **whole-body multichannel impedance cardiography**, which provides the time

[23] Frolec J, Králík T, Srnka A. *Low temperature thermal radiative properties of gold coated metals.* International Journal of Refrigeration, 82, 51-55 (2017).
Králík T, Musilová V, Hanzelka P, Frolec J, *Method for measurement of emissivity and absorptivity of highly reflective surfaces from 20 K to room temperatures.* Metrologia, 53, 743-753 (2016).
Frolec J, Králík T, Musilová V, Hanzelka P, Srnka A, Jelínek J. *A database of metallic materials emissivities and absorptivities for cryogenics.* Cryogenics, 97, 85-99 (2019).
[24] Brázdil M, Pail M, Halámek J, Plešinger F, Cimbálník J, Roman R, Klimeš P, Daniel P, Chrastina J, Brichtová E, Rektor I, Worrell GA, Jurák P. *Very High-Frequency Oscillations: Novel Biomarkers of the Epileptogenic Zone.* Annals of Neurology, 82, 299-310 (2017).
Rektor I, Doležalová I, Chrastina J, Jurák P, Halámek J, Baláž, M., Brázdil M, *High-Frequency Oscillations in the Human Anterior Nucleus of the Thalamus.* Brain Stimulation, 9, 629-631 (2016).
[25] U.S. Pat. No. 9,949,655, European Patent Application, EP 19212534.2. European Society of Cardiology and Computing in Caridology: Clinical Needs Translation Award (CTA) (2017)

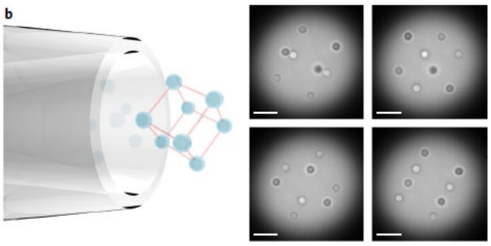
course of pressure-related waves from the whole body at a single time instant and indicates the condition of blood-vessel system [26],

The most remarkable achievements

- implementation of machine deep-learning on large data sets from deep brain structures to identify pathological and physiological areas (e.g., epileptic foci[27]), functional organization and mutual communication between brain structures[28],
- design of methods that can reliably identify heart activity in noisy data, e.g., from ECG Holter, using deep-learning methods[29],
- development of open-platform software SignalPlant for the analysis of large data sets in cardiology and neurology (used in 48 countries world-wide).

Microphotonics

- Forefront interdisciplinary research focused on the **interaction of laser light with matter** at the levels ranging from macroscopic down to quantum.
- Particular focus on:
  - employment of spatially shaped laser beams for various types of **optical micromanipulation**, e.g., sorting, self-arrangement, and delivery of nanoobjects, microobjects, and living cells of various material properties, shapes and sizes,
  - utilization of objects levitating in laser beams in vacuum for deeper understanding of the dynamics of **nonlinear stochastic systems** and **transition from classical to quantum behavior,**
  - development of **imaging and diagnostic methods for medicine, microbiology and biochemistry** that merge laser beams, optical detection, microfluidics and fiber technology and operate on the level of individual cells or even molecules,
  - design and manufacturing of complex, **custom-made experimental setups** for external academic and industrial partners.
- **The most remarkable achievements**
  - an elegant demonstration of detectability of the optical spin force using an optically levitated microparticle[30],
  - deployment of maneuverable arrays of holographic optical traps through hair-thin flexible optical fibers capable of penetrating through complex media, including living



[26] Soukup L, Hrušková J, Jurák P, Halámek J, Závodná E, Viščor I, Matějková M, Vondra V, *Comparison of noninvasive pulse transit time determined from Doppler aortic flow and multichannel bioimpedance plethysmography.* Medical & Biological Engineering & Computing, 57, 1151-1158 (2019).

[27] Nejedlý, P, Cimbálník J, Klimeš P, Plešinger F, Halámek J, Křemen V, Viščor I, Brinkmann B, Pail M, Brázdil M, Worell G, Jurák P, *Intracerebral EEG Artifact Identification Using Convolutional Neural Networks.* Neuroinformatics, 17, 225-234 (2019).

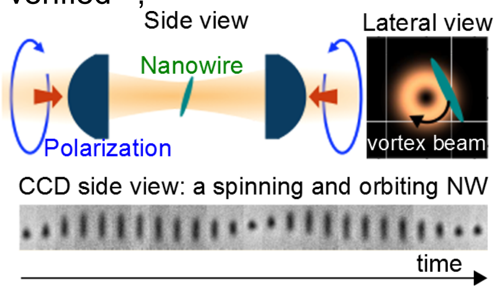
[28] Klimeš P, Jurák P, Halámek J, Roman R, Chládek J, Brázdil M, *Changes in connectivity and local synchrony after cognitive stimulation Intracerebral EEG study.* Biomedical Signal Processing and Control, 45, 136-143 (2018).

[29] Plešinger F, Nejedlý P, Viščor I, Halámek J, Jurák P, *Parallel use of a convolutional neural network and bagged tree ensemble for the classification of Holter ECG.* Physiol. Meas. 39, 094002 (2018).

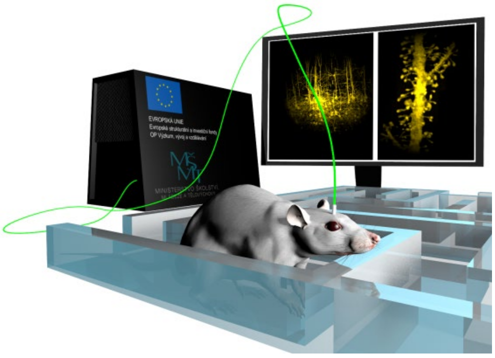
[30] Svak V, Brzobohatý O, Šiler M, Jákl P, Kaňka J, Zemánek P, Simpson SH. *Transverse spin forces and non-equilibrium particle dynamics in a circularly polarized vacuum optical trap*. Nature Communications, 9, 5453 (2018).

tissues, without causing any major damage[31],

- a new approach to simultaneous transport of many particles in any direction in a plane using an array of asymmetric optical potential wells – so called optical ratchets[32],
- investigation of optically trapped particles moving in strongly nonlinear-potentials[33] and proposal of a new theoretical approach for describing highly unstable systems, which was experimentally verified[34],
- theoretical and experimental studies leading to understanding of stochastic translational, rotational and synchronized behavior of silicon nanowires in shaped optical traps of various polarization properties[35,36,37],



- demonstration of the strong influence of size- and shape-dependent plasmonic resonances on the stability of 3D trapping of larger Au nanoparticles[38],
- development and testing of high-resolution ultra-thin endoscopes, capable of imaging biological structures deep inside the tissue of small animal models[39] and its extension to label-free non-linear microscopy with chemical contrast using coherent anti-Stokes Raman scattering (CARS) through a multimode fiber endoscope[40],



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[31] Leite IT, Turtaev S, Jiang X, Šiler M, Cuschieri A, Russell PSt. J, Čižmár T. *Three-dimensional holographic optical manipulation through a high-numerical-aperture soft-glass multimode fibre*. Nature Photonics, 12, 33–39 (2018).
[32] Arzola AV, Villasante-Barahona M, Volke-Sepúlveda K, Jákl P, Zemánek P. *Omnidirectional Transport in Fully Reconfigurable Two Dimensional Optical Ratchets*. Phys. Rev. Lett., 118, 138002 (2017).
[33] Šiler M, Jákl P, Brzobohatý O, Ryabov A, Filip R, Zemánek P. *Thermally induced micro-motion by inflection in optical potential.* Scientific Reports, 7, 1697 (2017).
[34] Šiler M, Ornigotti L, Brzobohatý O, Jákl P, Ryabov A, Holubec V, Zemánek P, Filip R. *Diffusing up the Hill: Dynamics and Equipartition in Highly Unstable Systems*. Phys. Rev. Lett., 121, 23601 (2018).
[35] Irrera A, Magazzu A, Artoni P, Simpson SH, Hanna S, Jones PH, Priolo F, Gucciardi P Giuseppe, Maragò OM. *Photonic Torque Microscopy of the Nonconservative Force Field for Optically Trapped Silicon Nanowires*. Nano Letters, 16, 4181-4188 (2016).
[36] Simpson SH, Zemánek P, Maragò OM, Jones PH, Hanna S. *Optical Binding of Nanowires*. Nano Letters, 17, 3485-3492 (2017).
[37] Donato MG, Brzobohatý O, Simpson SH, Irrera A, Leonardi AA, Faro MJLo, Svak V, Maragò OM, Zemánek P. *Optical Trapping, Optical Binding, and Rotational Dynamics of Silicon Nanowires in Counter-Propagating Beams*. Nano Letters, 19, 342-352 (2019).
[38] Brzobohatý O, Šiler M, Trojek J, Chvátal L, Karásek V, Paták A, Pokorná Z, Mika F, Zemánek P. *Three-Dimensional Optical Trapping of a Plasmonic Nanoparticle using Low Numerical Aperture Optical Tweezers*. Scientific Reports, 5, 8106 (2015).
[39] Flaes DEB, Stopka J, Turtaev S, de Boer JF, Tyc T, Čižmár T. *Robustness of Light-Transport Processes to Bending Deformations in Graded-Index Multimode Waveguides*. Phys. Rev. Lett., 120, 233901 (2018).
Turtaev S, Leite IT, Altwegg-Boussac T, Pakan JMP, Rochefort NL, Čižmár T. *High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging*. Light: Science & Applications, 7, 92 (2018).
Vasquez-Lopez SA, Turcotte R, Koren V, Ploschner M, Padamsey Z, Booth M, Čižmár T, Emptage NJ. *Subcellular spatial resolution achieved for deep-brain imaging in vivo using a minimally invasive multimode fiber*. Light: Science & Applications, 7, 110 (2018).
[40] Trägårdh J, Pikálek T, Šerý M, Meyer T, Popp J, Čižmár T. *Label-free CARS microscopy through a multimode fiber endoscope.* Optics Express, 27, 30055–30066 (2019).

- development of Raman micro-spectroscopic methods for fast identification of various microorganisms using machine learning algorithms[41], investigation of influence of antibiotic treatment on individual microbial cells[42] and monitoring of intracellular concentration and composition of biopolymers[43]
- optical printing (by laser radiation pressure) of surface-enhanced Raman scattering (SERS) active substrates directly in microfluidic chips for ultrasensitive SERS-based detection of proteins down to pM concentration[44].
- custom-initiated activities for external academic and industrial partners, including development of hyperspectral imaging cameras for repeatable non-contact investigation of plant physiology in the spectral regions 350-900 nm or 900-1700 nm and light-sheet microscope providing 3D fluorescent imaging of extended biological samples at 488 nm, 561 nm a 647 nm.

Coherence Optics

- Contribution to the **forefront research** in fundamental optical metrology and laser technologies and to the **competitiveness of the Czech industry,** especially of **innovative small and medium-sized** companies.
- **Particular focus on**
  - **quantum metrology based on laser cooled calcium ion clocks,**
  - **transfers of highly stable optical frequencies** over fiber-optic links in the CR and within the EU as part of two H2020 projects,
  - **development of highly coherent laser sources for** dimensional metrology (interferometry), laboratory applications, industrial practice, and telecom applications
  - development of **interferometric systems for nanometrology and precision positioning**
  - **development of optical sensors for length measurement**
  - monitoring and control of **laser-based welding process**
  - **design and deposition of optical thin films.**
- **The most remarkable achievements**
  - a unique three-axis (x, y and yaw) module for precise, high-resolution wafer positioning designed and built for electron-beam lithography systems of the Raith Nanofabrication, GmbH and TESCAN.
  - introduction of several novel methods of interferometry driven by optical frequency combs with femtosecond pulsed lasers that are appropriate for absolute measurement of long distances[45], e.g., for the calibration of global positioning systems within the EU program EMRP.

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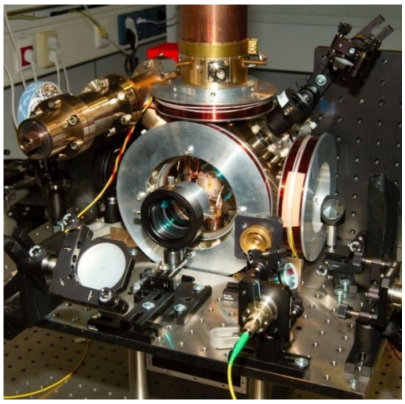
[41] Rebrošová K, Šiler M, Samek O, Růžička F, Bernatová S, Holá V, Ježek J, Zemánek P, Sokolová J, Petráš P. *Rapid identification of staphylococci by Raman spectroscopy*. Scientific Reports, 7, 14846 (2017).

[42] Pilát Z, Bernatová S, Ježek J, Kirchhoff J, Tannert A, Neugebauer U, Samek O, Zemánek P. *Microfluidic Cultivation and Laser Tweezers Raman Spectroscopy of E-coli under Antibiotic Stress.* Sensors, 18 1623 (2018).

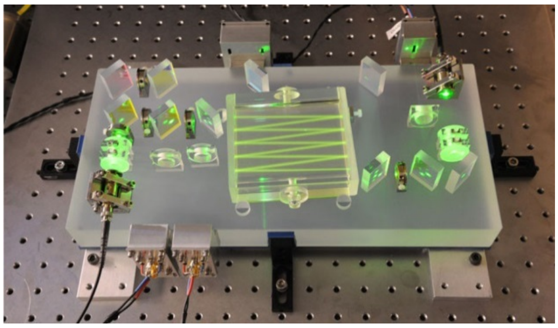
[43] Samek O, Obruča S, Šiler M, Sedláček P, Benešová P, Kučera D, Márová I, Ježek J, Bernatová S, Zemánek P. *Quantitative Raman Spectroscopy Analysis of Polyhydroxyalkanoates Produced by Cupriavidus necator H16*. Sensors, 16, 1808 (2016) plus 4 other publications

[44] Bernatová S, Donato MGrazia, Ježek J, Pilát Z, Samek O, Magazzu A, Maragò OM, Zemánek P, Gucciardi PG. *Wavelength-Dependent Optical Force Aggregation of Gold Nanorods for SERS in a Microfluidic Chip*. J. Phys. Chem. C, 123, 5608-5615 (2019).

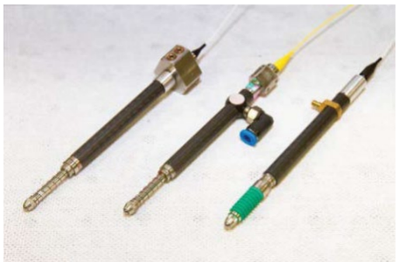
[45] Lešundák A, Voigt D, Číp O, van der Berg M, *High-accuracy long distance measurements with a mode-filtered frequency comb.,* Optics Express, 25, 32570-32580 (2017).



- assembling and putting into operation a very complex setup for trapping and laser cooling of Ca^{2+} ions, which serves as an experimental testing platform of quantum technologies[46], e.g., ion clocks or utilization of unique properties of large cold Coulomb crystals (in collaboration with several EU research groups).
- establishment and experimental operation of a network of coherent fiber-optic links for distribution of precise optical frequencies, which is planned to become a part of a Europe-wide infrastructure[47]



- the ISI is the leading provider of optical frequency references – absorption cells for the worldwide metrology community, including compact multi-pass cells for space programs (in the figure), one of which was launched as a part of TEXUS 54 mission.
- our system of fiber Bragg grating strain sensors was implemented in nuclear power plant in Temelín for the measurement of dimension changes of the containment of the nuclear reactor. The Czech State Office for Nuclear Safety has recently approved this technology and there is also a significant interest from operators of nuclear power plants equipped with similar containments in Ukraine and Russia.



- development of a laser interferometer displacement sensor with a small form-factor[48], compatible with that of the 8 mm pencil-gauge (in collaboration with Messing company).

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[46] Obšil P, Lachman L, Pham MT, Lešundák A, Hucl V, Čížek M, Hrabina J, Číp O, Slodička L, Filip, R. *Nonclassical Light from Large Ensembles of Trapped Ions.* Physical Review Letters, 120, 253602 (2018).
Obšil P, Lešundák A, Pham MT, Araneda G, Čížek M, Číp O, Filip R, Slodička L, *Multipath interference from large trapped-ion-chains.* New Journal of Physics, 21, 093039 (2019).
[47] Czech utility model no. 32014
[48] Czech utility model No. 33046

Research activity and characterisation of the main scientific results

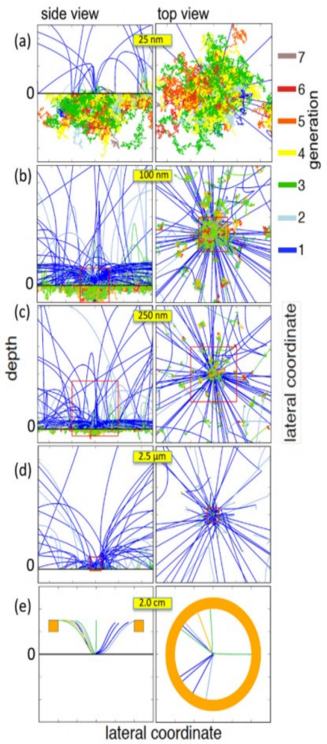
The electron microscopy department's research in the evaluated period was based on the previously established research topics of Low energy SEM, Cryo-SEM, and Environmental SEM. These research fields were further complemented with Quantitative and Hyperspectral imaging and Correlative microscopy techniques. Contrary to the previous evaluation period, we emphasize practical applications of the developed methods by increasing cooperation with partners both in the industry and academia. We found that unique applications of our methods require experts from specific fields, and so we hired specialists in chemistry and biology. We also established a new group for low energy SEM applications in metallography and materials sciences.

Electron optics and instrumentation

The development of cutting-edge electron microscopy methods with a high application impact is possible due to our extensive knowledge in electron microscopy, electron optics, and complementary instrumentation. Especially in low-energy SEM, environmental SEM, and quantitative microscopy, progress cannot be accomplished using standard instruments. We must be able to construct entire new specialized electron optical systems or add-ons such as novel electron-optical elements, detectors, and sample manipulation assemblies.

Signal formation in Scanning Field–emission Microscopy

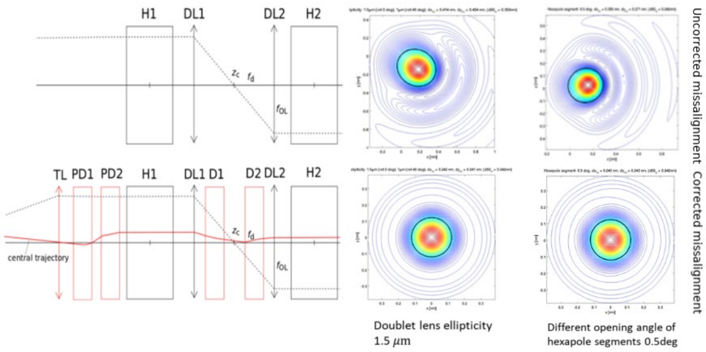
The Scanning Field-emission Microscope is essentially a Scanning Tunneling Microscope operated in the field emission regime. By applying a negative bias to the tip, field-emitted electrons are focused and accelerated toward the sample by the strong field between the tip and the surface. Subsequent interaction with the solid leads to the emission of a spectrum of electrons, which are detected at macroscopic distances. By scanning the tip, images of the topographic contrast and the magnetic signal of a sample have been obtained. The contrast mechanism had until now been far from understood. We described the signal generation mechanism via model calculations combining deterministic trajectory calculations in the field surrounding the field-emission tip in a vacuum, with Monte Carlo simulations of the electron transport inside the solid. This model gives rise to a two-dimensional electron cascade. Individual trajectories of detected backscattered electrons consist of repeated segments of travel in vacuum followed by re-entry into the solid and re-emission into the vacuum after being elastically or inelastically scattered.



Werner, W. S. M., Oral, M., Radlička, T., Zelinka, J., Müllerová, I., Bellissimo, A., … Gürlü, O. Scanning tunneling microscopy in the field-emission regime: Formation of a two-dimensional electron cascade. Applied Physics Letters, 115, 251604 (2019).

Electron-optical methods

The electron optics group uses commercially available software, Comsol and Simion, for the calculation of electromagnetic fields in 2D, such as round lenses and multipoles, and general 3-D fields, and with the EOD software for electron-optical calculations. We also write our own software, such as a program for general aberration calculations based on the differential-algebraic method, a program for wave-optical spot calculations, and a program for efficient computation of trajectories in general electromagnetic fields. This software combined makes it possible for us to calculate general electron optical systems. This is used to design electron-optical instruments at the ISI and in contractual research in cooperation with electron microscope manufacturers (with a budget of about 1 million CZK per year). We have successfully applied our expertise in designing a hexapole corrector for the SEM, in the design of a back-scattered electron detector with energy filtering, or simulation of space-charge limited emission.



Correction of misalignment aberration in the Rose hexapole corrector

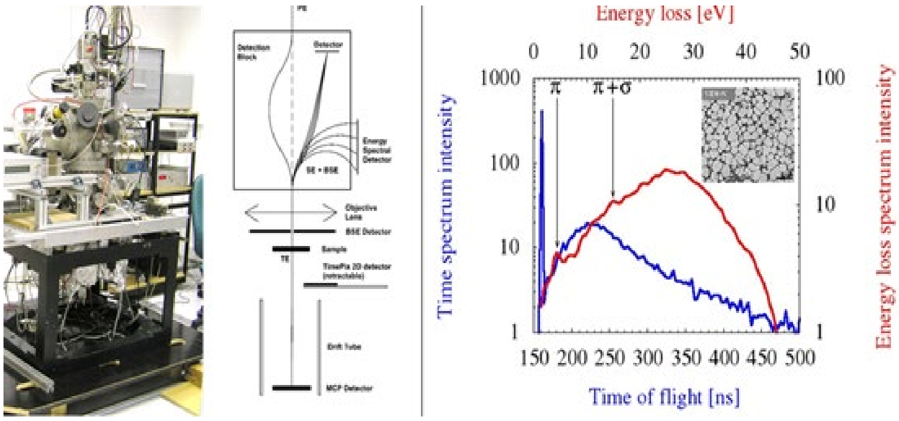
Radlička, T. Correction of parasitic aberrations of hexapole corrector using differential algebra method. Ultramicroscopy, 204, 81–90 (2019). https://doi.org/10.1016/j.ultramic.2019.05.006

Radlička, T., Unčovský, M., & Oral, M. In lens BSE detector with energy filtering. Ultramicroscopy, 189 (2018). https://doi.org/10.1016/j.ultramic.2018.03.015

Development and building of an UHV SLEEM/ToF tool

Detailed knowledge of the mechanisms of electron scattering and its practical consequences at very low energies are of prime importance not only for the advancement of measurement techniques but also for developing new materials for the next generation of electronic devices. Determining the inelastic mean free path (IMFP) of electrons in bulk materials is an ongoing spectroscopy topic. Available information on IMFP for very low electron energies, E ≤ 100 eV, is currently not satisfactory and even missing for 2-D materials, which emerge as promising in the semiconductor industry. The small thickness of 2-D crystalline materials motivated us to develop a unique ultra-high vacuum (UHV) instrument capable of analysing samples by transmitted electrons in a standard microscopic regime (energy range 0 – 5 keV) and also via the time-of-flight (ToF) method (E ≤ 300 eV).

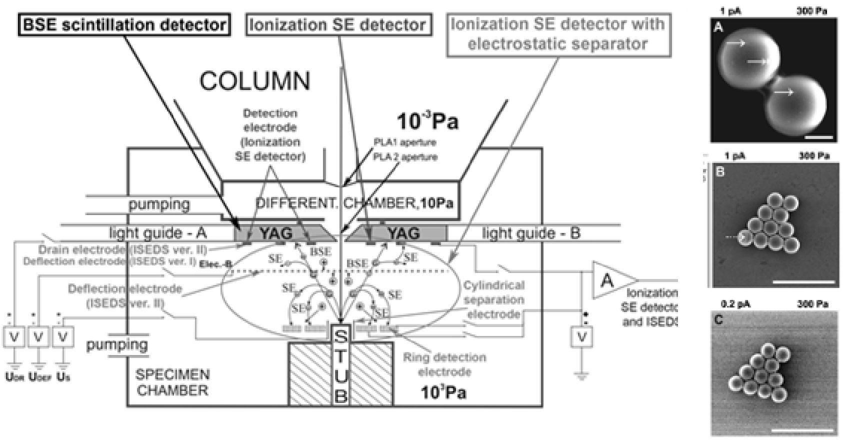
Konvalina, I., Daniel, B., Zouhar, M., at al. Studying 2D Materials by Means of Microscopy and Spectroscopy with Low Energy Electrons. Microscopy and Microanalysis, 25(S2), 482-483 (2019).



SLEEM/ToF developed at the ISI, and the energy loss spectrum of graphene

New high-efficiency detector for low-dose imaging in ESEM

Wet biological and biopolymer samples in their native state have a very low emissivity of signal electrons, high sensitivity to dehydration, and extremely high sensitivity to radiation damage in ESEM. Imaging these samples with higher resolutions under a high water vapor pressure environment and without damage places extreme demands on the detection system's efficiency. We built the unique Combined System for high-efficiency detection of Secondary and Backscattered Electrons (CSSBE) for ESEM, allowing detection of energy-filtered signal electrons in high-pressure conditions. It enables studying highly sensitive samples at low beam energies with the possibility of ultra-low beam currents of 0.2 pA at the pressure of hundreds of Pa, with the total electron dose about a hundred times smaller than when using commercial detectors.



New CSSBE detector for ESEM (above) and non-coated polystyrene spheres (below) observed at an ultra-low beam current, the beam energy of 5 keV, and the water vapor pressure of 300 Pa.

Neděla, V., Tihlaříková, E., Runštuk, J., & Hudec, J. High-efficiency detector of secondary and backscattered electrons for low-dose imaging in the ESEM. Ultramicroscopy, 184, 1–11 (2018). https://doi.org/10.1016/j.ultramic.2017.08.003

Nedela, V., Konvalina, I., Oral, M., & Hudec, J. Monte Carlo Simulations of Signal Electrons Collection Efficiency and Development of New Detectors for ESEM. Microscopy and Microanalysis, 21(S3), 1109–1110 (2015). https://doi.org/10.1017/s1431927615006339

Cathodoluminescence (CL) characterization of scintillators

The development of advanced scintillation materials used in electron detectors is one of the crucial parts of the development in electron microscopy. The electron microscopy team contributes to this field by testing the scintillation properties in the cathodoluminescence laboratory. We focus mainly on the kinetics of cathodoluminescence of Ce-activated, Mg-co-doped, and Pr-activated scintillators to reduce the afterglow of scintillators for electron detection in SEM. This is done in close cooperation with Charles University, Prague and with the CRYTUR company. We also concerned ourselves with determining the electron beam interaction volume in scintillators, studying the scintillator thermoluminescence, and the simulation of photon transport in optical crystals and light guides.

Kucera, M., Onderisinova, Z., Bok, J., Hanus, M., Schauer, P., & Nikl, M. Scintillation response of Ce3+ doped GdGa-LuAG multicomponent garnet films under e-beam excitation. Journal of Luminescence, 169, 674–677 (2016). https://doi.org/10.1016/j.jlumin.2015.01.034

Bok, J., Lalinský, O., Hanuš, M., Onderišinová, Z., Kelar, J., & Kučera, M. GAGG:ce single crystalline films: New perspective scintillators for electron detection in SEM. Ultramicroscopy, 163, 1–5 (2016). https://doi.org/10.1016/j.ultramic.2016.01.003

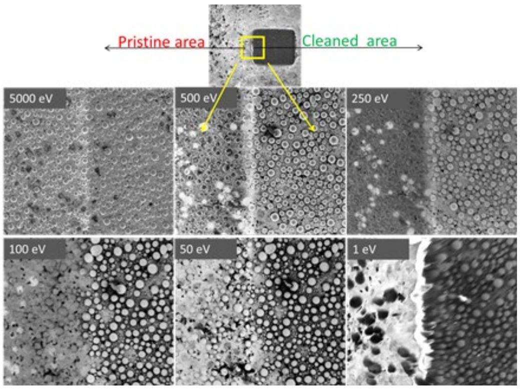
Low-energy SEM

Low-energy SEM is a group of techniques using slow and very slow electrons with energies down to the units of eV. In the evaluated period, we focused on developing new surface-sensitive techniques for a detailed study of the most advanced, mainly 2-D nanomaterials discovered in recent years. We have also developed new surface-cleaning techniques using low-energy electrons.

Development of a new in situ cleaning method for SEM

Surface studies of materials in SEMs equipped with electron spectroscopy techniques have not so far been common due to the significant problems associated with surface contamination. A range of surface-cleaning methods can be applied, but the usual ones, such as rinsing in a solvent, heating, ion bombardment, and plasma etching, have their limitations. Electron-induced in situ cleaning procedure is gentle, convenient, and very useful for a wide range of specimens. Even a small amount of hydrocarbon contamination can severely impact the results obtained with low-energy electrons, as illustrated in the figure below. During the scanning of surfaces by electrons, the image usually darkens because of a carbonaceous layer gradually deposited on the surface from adsorbed hydrocarbon precursors. This effect can be described as an electron-stimulated deposition. The surface diffusion of hydrocarbon molecules around the irradiated area serves as a source of additional precursors, responsible for the contaminated dark frame over the field of view. On the other hand, the effect of electron-stimulated desorption occurs simultaneously, especially at low energies, leading to a fundamental question as to what process, deposition or desorption, dominates.

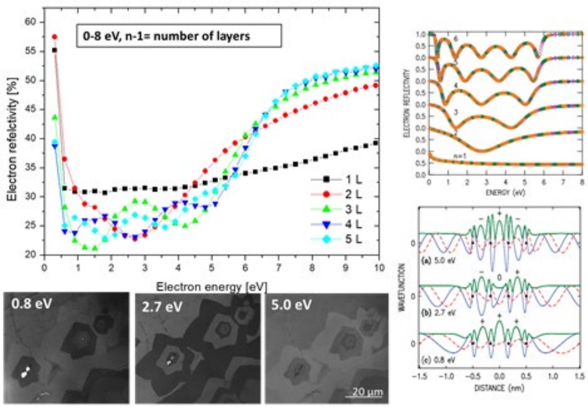
Frank, L., Mikmeková, E., & Lejeune, M. Treatment of surfaces with low-energy electrons. Applied Surface Science, 407, 105–108 (2017). https://doi.org/10.1016/j.apsusc.2017.02.131



Tin balls observed with 5000 eV, 500 eV, 250 eV, 100 eV, 50 eV, and 1 eV electrons (the left half of each frame shows the non-cleaned area covered with adsorbed molecules, while the right half is the area cleaned by slow electrons)

Study of advanced 2-D materials by low-energy electrons

The development of new types of materials such as 2-D crystals (graphene, MoS_2 , WS_2 , etc.) also requires the development of new surface-sensitive techniques for their characterization. The (ultra) low-energy electron microscopy can be a potent tool for the precise surface-sensitive study of these atomically thin materials. It can confirm the physical phenomena predicted to occur on the surfaces.



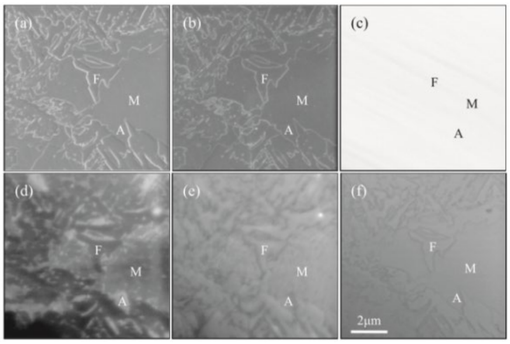
SLEEM study of cleaned CVD graphene on copper foil (left) supported by DFT simulations (right). Counting of graphene layers by slow electrons.

Frank, L., Mikmeková, E., Müllerová, I., & Lejeune, M. Counting graphene layers with very slow electrons. Applied Physics Letters, 106, 013117 (2015). https://doi.org/10.1063/1.4905221

Mikmeková, E., Frank, L., Müllerová, I., (2016). Study of multi-layered graphene by ultra-low energy SEM/STEM. Diamond and Related Materials, 63, 136–142. (2016).

Microscopy for Materials Science

The “Microscopy for Materials Science” team was established in June 2018 as a new research group in the Electron Microscopy department. Dr. Šárka Mikmeková founded the group after returning to ISI after a five-year stay at the Japanese steel giant, JFE Steel Company (Tokyo, Japan). The group is equipped with a new metallographic laboratory with high-end instruments, where ferrous and non-ferrous specimens of the highest-quality standards can be prepared for optical, laser, and electron microscopy. The unique samples prepared in our state-of-the-art metallographic laboratory are characterized by advanced scanning electron microscopy (SEM) techniques. The group's main research activity is the 0 eV project, which is a bilateral Japan-Czech project fully supported by JFE Steel Corporation. It aims to apply the Super Low Energy Electron Microscopy (SLESEM) technique, originally developed at the ISI, on advanced steels and other products produced by JFE Steel Corporation.



Micrographs of TRIP steel surface with the same area of view: conventional SEM images, and SLESEM images obtained at 0 eV (c), 1 eV (d), 2 eV (e), and 5 keV (f).

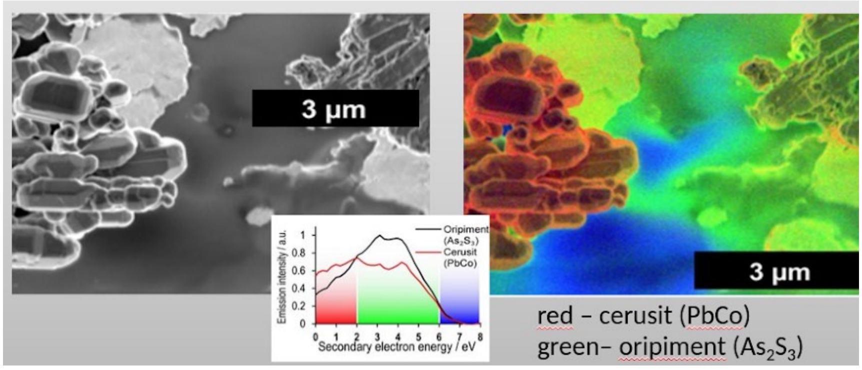
Aoyama, T., Mikmeková, Š., at al. Visualization of three different phases in multiphase steel by scanning electron microscopy at 1 eV landing energy. Ultramicroscopy, 204, 1–5 (2019).

Hyperspectral imaging

Hyperspectral imaging in SEM is based on filtering the energy of secondary electrons. Using secondary electron (SE) spectra taken at each image point, different chemical composition of nanoscale areas on the specimen can be distinguished. The method is fast (taking about one minute for a 512 x 512 pixel image). It provides a much better resolution (due to the shallow interaction volume of the SE) than standard energy-dispersive X-ray spectroscopy (EDX). The figure below shows an application of the method on a combination of pigments from a medieval wall painting, cerussite ($PbCO_3$), and orpiment (As_2S_3), obtained from our collaborator, a laboratory specialized in cultural heritage preservation and restoration. Cerussite and orpiment are notoriously difficult to discern from each other using standard methods such as EDX. Still, as each of them has a different SE spectrum, a map of chemical composition can be derived from the hyperspectral SE measurement. If a standard SEM without any specialized SE spectroscopy attachment is used, the development of the method requires detailed knowledge of SE trajectories in the microscope column. This was possible due to a close cooperation with an SEM manufacturer, who provided the design of the objective lens and SE detector so that SE trajectories and appropriate electrode settings could be calculated to fine-tune the SE energy filtering.

Abrams, K. J., Dapor, M., Stehling, N., Azzolini, M., Kyle, S. J., Schäfer, J., Quade, A., Mika, F., Kratky, S., Pokorna, Z., Konvalina, I., Mehta, D., Black, K., & Rodenburg, C. Making Sense of Complex Carbon and Metal/Carbon Systems by Secondary Electron Hyperspectral Imaging. Advanced Science, 6, 1900719 (2019). https://doi.org/10.1002/advs.201900719

Konvalina, I., Mika, F., Krátký, S., Materna Mikmeková, E., & Müllerová, I. In-Lens Band-Pass Filter for Secondary Electrons in Ultrahigh Resolution SEM. Materials, 12, 2307 (2019). https://doi.org/10.3390/ma12142307



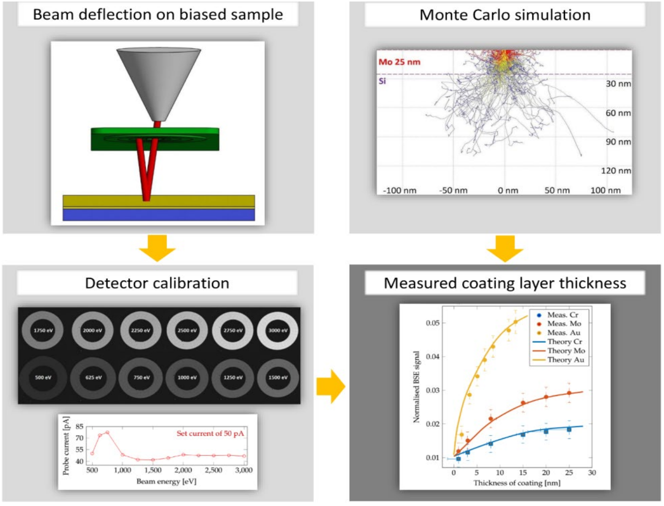
Visualization of sample consisting of cerusit and oripiment using hyperspectral imaging.

Quantitative imaging in SEM

Typical images acquired by an SEM are intensity images giving qualitative information about the signal, resulting from scattering of primary electrons after interaction with the sample. Quantitative imaging SEM is a technique that provides a physical value to each pixel of the sample (typically mass or thickness). The method development requires methodological and instrumental development combining appropriate calibration of measured signals and a theoretical description of electron scattering. We implemented the quantitative imaging for the transmitted scattered electrons detected by the STEM detector to obtain the local thickness and mass density using a single-electron counting detector developed in the framework of the CSF project. The idea of quantitative STEM imaging was further extended to commercial solid-state detectors, often included in modern SEMs. Another extension of this technique was done for back-scattered electrons (BSE), requiring a more complex calibration of the BSE detector (figure below), as well as the extension of the quantitative STEM imaging using a 2D-STEM detector (TimePix on FIB-SEM Helios G4 (ThermoFisher)) starting in 2019. The correlation of quantitative imaging with cathodoluminescence (CL) and X-ray at both room and cryogenic temperatures were applied to characterize and localize nanodiamonds in cells as a potentially applicable drug and probe carriers for *in vitro*/*in vivo* applications.

Skoupy, R., Nebesarova, J., Slouf, M., & Krzyzanek, V. Quantitative STEM imaging of electron beam induced mass loss of epoxy resin sections. Ultramicroscopy, 202, 44–50 (2019). https://doi.org/10.1016/j.ultramic.2019.03.018

Knötigová, P. T., Mašek, at al. Application of Advanced Microscopic Methods to Study the Interaction of Carboxylated Fluorescent Nanodiamonds with Membrane Structures in THP-1 Cells: Activation of Inflammasome NLRP3 as the Result of Lysosome Destabilization. Molecular Pharmaceutics, 16, 3441–3451 (2019). https://doi.org/10.1021/acs.molpharmaceut.9b00225



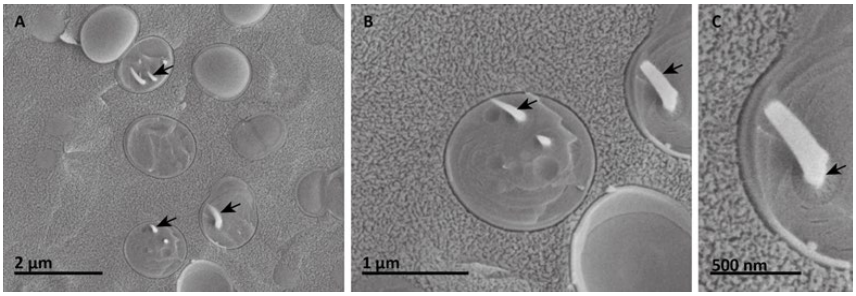
Scheme of quantitative imaging using the BSE signal leading to mapping of local thickness of thin films on a substrate

Cryo-SEM

Investigations of most hydrated samples, such as biological/medical samples or hydrogels, require cryogenic sample preparation and imaging at very low temperatures in SEM (cryo-SEM). Cryo-SEM is considered a well-established technique for some types of samples but often has relatively low reproducibility. Many samples require long testing to achieve appropriate conditions for their preparation, imaging, and interpretation. A new methodology for cryo-SEM combining high-pressure frozen samples on sapphire discs with perpendicular freeze-fracturing was developed and demonstrated on microbial biofilms. This technique was compared with other sample preparation methods and complemented with Raman spectroscopy on monitoring *Candida parapsilosis* and *Staphylococcus epidermidis* biofilms that allows a better understanding of biofilm structures. In 2018, a high-pressure-freezing (HPF) machine EM ICE (Leica microsystems) was purchased thanks to the CAS investment grant. Two main applications performed regularly use the combination of HPF and cryo-SEM: structure investigation of bioplastics production by bacteria and study of the structure of hydrogels. To increase cryo-SEM reproducibility, we developed a new cryo-stage and cryo-holder assemblies with an improved temperature controller. This is important mainly for the investigations of hydrogels that are more sensitive to sublimation than standard biological samples. Studies of the production of bioplastics (polyhydroxyalkanoates, PHA) by bacteria under various stress conditions showed unique properties of the PHA granules in cells that are amorphous and, therefore, elastic even at the temperature of liquid nitrogen (see figure below). For investigations of thin samples using the STEM detector, a new cryo-high-vacuum sample transfer system was developed.

Hrubanova, K., Krzyzanek, V., at al. O. Monitoring Candida parapsilosis and Staphylococcus epidermidis Biofilms by a Combination of Scanning Electron Microscopy and Raman Spectroscopy. Sensors, 18, 4089 (2018). https://doi.org/10.3390/s18124089

Tacke, S., Krzyzanek, V., Nüsse, H., Wepf, R. A., Klingauf, J., & Reichelt, R. A Versatile High-Vacuum Cryo-transfer System for Cryo-microscopy and Analytics. Biophysical Journal, 110, 758–765 (2016). https://doi.org/10.1016/j.bpj.2016.01.024



(A-B) Cryo-SEM of freeze-fractured cyanobacteria Synechocystis sp. PCC 6803 producing biopolymers in the cells. The bioplastics granules are elastic even at cryogenic temperatures

Environmental SEM

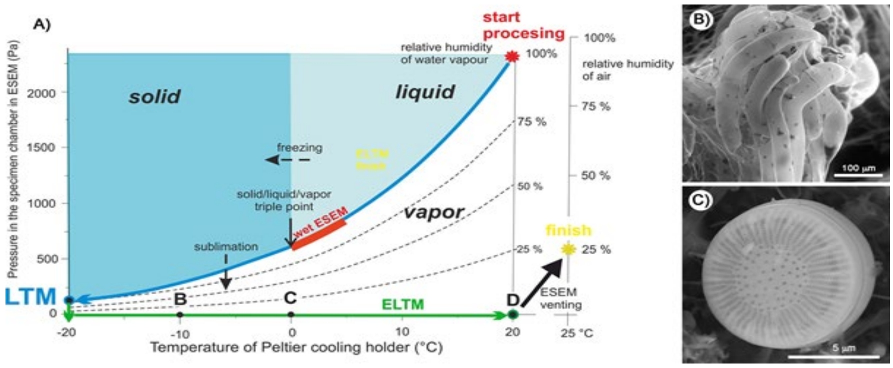
Microstructural and elemental analysis of sensitive wet samples in their native state represents a great challenge for electron microscopy. We focus on developing and modifying electron microscopes, unusual instrumentation, and unique methods to study these samples.

The Low-Temperature Method (LTM) for ESEM

Fresh wet samples are commonly observed in ESEM under temperature slightly above 0°C and thermodynamic equilibrium at 100% relative humidity. Usually, the sample surface is covered with a thin layer of liquid water, which makes its specific microstructure invisible. We developed Low-Temperature Method (LTM) for ESEM, which is based on the controlled decreasing of gas pressure from atmospheric pressure to hundreds of Pa and the decrease of specimen temperature to -20°C along the thermodynamic equilibrium curve, which causes evaporation of water and gentle sublimation of thin ice layer covering the specimen surface. The sample surface morphology stays intact, and its microstructure can be observed with higher resolution. The method is ideal for in vivo study of wet, mostly biological samples like plants, diatoms, or small water animals such as rotifers. For later repeated high-resolution SEM observation and energy-dispersive x-ray microanalysis, we can dry the sample by decreasing the water vapor and increasing the temperature to 20°C; we call the procedure Extended LTM (ELTM).

Michaloudi, E., Papakostas, S., Stamou, G., Neděla, V., Tihlaříková, E., Zhang, W., Declerck, S. A. J. Reverse taxonomy applied to the Brachionus calyciflorus cryptic species complex: Morphometric analysis confirms species delimitations revealed by molecular phylogenetic analysis and allows the (re) description of four species. PLoS ONE, 13, e0203168 (2018). https://doi:10.1371/journal.pone.0203168

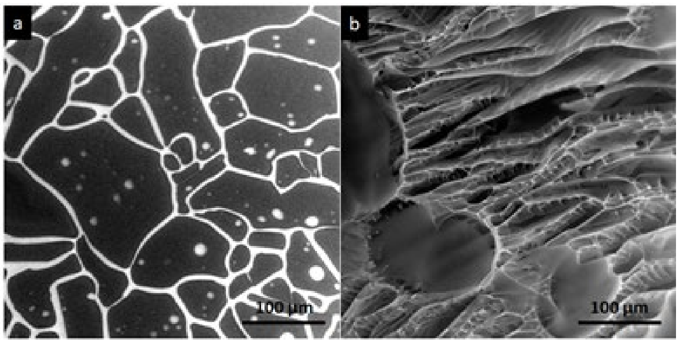
Tihlaříková, E., Neděla, V., Dordevic, B. In-situ preparation of plant samples in ESEM for energy dispersive x-ray microanalysis and repetitive observation in SEM and ESEM. Scientific Reports, 9, 2300 (2019). https://doi:10.1038/s41598-019-38835-w.



A) The saturated water vapor pressure curve with marked conditions of the LTM. B) Early embryogenic tissue (Pinus sylvestris L.). C) Intact diatom cell of Puncticulata balatonis

Dynamical in-situ studies of ice

We developed a method for imaging ice and frozen aqueous solutions in ESEM which, contrary to low-temperature SEM, provides several significant improvements: (a) moist gaseous environment in a specimen chamber of an ESEM prevents charging of an uncoated dielectric sample; (b) thermodynamic equilibrium of water vapor in the specimen chamber prevents sample sublimation and water vapor desublimation. Therefore, a frozen sample can be inspected at temperatures just below the freezing point (very low temperature is not needed); (c) by disturbing the equilibrium intentionally, we etch the sample in-situ. Our effort aims at understanding the fundamental properties of frozen solutions and its direct application in the fields of pharmaceutics (e.g. lyophilization of proteins) and environmental sciences (morphologies of salt-containing ices and their impact on the formation of salt aerosols in the atmosphere and potential of salts being oxidized to halogens diminishing the ozone layer).



In the morphology of a frozen 0.05 M solution of CsCl (fig. a), high contrast between veins of salty brine (white) and ice (dark grey) is achieved. Morphology of freeze-dried BSA protein layers (fig. b).

Yang, X., Neděla, V., Runštuk, J., at al. Evaporating brine from frost flowers with electron microscopy and implications for atmospheric chemistry and sea-salt aerosol formation. Atmospheric Chemistry and Physics, 17, 6291–6303 (2017).

Imrichová, K., Veselý, L., Gasser, T. M., Loerting, T., Neděla, V., & Heger, D. Vitrification and increase of basicity in between ice Ih crystals in rapidly frozen dilute NaCl aqueous solutions. The Journal of Chemical Physics, 151, 014503 (2019).

Vetráková, Ľ., Neděla, V., Runštuk, J., & Heger, D. The morphology of ice and liquid brine in an environmental scanning electron microscope: a study of the freezing methods. The Cryosphere, 13, 2385–2405 (2019).

Research activity and characterisation of the main scientific results

The team focuses on applied research. A significant part of the outputs comes from industrial funded projects and contractual research. We were involved in more than 20 funded projects. The team realised more than 450 projects of contract research from 2015 to 2019. The number of industrial and academic partners exceeded number 75. Among the most important companies, we can name Thermo Fischer Scientific, TESCAN, IQ Structures, Honeywell, Nuclear Research Institute Řež (UJV), Focus GmbH, Charles University - Prague, Siemens Industrial Turbomachinery, Rigaku Innovative Technologies, ČKD Group and many more. The total volume was around 880.000 Euro during the last five years. In the following text, the most important topics are briefly described.

Electron-beam lithography

Origination and technology

We maintained the operability of both e-beam pattern generators: the older e-beam writer with the shaped beam (Tesla BS600+) and the Raith's Gaussian system (Vistec EBPG5000plusES). These systems were intensively used for the research topics of the New Technologies team as well as for the cooperation with scientific and commercial partners. The focus on the greyscale lithography brought case-oriented challenges in both the data preparation domain (i.e. writing strategy, partitioning, huge data amount handling, proximity effect correction) and the processing technology (including the structural and functional evaluation of the exposure results). Technology combining exposure of two different energies was developed[1] (see Figure **1**).

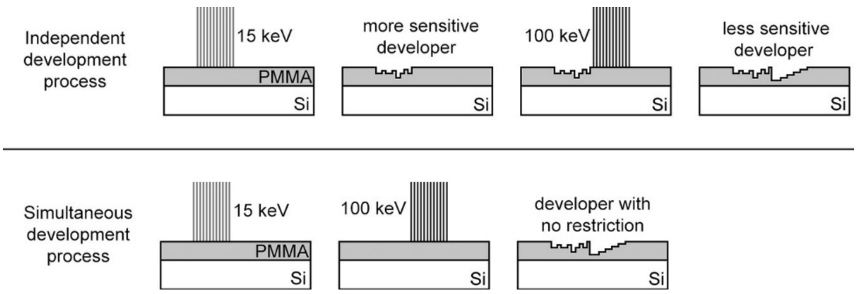


Fig. 1 Combined e-beam lithography: two-energy exposure process

The topic was addressed in the funded projects TN01000008[2], TE01020233[3], and FV40197[4], and several contractual research projects.

Microscopy calibration samples

Previously developed planar dimension standards for SEM (Scanning Electron Microscopy) were extended to samples combining precise lateral and depth artefacts. Also, samples combining different metal artefacts for the microscopy material analysis

[1] Krátký, S. - Kolařík, V. - Horáček, M. - Meluzín, P. - Král, S., *Combined e-beam* lithography *using different energies*, Microelectronic Engineering, 177, 30-34 (2017).

[2] Project TN01000008 *Centre of Electron and Photonic Optics* funded by Technology Agency of the CR (2015 – 2019).

[3] Project TE01020233 *Advanced Microscopy and Spectroscopy Platform for Research and Development in Nano and Microtechnologies – AMISPEC* funded by Technology Agency of the CR (2012 – 2019).

[4] Project FV40197 Design and manufacturing of advanced diffractive optical elements and their application to the industry funded by Ministry of Industry and Trade CR (2019 – 2022).

were prepared. A big challenge presented the development of electrically conductive transmission nano-technology samples for TEM and electron diffraction analysis.

Further research and development activities were carried in the field of microtechnology for two-quadrant plan parallel phase filter on a glass substrate and in the field of zonal structures for surface micro-roughness measurements, utility model 33047. In the field of light microscopy, a customised calibration target was developed. The target sample (coined *CALISI*) is composed of any subset (or the whole set) of various common microscopic patterns in very high resolution (see Figure **2**), e.g. USAF, knife-edge, Siemens star, rulers, concentric geometry, orthogonal gratings, pinholes, zonal patterns, etc.

The topic was addressed in the funded projects FV40197, TN01000008[2], and TE01020118[5] and several contractual research projects.

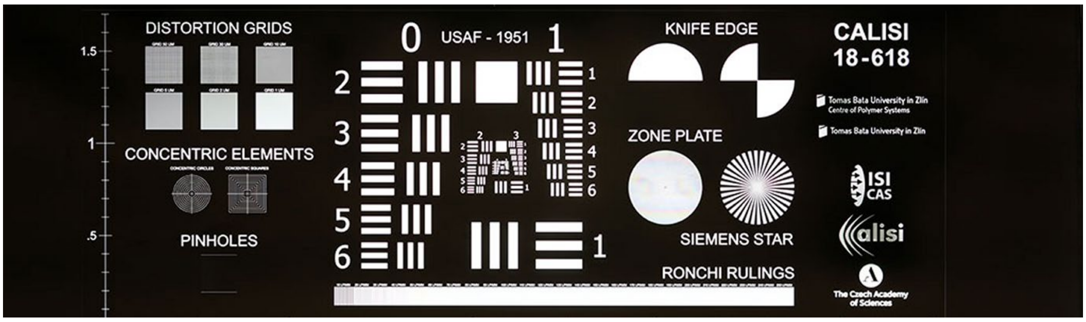


Fig. 2 CALISI — Customized Optical CALibration ISI target

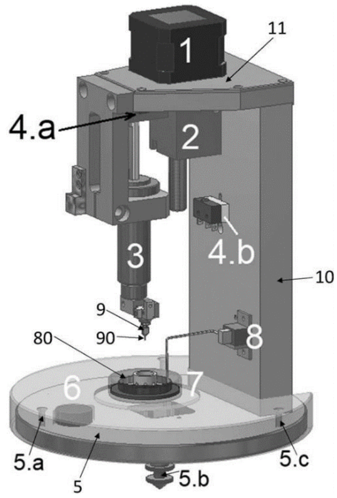


Fig. 3 Equipment for reproducible preparation of sharp tips

E-beam microscopy and lithography: gun development

Development of advanced electron sources was carried on. Particularly, within the framework of the project TE01020118[5], we participated in the research of carbon nanotubes electron sources for both the electron beam microscopy and the electron beam lithography[6].

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5 Project TE01020118 *Electron microscopy* funded by Technology Agency of the CR (2015 – 2019).

6 Knápek, A. - Radlička, T. - Krátký, S., *Simulation and Optimization of a Carbon Nanotube Electron Source*, Microscopy and Microanalysis, Volume 21, 60-65 (2015).

A set of related works comprises STM probe from graphite material and methods of its preparation, testing of field emission orthodoxy by using a simple web application, the utilization of plasma to produce barrier coatings on cold field emission cathodes, fluctuations of the focused electron beam in a conventional SEM, and noise behaviour of field emission cathode based on lead pencil graphite[7].

A programmable set-up for electrochemical preparation of STM (Scanning Tunneling Microscopy) tips and ultra-sharp field emission cathodes was developed (see Figure **3**)[8,9] and related utility model was granted[10].

The topic was addressed in the funded projects TE01020118[5] and TG03010046[11].

Custom photolithography masks

Recent success in the field of amplitude and phase masks for the photolithography process led us to push this technology to a better resolution and a larger scope. One line focused on the phase masks for the UV exposure of fibre Bragg gratings for the measurement of the containment construction of nuclear power stations to guarantee safety in case of hard accidents.

New technology for the laser exposure of volume holographic grating was developed. These gratings, in combination with planar optical structures, are intended to enhanced light distribution from LED sources in the lighting industry.

Within the frame of contractual research, several specific custom projection masks were developed e.g. combined amplitude- and phase- masks for the generation of vortex beams, large-area structures with a specific non-binary relief, and pinhole masks with specific requirements on pinhole size, low residual transparency and precise reticle layout.

The topic was addressed in the funded projects FV10618[12], VI20172020099[13], and VG20132015124[14] and several contractual research projects.

Diffractive optically variable image devices (DOVIDs)

The research in this period was focused on recent and new concepts in this field (e.g. zero-order diffractive gratings, spiral gratings[15], plasmonic structures, fractal-based

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[7] Knápek, A. - Sobola, D. - Burda, D. - Daňhel, A. - Mousa, M. - Kolařík, V., *Polymer Graphite Pencil Lead as a Cheap Alternative for Classic Conductive SPM Probes,* Nanomaterials, 9, 1756 (2019).

[8] Knápek, A. - Horáček, M. - Chlumská, J. - Kuparowitz, T. - Sobola, D. - Šikula, J., *Preparation and noise analysis of polymer graphite cathode*, Metrology and Measurement Systems, 25, 451–458 (2018).

[9] Knápek, A. - Sýkora, J. - Chlumská, J. - Sobola, D., *Programmable set-up for electrochemical preparation of STM tips and ultra-sharp field emission cathodes*, Microelectronic Engineering, Volume 173, 42-47 (2017).

[10] Knápek, Alexandr - Klein, Pavel - Delong, A. *Equipment for repeated production of sharp tips*. Czech utility model no. 33278. http://spisy.upv.cz/UtilityModels/FullDocuments/FDUM0030/uv030627.pdf

[11] Project TG03010046 *Commercialization of the research results of the Institute of Scientific Instruments of the CAS, v. v. i.* funded by Technology Agency of the CR (2016 – 2019).

[12] Project FV10618 *Micro and Nano Optics for Controled Light Directing from LED Sources* supported by Ministry of Industry and Trade (2016 – 2019).

[13] Project VI20172020099 *Fibre optic sensors for safety measurements in nuclear power plants during severe accidents* supported by Ministry of Industry and Trade (2017 – 2020).

[14] Project VG20132015124 *New method of themeasurement of the construction of containment of nuclear power station Temelin to guarante of safety in case of hard accidents supported* by Ministry of Industry and Trade (2013 – 2015).

[15] Kolařík, V. - Horáček, M. - Knápek, A. - Krátký, S. - Matějka, M. - Meluzín, P., *Spiral arrangement: From nanostructures to packaging, Journal of Electrical Engineering,* 70, 74–77 (2019).

structure design, single line gratings, generalised Fresnel relief structures, planar total internal reflection devices, advanced laser readable devices, etc). One utility model[16] and one patent[17] were granted. The combination of various approaches for e-beam origination - using both the shaped beam writer and the Gaussian beam writer - was also elaborated. The reproducibility of a DOVID functional sample with generalised zonal structures, line gratings and the spiral arrangement was verified by standard electroforming and embossing processes (see Figure **4**).

Fig. 4 Functional sample of a DOVID under different lighting conditions

The topic was addressed in the funded projects TE01020233[3], VI20192022147[18] and several contractual research projects.

Thin layers

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

The project 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. The obtained absolute values of the heat transfer in a range of at least four orders of the magnitude were 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[19,20].

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[21]

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[16] Horáček, M. - Matějka, M. - Krátký, S. - Meluzín, P. - Kolařík, V., *Diffractive Optically Variable Image Device* (2018). Czech utility model no. 30627. http://spisy.upv.cz/UtilityModels/FullDocuments/FDUM0030/uv030627.pdf

[17] Horáček, M. - Kolařík, V., *An optically variable imaging device and the method of its preparation* (2018). Czech patent no. 306956. http://spisy.upv.cz/Patents/FullDocuments/306/306956.pdf

[18] Project VI20192022147 *Diffractive and refractive optics for advanced document security* funded by Ministry of Industry and Trade (2019 – 2022).

[19] Urban, P. - Hanzelka, P. - Vlček, I. - Schmoranzer, D. - Skrbek, L. *Convective heat transport in two-phase superfluid/vapor 4He system,* Low Temperature Physics, 44, 1001-1004 (2018).

[20] Musilová, V. - Králík, T. - Fořt, T. - Macek, M., *Strong suppression of near-field radiative heat transfer by superconductivity in NbN*, Physical Review B, 99, 024511 (2019).

[21] Martínek, T. - Kudelka, J. - Navrátil, M. - Fořt, T. - Křesálek, V. *Characterization of ultra-thin tungsten layers*, International Journal of Applied Engineering Research, Volume 11, 7523-7525 (2016).

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. The topic was addressed in the funded project GA14-07397S[22].

Dynamic impact test as a method of analyses of mechanical properties of protective coatings used in industry

The aim of this running project is a dynamic impact analysis of protective coatings[23]. This unique method can be used to evaluation of impact resistance and impact wear of the protective coatings used in a wide range of industry sectors, such as a mechanical engineering, automotive industry or energetics. Nowadays, there are only two institutes in Europe with research and development focused on the dynamic impact testing - Aristotle University in Thessaloniki and Institute of Scientific Instruments, CAS (ISI). This special instrument, developed in ISI, can test coatings using less than one hundred impacts. Thus, it is possible to study the beginning phase of the impact-fatigue. Another goal is the construction of the second instrument to gain more effective testing of the protective coatings. Already established collaboration with academicals partners (ZČU Plzeň, MUNI Brno, CEITEC, VUT Brno) and industry partners (PLATIT AG, HVM Plasma spol. s.r.o., VZÚ s.r.o.) is essential for further development of the dynamic impact testing and its implementation in the industry.

The postdoc project[24] founded by the Czech Academy of science is focused on the development of the impact testing and extension application of this method on the new materials where the New Technology team is the principal investigator.

Local microstructural changes induced by a static and dynamic indentation in nanostructured and nanolaminate coatings

The main subject of the project[25] was the preparation of novel nanostructured and nano-laminate coatings using magnetron sputtering, and complex analysis of coating response to local static and dynamic indentation tests. Original data were obtained from indentation tests in a broad range of applied loads. The microstructure of deformed volume was studied by advanced methods of electron microscopy (SEM including 3D mapping using FIB, TEM including high resolution). Deformation mechanisms and failure onsets were assigned. Various effects detected on the indentation curves (such as pop-ins and slope discontinuities) were interpreted in terms of observed microstructural changes and results of computer modelling of indentation response. The method was applied for optimization of deposition conditions and novel nanolaminate coatings with superior mechanical properties were obtained.

The role of our team was the analysis and investigation of the response of nanostructured and nano-laminated coatings on the repetitive dynamic load. The Mo-B-C and W-B-C coatings were studied using dynamic impact tester and laser confocal microscope. The results of the analyses were presented in scientific papers and

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[22] Project GA14-07397S *Effect of near field on radiative heat transfer at low temperatures* funded by Czech Science Foundation (2014 – 2016).

[23] Fořt, T. - Grossman, J. - Sobota, J. - Daniel, J. *Dynamical impact test – a unique method of protective coatings analysis,* Fine Mechanics and Optics, 62, 257-260 (2017), in Czech.

[24] Project L100651901 *Dynamic impact test as a method of analyses of mechanical properties of protective coatings used in industry* funded by Czech Academy of Science (2019 – 2020).

[25] Project GA15-17875S *Local microstructural changes induced by static and dynamic indentation in nanostructured and nanolaminate coatings* supported by Czech Science Foundation (2015 – 2018).

conference contributions. The participation of the New Technology team on this project was in the contracted form.

Quantitative imaging in SEM with elastically scattered electrons

The main goal of the project[26] was focused on the instrumental development and establishment of the methods for quantitative imaging of very thin specimens by transmitted scattered electrons. This technique improved the classical image which is acquired by a scanning electron microscope (SEM). This enables measurement of the mass of imaged nanoparticles or thickness mapping, and on the other hand simultaneously high-resolution imaging. It requires an extension of a commercial high-resolution SEM without any restriction of its inherent features. The aimed quantitative studies utilize elastically scattered electrons and are applied to individual nanoparticles or macromolecules to measure their total mass, mass distribution and mass-thickness mapping. To lower the electron beam damage of sensitive samples, it is necessary to use cryo-techniques adapted for quantitative imaging.

Our team was part of the investigators. During the project we prepared radiofrequency magnetron sputtered metal and indium tin oxide coatings with low roughness, high electrical conductivity, and required optical properties.

Reduction of the afterglow of scintillators for electron detectors in SEM

The goal of the project[27] was the extensive study of the cathode-luminescence kinetics, especially of the fast scintillation materials. Methods for research of existing and looking for new scintillation materials that investigate allowed 5d-4f transitions in Ce activated oxide-based solids, was developed and used. The project was motivated by an effort to analyse a bad decay of current scintillators resulting in a low contrast and a bad spatial resolution of scanning electron microscope (SEM) images. The investigation took advantage of the experiences of the specialized laboratory, to obtain formerly inaccessible qualitatively new knowledge, which allowed designing superior electron detectors. These can be applied especially in the fast Everhart-Thornley detectors in the SEM.

Our team was part of the investigators. During the project we prepared radiofrequency magnetron sputtered metal and indium tin oxide coatings with low roughness, high electrical conductivity, and required optical properties.

Deposition technology and implementation of EUV multilayer system

We have developed deposition technology and the implementation of multilayer systems of Mg/Si or molybdenum and silicon prepared by magnetron sputtering. Interface roughness has to be smaller than 0.1 nm and the reproducibility of bilayer thickness (i.e. molybdenum or Mg and silicon) must be better than 0.1nm. This multilayer system was used as a EUV mirror.

This unique technique of production of extremely accurate multilayer systems x-ray and EUV optics was mastered in the Czech Republic by ISI only. There are only a few laboratories with the required know-how in Europe.

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[26] Project GA14-20012S *Quantitative imaging in SEM with elastically scattered electrons* supported by Czech Science Foundation (2014 – 2016).

[27] Project GA16-05631S *Reduction of afterglow of scintillators for electron detectors in SEM* supported by Czech Science Foundation (2016 – 2018).

Electron-beam technologies

Electron-beam welding gun design

The research of electron beam processing technologies in the Institute of Scientific Instruments started in the 1960s. Use of a focused intensive electron beam for welding and other technologies led to the development of several welding machines in subsequent years. The expertise we gathered up today helped us to start cooperation with several industrial companies.

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. The development of the MEBW continues on both sides. Other partners willing to incorporate e-beam technologies come from nuclear research. Besides the R&D of the welding equipment, the welding technology was continuously improved according to ISI and industrial partners' requests.

During the evaluation period, we worked mainly on our electron gun design to match the requirements of the customers. Our former e-gun design, which was tailored for welding in a vacuum with maximum beam power up to 2 kW and maximum acceleration voltage 60 kV, was redesigned and has now beam power raised to 6 kW and can operate in radiation environment in a hot-chamber in nuclear research facilities. The e-gun design was also refined to remove previous design flaws, to improve manufacturability. The X-ray shielding was improved for higher user safety. A brand new ceramic high-voltage isolator, based on numerical simulation of the electric field distribution, was designed (see Figure **5**). The test batch of the isolators was produced for verification.

The research led from 2015 to 2019 was financed by several contractual research projects.

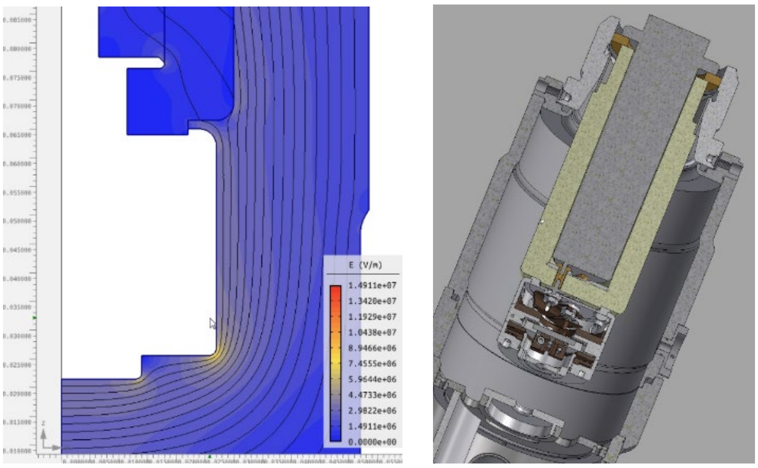


Fig. 5 Electric field distribution around the isolator (left) and top part of the gun cross-section (right).

Precise high-voltage power supply for FEG

For the TESCAN company, an important producer of electron microscopes, we developed a precise 100 kV high voltage supply intended for electron microscopy and/or lithography with field emission electron guns. The work was split into a series of smaller projects funded by TESCAN. The work was a continuation of a project funded by the Czech Ministry of Trade which was solved in 2011 – 2013.

In the first phase, former design of the supply was thoughtfully revised to meet series production needs, improvement of the supply features, better control and system integration. Complete manufacturing documents were prepared and delivered to the customer.

In the next phase, the prototype of the HV supply was extensively tested and its properties evaluated. The measurements confirmed the stability of the supply better than 2 ppm at 100 kV over 24 hours. The short-term ripple and noise were better than 0.25 ppm at 100 kV. In the last phase, new software for the supply's control CPU was developed. The result is a more robust, reliable and expandable system, which was successfully integrated into TESCAN's microscope infrastructure. The supply was tested with a prototype of TESCAN's FEG and is now a part of their new state-of-the-art transmission electron microscope (see Figure **6**).

The research led from 2015 to 2019 was financed by a series of contractual research projects.

Fig. 6 Precise 100 kV supply for FEG (right) and reference divider (left) during testing in our lab.

The E-field detector for the ExoMars mission

ExoMars (Exobiology on Mars) is an astrobiology programme led by the European Space Agency (ESA) and the Russian space agency Roscosmos. The goals of ExoMars are to search for signs of past life on Mars, investigate how the Martian water and geochemical environment varies, investigate atmospheric trace gases and their sources and by doing so demonstrate the technologies for a future Mars sample-return mission.

The ExoMars Kazachok (formerly ExoMars 2020 Surface Platform) is a planned robotic Mars lander led by Roscosmos, part of the ExoMars 2022 joint mission with the European Space Agency. Part of the scientific payload is the Wave Analyzer Module (WAM) for measuring of changes in a magnetic and electric field in the planetary atmosphere which was developed by Space Physics department on the Institute of Atmospheric Physics ASCR.

An external detector of the electric field was co-developed by our team. Spring-powered self-erecting rod antenna with remotely activated trigger together with magnetometer is held by lightweight aluminium structure and is mounted on one of the photovoltaic panels of the lander (see Figure **7**).

The ExoMars mission was delayed due to the pandemic and the start is postponed to 2022. However, we are looking forward to the first data sent back from the Mars surface. The project was supported by AV21 programme[28].

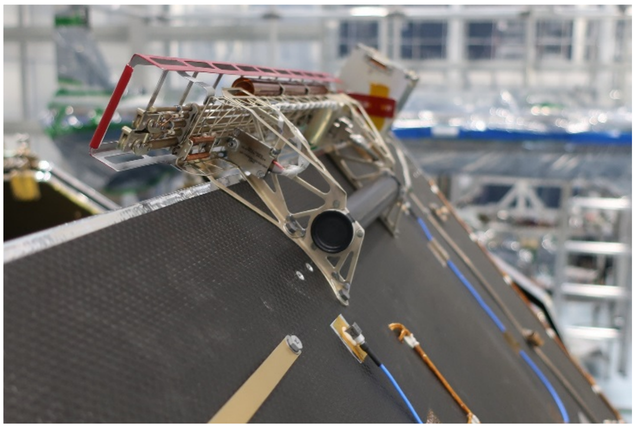


Fig. 7 The detector of the electric and magnetic field mounted on the ExoMars lander module

Metal joining technologies

Our team is equipped with two electron-beam welders (built completely in-house) and two vacuum brazing furnaces. We can make vacuum tight joints of metal parts from 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 (see Figure **8** for one example).

We provide these technologies for our team but also colleagues from other ISI teams and external customers. We can 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.

These 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.

The research led from 2015 to 2019 was financed by more than 600 individual contractual research projects.

[28] Supported by Space for mankind programme, AV21, main investigator: Institute of Atmospheric Physics of the CAS, O. Santolík (2018 – 2019).

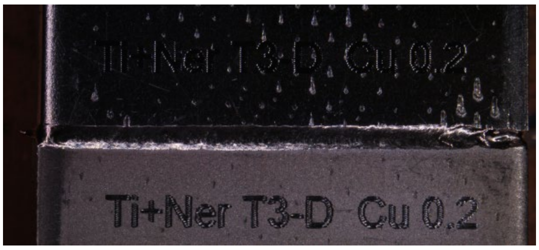


Fig. 8 A heterogeneous joint of titanium alloy and stainless steel using filler metal
Fig. 9 Large-scale deep microstructured period gratings (for Optometrics company).

Research activity and characterisation of the main scientific results

Magnetic Resonance

Since the 1990s, the core activities have been directed to quantitative MR techniques for biomedical research, for which appropriate equipment is available:

- NMR equipment: MR scanner Bruker BioSpec 94/30 USR Avance III HP with AutoPac positioning unit (acquired in 2012 under a project establishing Application Laboratories of the Institute of Scientific Instruments – ALISI), with 9.4 T field and 30 cm diameter bore, 660 mT/m gradients, 2 transmit and 8 receive channels, a set of volume/surface linear/quadrature/array RF coils for excitation and detection of ^{1}H, ^{13}C, ^{19}F, ^{23}Na, ^{31}P, and ^{129}Xe nuclei, best supporting proton imaging of the mouse and rat brain. An old 4.7T/20cm system was decommissioned in 2020.
- Animal experimentation support: heated animal beds, ECG-respiration-temperature monitoring (SAII 1030) and inhalation anesthesia during scanning, a surgery room, animal house for about 200 mice and 100 rats in individually ventilated cages, 2 laminar flow boxes, cage washer, hygienic loop, sterilization chamber, autoclave. All animal operations follow strict standards so that even immunodeficient genetically modified mice are sufficiently protected.

Because of the uniqueness of such equipment in Czechia, the MR Group has become a busy hub for animal research.

The origin in ALISI determined the group's double orientation to

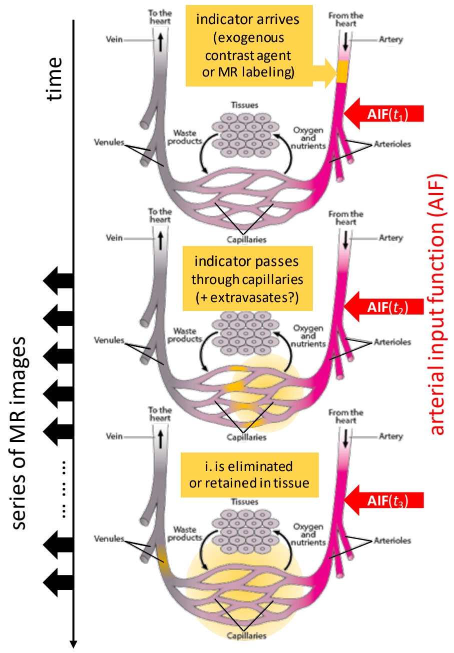
- **own biomedical-MR research**, aimed at progressive experimental techniques,
- **support of external research** by providing access to MR imaging and spectroscopy in small animals, ex vivo organs or other objects.

The MR Group participates in large research **infrastructures Czech-BioImaging and Euro-BioImaging** by offering open access to about 50% of its capacity. A 7-year experience proves that this duality is essential for sustainability of high quality of service and research: the applications stimulate a biomedically relevant research orientation and provide immediate feedback, the users get highly qualified and motivated support. Thus, the research is partly method-, partly application-oriented.

(A) Perfusion

Perfusion is the physiologic process in which blood is carried by capillaries (diameter 5-15 μm) to close vicinity (~15 μm) of cells, providing them, among other, with water, gases, nutrients, hormones, waste removal, immune system services or thermo-regulation. Therefore, it is essential for animal cell homeostasis. Hypoperfusion is associated with conditions such as artery disease, low blood pressure, heart failure, loss of blood volume or tumor necrosis; changed characteristics may be indicative of blood-brain barrier disruption or tumor neoangiogenesis or necrosis. Perfusion also enables delivery of medical drugs to diseased cells. For these reasons, quantitative assessment of local perfusion parameters can support pathophysiology research, drug development, diagnostics and therapy planning and monitoring, and is a subject of external demand. For the physiology-dictated temporal resolution of ~1 s, the achievable MRI resolution is ~300 μm, which is too coarse to show individual capillaries. However, the aggregate signal dynamics can provide insight in the perfusion processes if suitable experimental and data-modeling techniques – including NMR and pharmacokinetics (PK) – are applied to estimate local parameters such as blood flow, mean transit time, intravascular and interstitial volumes, and vascular permeability. With the 2-6 parameters used in various PK models, different levels of biophysical realism are achieved. The parameters in simpler, underparametrized phenomenological models may appear more stable but they obscure properties treated as independent in advanced, more physically grounded models; these appear as overparametrized and unstable unless high quality MR data (SNR, temporal resolution) are available.

To support realistic insight into microphysiology, we focused on the advanced PK models and on improving the physical specificity, robustness, accuracy and precision of the perfusion characteristics obtained from MR imaging, with emphasis on cancer and stroke biomarkers and drug delivery.



MR detection of perfusion utilizes the dynamic changes of image intensity due to the passage of an indicator through the blood stream and tissues. The indicator may be an injected exogenous agent (shortening water relaxation times T_1 or T_2/T_2^* for DCE or DSC methods, respectively), or magnetically labeled blood plasma itself (in method ASL). The information provided by these approaches is similar, but not identical. While DCE/DSC can test targeted drug delivery, ASL reports on perfusion changes due to disease or therapy.

Firstly we focused on 2D slices, yielding high-quality data, and analyzed the results of different advanced PK models with human and animal data[1 2 3]. To overcome the lack of ground truth, we developed a method applying different contrast agents[4]. We found that estimates of homologous parameters in different models were often different and even weakly correlated, arguably due to estimator bias and sensitivity to SNR, image artifacts, bolus administration time, animal physiology or MR-excitation model inaccuracy. All models are crucially sensitive to accurate and precise knowledge of the global arterial input function (AIF), whose time-domain convolution with a local parametrized tissue residual function is used to describe the indicator concentration.

We addressed these problems by utilizing several types of prior knowledge:

- We eliminated the need for separate AIF determination by **blind deconvolution**, linking all local time evolutions to a common AIF. We showed compatibility even with advanced PK models. [5 6]

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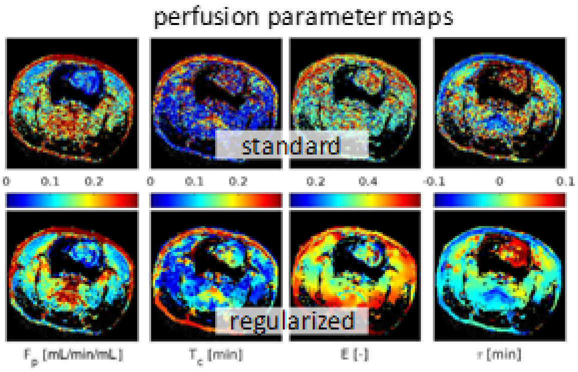
[1] Eskilsson E - Jiřík R et al. EGFRvIII mutations can emerge as late and heterogenous events in glioblastoma development and promote angiogenesis through Src activation. Neuro-Oncology 18, 12, 1644-1655 (2016)
[2] Obad N - Jiřík R et al. *Lack of functional normalisation of tumour vessels following anti-angiogenic therapy in glioblastoma.* Journal of Cerebral Blood Flow and Metabolism 38, 10, 1741-1753 (2018)
[3] Taxt T - Jiřík R et al. Using Single-Channel Blind Deconvolution to Choose the Most Realistic Pharmaco-kinetic Model in Dynamic Contrast-Enhanced MR Imaging. Applied Magnetic Resonance 46, 6, 643-659 (2015)
[4] Jiřík R - Taxt T - Macíček O - Bartoš M - Kratochvíla J - Souček K - Dražanová E - Krátká L - Hampl A - Starčuk jr. Z. *Blind deconvolution estimation of an arterial input function for small animal DCE-MRI.* Magnetic Resonance Imaging 62, 46-56 (2019)
[5] Kratochvíla J - Jiřík R - Bartoš M - Standara M - Starčuk jr. Z - Taxt T. *Distributed capillary adiabatic tissue homogeneity model in parametric multi-channel blind AIF estimation using DCE-MRI.* Magnetic Resonance in Medicine 75, 3, 389-396 (2016)
[6] Taxt T - Jiřík R et al. Semi-parametric arterial input functions for quantitative dynamic contrast enhanced magnetic resonance imaging in mice. Magnetic Resonance Imaging 46, 10-20 (2018)



- We suppressed the sensitivity to noise and image artifacts by **spatial regularization** preferring solutions with lower spatial variation, assuming the prevalence of similar perfusion in adjacent voxels[7]. This allowed us to handle 3D data. We implemented 2D and 3D **compressed sensing MR acquisition and image sequence reconstruction** based on the low-rank-plus-sparse (L+S) model (collaboration with VUT and ÚTIA) [8], and a new method of perfusion analysis **processing all voxel time courses simultaneously**, which was essential for the spatial regularization. For the first time was such a model used for advanced PK models.
- We improved the model conditioning by **simultaneous modeling** of DCE and DSC measurements, i.e. by enforcing identity of shared parameters of blood flow and compartmentation[9] [10]. A similar initial study combined DCE and ASL.[11]
- Some of these principles were also applied in **ultrasonography**.[12] [13] [14] [15]

For the perfusion analysis, we have been developing **software PerfLab**, including its web-interfaced version, aimed at providing these tools to the public.

These boundary-shifting results were achieved with collaborators providing human data (MOÚ, Univ. Bergen), tumour models (BFÚ, FNUSA-ICRC), and software implementation (VUT, ÚTIA). Problem analysis, solution ideas, animal data and coordination came from the MR Group. The ultrasound work by R. Jiřík is mostly attributed to his engagement at VUT.[16]

Perfusion analysis was the key technique applied in several instances of biomedical research in the pathophysiology and therapy of

- **schizophrenia**: With ASL we tested several hypotheses concerning the involvement of perfusion alteration in the pathophysiology of schizophrenia (SCZ). We tested regional changes of cerebral perfusion in 3 animal models (MAM, THC, polyI:C)

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[7] Bartoš M - Rajmic P - Šorel M - Mangová M - Keunen O - Jiřík R. *Spatially regularized estimation of the tissue homogeneity model parameters in DCE-MRI using proximal minimization.* Magnetic Resonance in Medicine 82, 6, 2257-2272 (2019)

[8] Walner H - Bartoš M - Mangová M - Keunen O - Bjerkvig R - Jiřík R - Šorel M. *Iterative Methods for Fast Reconstruction of Undersampled Dynamic Contrast-Enhanced MRI Data.* World Congress on Medical Physics and Biomedical Engineering 2018. Singapore: Springer, 2019

[9] Macíček O - Jiřík R - Starčuk jr. Z. *Comparison of pharmacokinetic models for joint DCE/DSC-MRI.* Magma 29, S1, 286-288. Berlin: Springer, 2016.

[10] Macíček O - Jiřík R - Mikulka J - Bartoš M - Šprláková A - Keřkovský M - Starčuk jr. Z - Bartušek K - Taxt T. *Time-Efficient Perfusion Imaging Using DCE- and DSC-MRI.* Measurement Science Review 18, 6, 262-271 (2018)

[11] Krátká L - Jiřík R - Dražanová E - Souček K - Starčuk jr. Z. *Direct comparison of ASL and DCE-MRI in a mouse model of cancer.* Magma 29, S1, S289-S290 (2016)

[12] Engjom T - Jiřík R et al. Contrast-enhanced ultrasonography of the pancreas shows impaired perfusion in pancreas insufficient cystic fibrosis patients. BMC Medical Imaging 18, 14, 8 p (2018)

[13] Mézl M - Jiřík R et al. - Harabiš V - Kolář R - Standara M - Nylund K - Gilja OH - Taxt T. *Absolute Ultrasound Perfusion Parameter Quantification of a Tissue-Mimicking Phantom Using Bolus Tracking.* IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control 62, 5, 983-987 (2015)

[14] Schafer S - Jiřík R et al. Semi-automatic motion compensation of contrast-enhanced ultrasound images from abdominal organs for perfusion analysis. Computers in Biology Medicine 63, 229-237 (2015)

[15] Stangeland M - Jiřík R et al. Interobserver Variation of the Bolus-and-Burst Method for Pancreatic Perfusion with Dynamic – Contrast-Enhanced Ultrasound. Ultrasound International Open 3, 3, E99-E106 (2017)

[16] For acronyms see box in section Cooperation.

exposed to 3 treatments (cannabidiol, aripiprazole, olanzapine). We demonstrated a positive effect of cannabidiol in the MAM model [17], a difference in phenotypes developed in the MAM and THC models [18], a likelihood that aripiprazole causes proneness to the metabolic syndrome [19], and that olanzapine alone diminishes perfusion and decreases sensorimotor cortex volume in rats [20]. All these findings tend to support the hypothesis that in SCZ functional changes precede structural ones.

- **cancer**: We applied DCE analysis to data testing an anti-angiogenic therapy (by VEGF-blocking agents) and found that normalization of the vascular morphology is no guarantee of improvement of the vascular function; poor blood flow and hypoxia may explain the disappointing results of previous clinical trials. [21]

The publications on SCZ report on experiments proposed by the MUNI collaborators; we collaborated on the design and executed the MR parts of the studies. In the VEGF study, we analyzed the collaborator's (Univ. Bergen) human data with our algorithms.

Our perfusion research has been supported by several **grants** [22] [23].

(B) Spectroscopy

With in vivo ^{1}H MR spectroscopic techniques, concentrations of water, fat, and of up to about 20 low-molecular-weight metabolites can be quantified. The detected metabolic changes are most valuable in the exploration of brain diseases and tumours. With lower sensitivity, spectroscopy of ^{31}P can report on high-energy phosphates, ^{13}C on TCA-cycle metabolic processes. Unfortunately, these techniques are only marginally supported by the commercial human and animal MR scanners. The low concentrations of metabolite molecules, presence of strong nuisance signals and interference of spatial and spectral encoding result in low SNR and often signal artifacts. This complicates inference of accurate and precise molecule concentrations.

Our research aimed at analyzing the pitfalls, at collaborative development of software for better quantitative analysis of spectroscopic signals, and developments towards fast spectroscopic imaging in humans and animals.

The **software work** was supported by two European projects including ~10 partners (from FR, ES, UK, BE, NL, UK, CH, DE, CZ). The resulting software, **jMRUI** [24], now has about 5000 licensees worldwide. In 2018, J. Starcukova was appointed the coordinator of the development. The main independent contribution of the MR Group is the quantum-mechanics based signal simulator module **NMRScopeB**, producing the spectral patterns needed for signal decomposition into single-metabolite components, for any specific type of excitation of molecules with up to 10 coupled spins. Some

[17] Štark T - Dražanová E - Starčuk jr. Z et al. Peripubertal cannabidiol treatment rescues behavioral and neurochemical abnormalities in the MAM model of schizophrenia. Neuropharmacology 146, 212-221 (2019)

[18] Dražanová EJ - Rudá-Kučerová J - Krátká L - Štark T - Kuchař M - Maryška M - Drago F - Starčuk jr. Z - Micale V. Different effects of prenatal MAM vs. perinatal THC exposure on regional cerebral blood perfusion detected by Arterial Spin Labelling MRI in rats. Scientific Reports 9, 1, 6062 (2019)

[19] Horská K - Dražanová E et al. Aripiprazole-induced adverse metabolic alterations in polyI:C neurodevelopmental model of schizophrenia in rats. Neuropharmacology 123, 1, 148-158 (2017)

[20] Dražanová E - Krátká L - Vaškovicová N - Skoupý R- Horská K - Babinská Z - Kotolová H - Vrlíková L - Buchtová M - Starčuk jr. Z - Rudá-Kučerová J. *Olanzapine exposure diminishes perfusion and decreases volume of sensorimotor cortex in rats.* Pharmacological Reports 71, 5, 839-847 (2019)

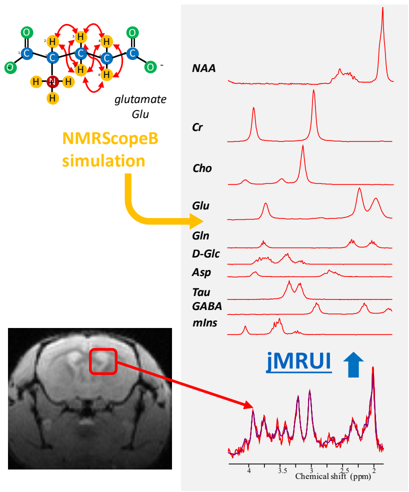
[21] Obad N - Jiřík R et al. *Lack of functional normalisation of tumour vessels following anti-angiogenic therapy in glioblastoma.* Journal of Cerebral Blood Flow and Metabolism 38, 10, 1741-1753 (2018)

[22] Grants: MŠMT LM2015062 (Czech-BioImaging service 2016-2019), MŠMT CZ.02.1.01/0.0/0.0/16_013/-0001775 (Czech-BioImaging modernization 2017-2020)

[23] GA ČR GA16-13830S (robust data analysis 2016-2018), MZ NV16-30299A (stroke 2016-2020)

[24] http://www.jmrui.eu

features make it globally unique: the user can program pulse sequences and molecule properties, handle heteronuclear spin systems, study spatial and frequency selectivity, or use it in pulse sequence development and education. In 2015-2019, extensions for high-field spectroscopy were implemented (e.g., simultanous RF and gradient modulation, modern pulse sequences[25]). For all jMRUI, our team provided **support** to users worldwide. The principles and limits of MRS simulation were analyzed in a publication [26]; certain practical improvements were described in two technical papers [27] [28].



For **quantification of the fat/water and brown/white fat ratios** we developed FTSED, a method expanding the Dixon method to fast multi-echo acquisition [29]. The method combines spectral undersampling with prior knowledge of the fat spectra, which suggests a way for future fast spectroscopic imaging in general. The method was designed specifically for fatty liver diseases and ultra-high-field MR scanners, and was tested at the collaborator's (Med. Univ. Wien) 7 T human scanner.

The spectroscopy work has been supported by several Czech and European **grants** [22] [30].

<u>(C) Diffusion</u>

MR is the only modality that can noninvasively characterize the local mobility (directionality, restrictedness, intensity) of water molecules in vivo, which may be used for tracking neurons or disease progression. E.g., we supported a search for objective biomarkers of Parkinson's disease. [31] [32] [33] [34] As this disease is associated with over-expression of protein α-synuclein, which aggregates in some neurons and forms Lewy bodies, reduction of the cytosolic water diffusion was hypothesized. By diffusion kurtosis imaging, DKI, we indeed observed diffusion restriction in affected brain areas of model animals.We used protocols adapted to our instrument and evaluated the data by 3rd party software; interpretation was the responsibility of MUNI. The project was supported by the Czech-BioImaging infrastructure **grants** [22].

[25] Sequences – [semi-]LASER, MEGA-PRESS, SPECIAL

[26] Starčuk jr. Z - Starčuková J. Quantum-mechanical simulations for in vivo MR spectroscopy: Principles and possibilities demonstrated with the program NMRScopeB. Analytical Biochemistry 529, 79-97 (2017)

[27] Jabłoński M - Starčuková J - Starčuk jr. Z. Processing tracking in jMRUI software for magnetic resonance spectra quantitation reproducibility assurance. B M C Bioinformatics 18, 56, 11 p (2017)

[28] Mocioiu V - Ortega-Martorell S - Olier I - Jabłoński M - Starčuková J - Lisboa P - Arús C - Julia-Sapé M. *From raw data to data-analysis for magnetic resonance spectroscopy – the missing link: jMRUI2XML.* B M C Bioinformatics 16, 378-388 (2015)

[29] Kořínek R - Bartušek K - Starčuk jr. Z. *Fast triple-spin-echo Dixon (FTSED) sequence for water and fat imaging.* Magnetic Resonance Imaging 37, 164-170 (2017)

[30] Grants: EC 316679 (TRANSACT 2013-2017), EC 813120 (INSPiRE-MED 2019-2022), MŠMT 8J18AT023 (water/fat 2018-2020), AV ČR L100651651 (water/fat 2017-2018), AV ČR MSM100651801 (water/fat 2018-2019)

[31] Arab A - Rudá-Kučerová J - Minsterová A - Dražanová E - Szabó N - Starčuk jr. Z - Rektorová I - Khairnar A. *Diffusion Kurtosis Imaging Detects Microstructural Changes in a Methamphetamine-Induced Mouse Model of Parkinson's Disease.* Neurotoxicity Research 36, 4, 724-735 (2019)

[32] Khairnar A - Dražanová E - Starčuk jr. Z et al. Early and progressive microstructural brain changes in mice overexpressing human alpha-Synuclein detected by diffusion kurtosis imaging. Brain Behavior and Immunity 61, 197-208 (2017)

[33] Khairnar A - Dražanová E - Starčuk jr. Z et al. Late-stage alpha-synuclein accumulation in TNWT-61 mouse model of Parkinson's disease detected by diffusion kurtosis imaging. Journal of Neurochemistry 136, 6, 1259-1269 (2016)

[34] Khainar A - Dražanová E - Starčuk jr. Z et al. Diffusion Kurtosis Imaging Detects Microstructural Alterations in Brain of alfa-Synuclein Overexpressing Transgenic Mouse Model of Parkinson's Disease: A Pilot Study. Neurotoxicity Research 28, 4, 281-289 (2015)

(D) Other

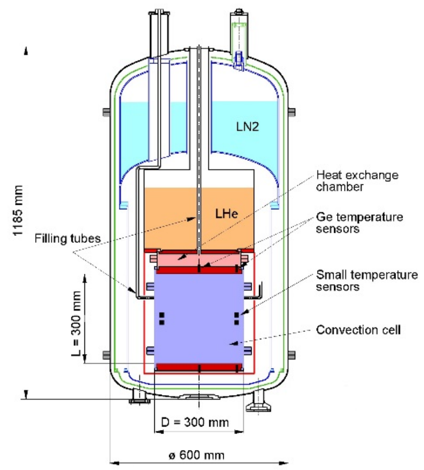
- In collaboration with MUNI we developed for a human 3 T scanner an **imaging technique with extremely short echo-time** of 60 μs, allowing us to detect the fast relaxing signals of **myelin**, which plays an important role in some neurodegenerative diseases [35] [36] (GA ČR grant GA15-12607S). It is now successful in imaging tendons.
- We tested in vitro and in vivo experimental **nanoparticle** contrast agents and potential drug carriers based on graphene [37] as a subcontract of UPOL research.
- In a test tube, we observed relaxometrically the othewise invisible dynamics of a **diffusion-reaction process** simulating the geologically interesting formation of visible Liesegang patterns. [38]

Cryogenics and Superconductivity

Basic research

(A) *Turbulent thermal (thermally-generated) convection*

plays a vital role in heat transfer in the nature – in the circulation in the atmosphere or the oceans, the flows under the surface of stars, or in diverse branches of industry. The **Rayleigh-Bénard convection** (RBC) is a simplified physical model used for fundamental studies of thermally-generated flows in an Oberbeck-Boussinesq (OB) fluid layer confined between two horizontal perfectly conducting plates heated from below and cooled from above in the gravitational field.



Several years ago we designed and constructed a **helium cryostat for experimental study of RBC**, which we now use. The cryostat contains a cylindrical (30-cm diameter) aspect-ratio-one Rayleigh-Bénard cell of arguably one of the best designs realized so far, with the smallest parasitic effects. This apparatus allows us to study thermally-generated flows of up to about Ra ~10^{15} under Boussinesq conditions, utilizing the cryogenic helium gas as a working fluid with well-known and in-situ tunable physical properties. The cell allows easy modifications including a change of the aspect ratio. To our best knowledge, we run currently the only high-Rayleigh-number cryogenic experiments in the world. We closely collaborate with the group of prof. L. Skrbek (Charles University, Prague), as evidenced by two joint projects funded by GAČR and one by MŠMT during 2015-2019.

Outline of the main results obtained in these projects:

(i) One of the most discussed topics in the field of turbulent RBC is the existence of the **ultimate state of OB RBC**, predicted by R. Kraichnan in 1962 as the most efficient-ballistic-heat transport. Proving the existence of the ultimate state would enable extrapolation of laboratory heat-transfer results to extremely large-scale turbulent natural phenomena, not observable in a laboratory. Observation of the transition to the

[35] Latta P - Starčuk jr. Z - Gruwel MLH - Lattova B - Lattova P - Štourač P - Tomanek B. Influence of k-space trajectory corrections on proton density mapping with ultrashort echo time imaging: Application for imaging of short T2 components in white matter. Magnetic Resonance Imaging 51, 87-95 (2018)

[36] Latta P - Starčuk jr. Z - Gruwel MLH - Weber MH - Tomanek B. *K-space trajectory mapping and its application for ultrashort echo time imaging.* Magnetic Resonance Imaging 36, 68-76 (2017)

[37] Tuček J - Malá A et al. Air-stable superparamagnetic metal nanoparticles entrapped in graphene oxide matrix. Nature Communications 7, 12879, 11 p (2016)

[38] Klanicová N - Malá A - Macíček O - Zeman J - Starčuk jr. Z. MRI *Study of Liesegang Patterns: Mass Transport and Banded Inorganic Phase Formation in Gel.* Appl. Magnetic Resonance 48, 6, 545-557 (2017)

ultimate state has been claimed several times in cryogenic helium experiments in Grenoble and more recently in the room-temperature SF_6 experiments in Göttingen. Our theoretical analysis of the published results strongly suggests that such **claims are not justified** and that the empirical findings are **caused by non-OB effects** [39]. We contributed 60% by published experimental data analysis and paper co-authorship.

(ii) Transitions between different states of convection cause substantial changes in the heat transfer laws and in the turbulent velocity field, affecting the structure and the dynamical modes of the mean large-scale 'winds' (observable after averaging-out turbulent fluctuations). They can be quantified by the Reynolds number (Re), which we determine experimentally from correlations of 1- and 2-point time records of temperature fluctuations in the turbulent flow. We observed a **transition between different regimes of turbulent RBC** at Raleigh number (Ra) of 10^{10}-10^{11} and the Prandtl number Pr ≈ 1 [40], including the power law scaling of the global heat transfer, and linked the transition to the re-shaping of the large-scale circulation. We contributed 85% by experiment design and execution, data analysis and paper co-authorship.

(iii) In our RBC cell, we previously observed a seeming **violation of the second law of thermodynamics** in liquid-vapour helium, heat flow against the temperature drop between the top and the bottom plates. We explained this effect by the presence of liquid boil at the bottom plate [41]. In an analogous experiment, we used superfluid He II instead of the classical viscous liquid He I, where boiling hardly occurs. In this case, the cell followed closely the equilibrium saturated vapour curve temperature corresponding to the pressure. We explained the observed behaviour by the heat conduction short-circuit by a superfluid He II film covering the entire cell interior [42]. We contributed 70 % by experiment design and execution, data analysis and paper co-authorship.

(iv) We have shown that a transition to an **ultimate-like regime of turbulent RBC** in cryogenic helium fluid can be observed even in our experiment. However, it is spurious, caused by **non-OB effects** when the ^{4}He gas near the top plate of the RBC cell closely approaches the ^{4}He saturation vapor curve (SVC). In particular, our analysis suggests that the Göttingen findings were most likely caused by non-OB effects in the SF_6 fluid in the proximity to the SVC (which we theoretically analyzed earlier [39]). Thus, the important question of the transition to the ultimate state of RBC remains open [43]. We contributed 75% by experiment design and execution, data analysis and paper co-authorship.

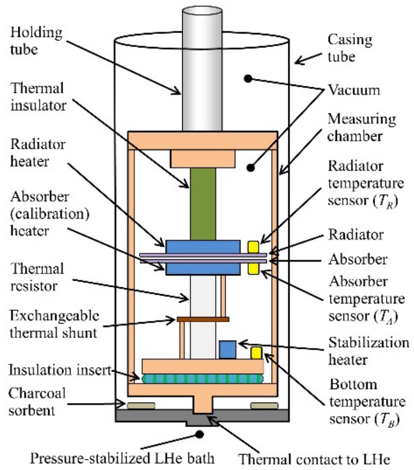
(v) A joint project with TU Ilmenau, Germany, under the European High Intensity Turbulence (**EuHIT**) infrastructure program “Scaling and temperature profiles in turbulent Rayleigh-Bénard convection (**ScaRaB**)” enabled us to extend the knowledge about non-OB effects in RBC. The experiments took place in the Barrel of Ilmenau in a cylindrical cell (diameter 7 m, height 6.3 m) filled with dry air. The new optical fibre system LUNA ODiSI-B allowed us to measure temperature profiles with high spatial

[39] Skrbek L - Urban P. Has the ultimate state of turbulent thermal convection been observed? Journal of Fluid Mechanics 785, 270-282 (2015)
[40] Musilová V - Králík T - La Mantia M - Macek M - Urban P - Skrbek L. Reynolds number scaling in cryogenic turbulent Rayleigh-Benard convection in a cylindrical aspect ratio one cell. Journal of Fluid Mechanics 832, 721-744 (2017).
[41] Urban P - Schmoranzer D - Hanzelka P -Sreenivasan KR - Skrbek L. *Anomalous heat transport and condensation in convection of cryogenic helium*. Proc. Nat. Acad. Sci. USA 110, 8036 (2013)
[42] Urban P - Hanzelka P - Vlček I - Schmoranzer D - Skrbek L. Convective heat transport in two-phase superfluid/vapor 4He system. Low Temperature Physics 44, 10, 1001-1004 (2018)
[43] Urban P - Hanzelka P - Králík T - Macek M - Musilová V - Skrbek L. Elusive transition to the ultimate regime of turbulent Rayleigh-Benard convection. Physical Review E 99, 1, 011101 (2019)

resolution and at a high frequency simultaneously along the whole vertical axis of the cell, in the Ra number range of 10^{11}-10^{12}. The results were partially published [44].

(B) *Near-field radiative heat transfer*

Electromagnetic interaction of closely spaced bodies via thermal radiation (van der Waals force, Casimir force, near-field radiative heat transfer) attracts research interest in connection with the development of micro-electro-mechanic and optoelectronic systems. Radiative heat transfer over distances shorter than the characteristic wavelength of radiation, the so called near-field radiative heat transfer (NFRHT), can exceed the classical black-body limit for far-field radiation by several orders of magnitude. Distances typical for cryogenic NFRHT are of the order of micrometres, thus NFRHT may be relevant to cryogenic design. Previously, we designed a cryogenic apparatus for the measurement of **NFRHT between plane-parallel samples** and presented the results obtained with tungsten samples. We extended this successful experiment to low-temperature superconductors Nb [45] and NbN [46]. We showed that the **effect of superconductivity on NFRHT**, unlike the far-field RHT, is surprisingly strong also for radiation with the characteristic energy of photons higher than the superconducting energy gap.



A challenging experimental setup with plane-parallel sample surfaces enabled us to test the theory more directly than other geometries could. Using the classical theory of superconductivity (BCS) for the calculation of optical constants of a NbN superconductor, we showed good agreement with the theory of NFRHT. We also measured NFRHT between copper surfaces to test for possible parasitic effects of NFRHT on cryogenic experiments measuring critical Rayleigh number of convection in cold helium gas [47]. In a paper submitted to Cryogenics in Nov 2019 we enhanced our previous results on NFRHT by presenting the dependences of thermal conductivities of a vacuum gap in the NFRHT regime on distance. To all three NFRHT papers we contributed 80% by experiment design and execution, data analysis and paper authorship.

(C) *Quantum phase transitions (QPT)*

occur as a sharp change of properties in a wide range of physical systems (e.g. atomic nuclei, molecules, ultra-cold gases etc.) and in many aspects resemble standard thermal phase transitions; unlike them, they are triggered by quantum (vacuum) fluctuations at zero absolute temperature. QPTs as well as their novel extension – the **excited-state quantum phase transitions** (ESQPTs) – are a non-linear quantum phenomenon, which besides its fundamental appeal is expected to be relevant for future quantum technologies, such as quantum simulators. This topic, studied by the new team member M. Macek in his previous career, opened new perspectives for ÚPT development thanks to

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[44] Drahotský J - Hanzelka P - Musilová V - Macek M - Du Puits R - Urban P. Temperature profiles measurements in turbulent Rayleigh-Bénard convection by optical fibre system at the Barrel of Ilmenau. EPJ Web of Conferences 180, 02020 (2018)

[45] Králík T - Musilová V - Fořt T - Srnka A. Effect of superconductivity on near-field radiative heat transfer. Physical Review B 95, 6, 060503, 5 p (2017)

[46] Musilová V - Králík T - Fořt T - Macek M. Strong suppression of near-field radiative heat transfer by superconductivity in NbN. Physical Review B 99, 2, 024511 (2019)

[47] Urban P - Králík T - Hanzelka P - Musilová V - Věžník T - Schmoranzer D - Skrbek L. *Thermal radiation in Rayleigh-Bénard convection experiments.* Physical Review E 101, 043106 (2020)

the possible experimental-theoretical synergy with the ÚPT's team of coherence optics. So far, two papers were published:

(i) We extended [48] our systematic theoretical approach to ESQPTs in systems with two degrees of freedom [49] [50] to truly **many-body quantum systems**. As an example, we took the interacting **boson model** of nuclear collective dynamics, with a Hamiltonian describing atomic nuclei undergoing first-order QPTs between spherical and prolate deformed ground state shapes. ESQPTs occurring in the system result from various classical stationary points of the model Hamiltonian, whose complexity exceds the existing theory [49] [50] because of (a) nontrivial mixing of kinetic and potential energy terms and (b) boundedness of the classical phase space. The features studied here are inherent in a great majority of interacting boson systems. Our contribution was 50 %: discovery and interpretation of the effects, calculations, co-authorship.

(ii) We generalized the notion of ESQPTs for one- and two-dimensional **crystals** and studied (numerically for square and hexagonal lattices) the localization properties of the wave functions of vibrations in two-dimensional (2D) crystals [51]. The wave functions of 2D lattices have remarkable localization properties, especially at the van Hove singularities. We compared the wave localisation in both structures and found unique features in graphene-like structures. Our contribution was 33%: design and execution of numerical calculations, paper co-authorship.

(iii) We dealt with various aspects of **QPTs in mesoscopic systems** (symmetry-breaking and chaos, possible experimental signatures in microwave resonators, analogy between QPT in few-body lattice systems and single-site many-body systems) in a series of the proceedings of the Yale-University "Workshop Symmetries and Order: Algebraic Methods in Many-Body Systems", concluded by a review paper [52].

Applied research

(a) *The radiative heat flow*, crucial in cryogenic design, depends on the emissivity and absorptivity of materials. Accurate measurements of these quantities are difficult, especially with highly reflecting metals at low temperatures. Moreover, their values are difficult to predict due strong dependence on the particular state of the material surface.

During 2015-2019, we kept improving our cryogenic apparatus (**Emister**) and used it for **measurement of total hemispherical emissivity and absorptivity** [53]. The surface characteristics of various materials are often altered by deposition of a thin layer of a different material; we found the optimum technology achieving the lowest emissivity and absorptivity of gold-coated material [54]. In a key paper [55] we summarized two decades of our experience by presenting the results of almost 60 experiments in the associated

[48] Macek M - Stránský P - Leviatan A - Cejnar P. Excited-state quantum phase transitions in systems with two degrees of freedom. III. Interacting boson systems. Physical Review C 99, 6, 064323 (2019)
[49] Macek M - Stránský P - Leviatan A - Cejnar P. Excited-state quantum phase transitions in systems with two degrees of freedom:III. Interacting boson systems. Ann. Phys. 345, 73-87 (2014)
[50] Stránský P - Macek M - Leviatan A - Cejnar P. Excited-state quantum phase transitions in systems with two degrees of freedom: II. Finite-size effects. Ann. Phys. 356, 57-82 (2015)
[51] Dietz B - Iachello F - Macek M. Algebraic Theory of Crystal Vibrations: Localization Properties of Wave Functions in Two-Dimensional Lattices. Crystals. Roč. 7, 8, 246 (2017)
[52] Cejnar P - Stránský P - Kloc M - Macek M. Static vs. dynamic phases of quantum many-body systems. Symmetries and Order: Algebraic Methods in Many-Body Systems 2150, 020017 (2019)
[53] Králík T - Musilová V- Hanzelka P - Frolec J. Method for measurement of emissivity and absorptivity of highly reflective surfaces from 20 K to room temperatures. Metrologia 53, 2, 743-753 (2016)
[54] Frolec J - Králík T - Srnka A. Low temperature thermal radiative properties of gold coated metals. International Journal of Refrigeration 82, 51-55 (2017)
[55] Frolec J - Králík T - Musilová V - Hanzelka P - Srnka A - Jelínek J. A database of metallic materials emissivities and absorptivities for cryogenics. Cryogenics 97, 85-99 (2019)

database describing emissivities and absorptivities in a wide range of cryogenic temperatures, including the influence of the material surface treatment and coating. We also used the Emister apparatus in a study on temperature dependence of niobium sample emissivity at very low temperatures. In cooperation with Frentech Aerospace Company, Brno, we assessed thermal radiative properties of the gold coatings used for the parts of the third generation of the meteorological satellites (MTG mission).

(b) *Multilayer insulation* (MLI, a stack of highly reflective foils interleaved with thermally insulating layers of fabrics, the spacers) is one of the basic thermal insulating systems for cryogenic devices. In collaboration with our industrial partner (RUAG Space, Vienna), we built a special cryogenic apparatus (**Spaceman**) and used it for measurements of crucial properties of MLI spacers under conditions similar to those in a practical MLI. The heat transfer across a spacer dependence on temperature, temperature difference and compression were used for the optimisation of insulations. The apparatus was further enhanced by a system of direct measurement of the spacer thickness based on the electrical capacitance. Besides this feature, our apparatus for MLI tests is unique by providing a wide temperature range of 10-300 K and a precisely controlled weight load 0.1-5.0 g applied on the spacer (maintained constant in the whole temperature range via a feedback loop with a load sensor). Our data on thermal properties of MLI components were used for the optimization of the insulations in numerous applications where thermal radiative heat flow between hot and cold parts of a system is required to decrease substantially, e.g. cryogenic industry, medicine or space applications. The results are described in internal research reports.

(c) *Cryogenic components for electron microscopy*:
For the R&D project **AMISPEC**[56] we developed the low-temperature parts of the UHV SEM/SPM modular system for in situ fabrication and characterisation of nanostructures under UHV conditions in a wide temperature range of 20-700 K. Our main scientific achievements are two prototypes, a **LHe/LN2 flow cryostat** for cooling the sample holder using with helium or liquid nitrogen, and a **thermal insulation pad** (InBallPad) for placing the sample holder. Our third important result is a utility model of the **cryogenic sample holder** electrically connectible to a removable transport pallet with the sample. The function of all parts was successfully verified down to the temperature of 20 K, reached in a test vacuum chamber with a helium flow cooling system. No other holders for the intended use have been found available on the market.

Our team further collaborates on the GAČR project No. GA19-08239S with the team of assoc. prof. D. Heger from Department of Chemistry, Masaryk University and Dr. V. Neděla‘s ÚPT team of environmental electron microscopy, focusing on the the application of optical spectroscopies coupled with environmental scanning electron microscopy (ESEM) to **studies of water ice**. The CS group will design a low-temperature system for cooling the water ice samples studied in the ESEM and install it inside the current vacuum chamber of the microscopy.

[56] Project AMISPEC (Advanced Microscopy and Spectroscopy Platform for Research and Development in Nano and Micro¬technologies), supported by the Technology Agency of the Czech Republic from the program of Competence Centres (Grant No.TE01020233, 2012-2019)

Research activity and characterisation of the main scientific results

The selected results that best characterize and summarize the team's activities and MediSig contributions are also presented in the first phase of evaluation.

Breakthrough technologies

Ultra-high-frequency electrocardiography – UHF-ECG (100-1000 Hz)

UHF-ECG is a worldwide new field of cardiac electrophysiology, which our team introduced. Using UHF-ECG, we can localize the course of the propagation of depolarization wavefront in the heart ventricles. The basic principle is shown in Figure 1.

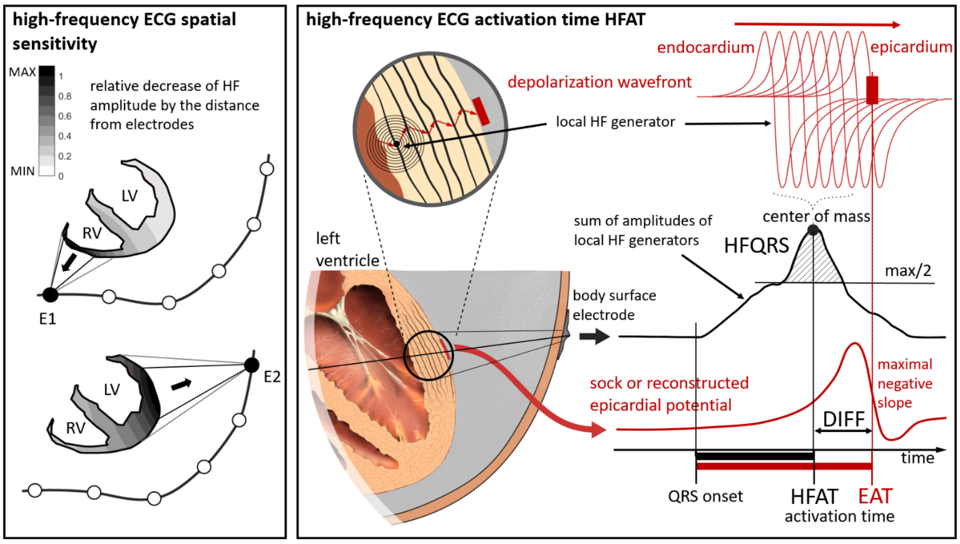


Figure 1. ***Ultra-high-frequency ECG principle.*** *UHF-ECG measures the temporal and spatial distribution of myocardial cell depolarization.*

UHF-ECG differs from standard ECG, which measures the projection of the main electric vector into individual body surface electrodes. UHF-ECG reflects volumetric activation linked with mechanical contraction. This feature provides essential information, especially in the field of cardiac pacing.

Ventricular Dyssynchrony Imaging (VDI) technology

VDI is the original UHF-ECG technology for cardiac pacing treatment. The VDI monitor uses 5kHz data acquisition to measure the depolarization activation properties:

- Image of electrical depolarization pattern of the ventricles (Figure 2).
- A set of numerical parameters that measure the electrical interventricular dyssynchrony (e-DYS) and the local depolarization duration (Vd).
- Results are computed automatically in real-time, without the operator's intervention.

- VDI utilization is cheap and easy to implement into clinical care through standard 12(14)-lead ECG setup.

The field of application of VDI includes above all:

- Identification of ventricular conduction abnormalities in general, especially in patients with heart failure before pacemaker implantation.
- Determination of immediate pacing effect on ventricular depolarization during the pacing lead placement – biventricular, His bundle, right ventricular septal, LBB pacing and more.
- Optimization of cardiac resynchronization therapy (CRT) device settings (AV and VV delay; LV fusion pacing, vector selection of a quadripolar pacing lead).
- Long-term evaluation of changes in ventricular electrical activation.

For these purposes, VDI technology is currently being tested in 6 hospitals in the Czech Republic, Poland, and England. Basic principles were validated in the Maastricht, Nederland, and Bordeaux, France.

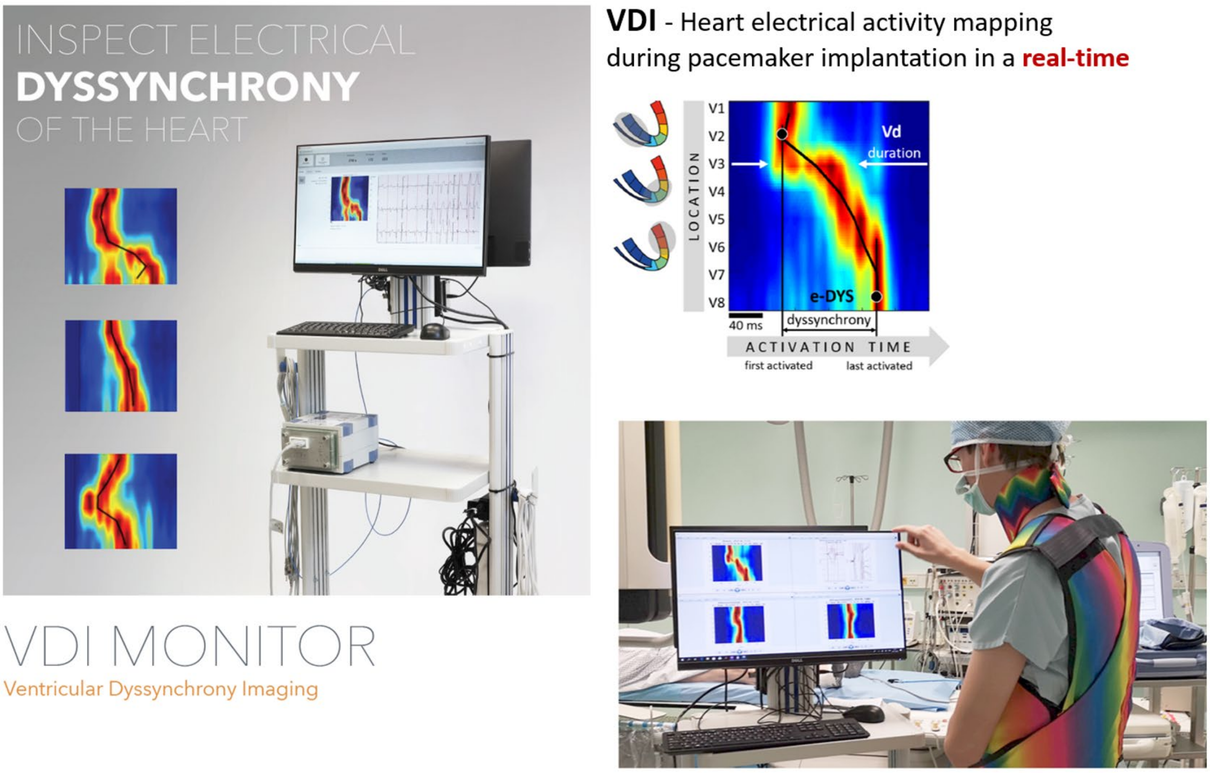


Figure 2. ***VDI monitor.*** *U.S. Pat. No. 9,949,655, European Patent Application, EP 19212534.2. European Society of Cardiology and Computing in Caridology: Clinical Needs Translation Award (CTA) 2017*

Research is supported by publications and projects:

- Jurak P, Curila K, Leinveber P, Prinzen FW, Viscor I, Plesinger F, Smisek R, Prochazkova R, Osmancik P, Halamek J, Matejkova M, Lipoldova J, Novak, M., Panovsky R, Andrla P, Vondra V, Stros P, Vesela J, Herman D. *Novel ultra-high-frequency electrocardiogram tool for the*

description of the ventricular depolarization pattern before and during cardiac resynchronization, Journal of Cardiovascular Electrophysiology, 31, 300-307 (2020), *Finalist EHRA 2020 innovation award (EHRA cancelled due to COVID-19).*

- Curila K, Prochazkova R, Jurak P, Jastrzebski M, Halamek J, Moskal P, Stros P, Vesela J, Waldauf P, Viscor I, Plesinger F, Sussenbek O, Herman D, Osmancik,P, Smisek R, Leinveber, P, Czarnecka D, Widimsky P. *Both selective and nonselective His bundle, but not myocardial, pacing preserve ventricular electrical synchrony assessed by ultra-high-frequency ECG*, Heart Rhythm, 17, 607-614 (2020), b*est of Heart Rhythm Journal 1-6 2020.*
- Halamek J, Leinveber P, Viscor I, Smisek R, Plesinger F, Vondra V, Lipoldova J, Matejkova M, Jurak P. *The relationship between ECG predictors of cardiac resynchronization therapy benefit,* PLOS One, 14, e0217097 (2019).
- Plesinger F, Jurak P, Halamek J, Nejedly P, Leinveber P, Viscor I, Vondra V, McNitt S, Polonsky B, Moss AJ, Zareba W, Couderc JP. *Ventricular Electrical Delay Measured From Body Surface ECGs Is Associated With Cardiac Resynchronization Therapy Response in Left Bundle Branch Block Patients From the MADIT-CRT Trial*, Circulation Arrhythmia and Electrophysiology, 11(5), (2018).
- Jurak P, Halamek J, Meluzin J, Plesinger F, Postranecka T, Lipoldova J, Novak M, Vondra V, Viscor I, Soukup L, Klimes P, Vesely P, Sumbera J, Zeman K, Asirvatham RS, Tri J, Asirvatham SJ, Leinveber P. *Ventricular dyssynchrony assessment using ultra-high frequency ECG technique,* Journal of Interventional Cardiac Electrophysiology, 49, 245-254 (2017).
- Leinveber P, Halamek J, Jurak P. *Ambulatory monitoring of myocardial ischemia in the 21st century-an opportunity for high frequency QRS analysis,* Journal of Electrocardiology, 49, 902-906 (2016)
- OP PIK, VDI monitor project EG15_019/0004993. Cardion company, ISI Brno (Jurak P et. al.), The Czech business project of 2020

Collaboration:

- International Clinical Research Center, St. Anne’s University Hospital, Brno, CZ.
- University Hospital Kralovske Vinohrady, Prague.
- Cardiovascular Research Institute Maastricht, NL.
- 3rd Faculty of Medicine, Charles University, Prague, CZ.
- Imperial College, London.
- University Bordeaux, France.
- University of Rochester, Rochester, NY, USA
- Cardion company, CZ

Fast, handheld arrhythmia detector with AI – HACT

HACT is highly innovative technology that combines cutting-edge design with research in the field of artificial intelligence.

A handheld Heart Activity monitor (HACT) is aimed for anybody needing a fast analysis of heart rhythm. It measures ECG signal and using on-chip artificial intelligence, and it decides whether the ECG contains atrial fibrillation, other arrhythmias, or just regular sinus rhythm. When the user needs to see the measured ECG curve, it can be connected to a tablet where the curve is displayed after the 15-seconds long measurement.

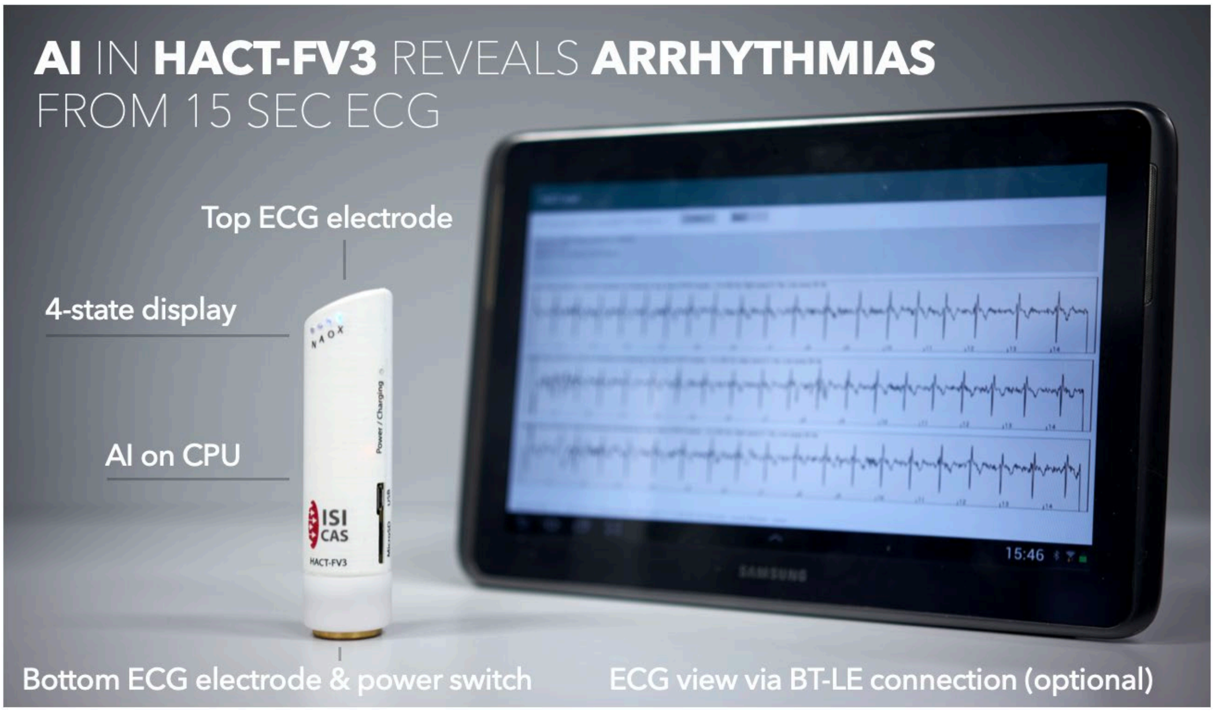


Figure 3. ***HACT, ECG based automatic arrhythmia detector.*** *HACT is an apparatus intended for cardiac arrhythmias identification. It is designed to be held in one hand and attached to the other hand or chest. The measured signal is automatically processed using artificial intelligence methods.*

Research is supported by publication and project:

- Plesinger F, Andrla P, Viscor I, Halamek J, Bulkova V, Jurak P. *Shape Analysis of Consecutive Beats May Help in the Automated Detection of Atrial Fibrillation*, Computing in Cardiology, 45, 8743764 (2018).
- TG03010046-2019V002 project, the Czech Technological Agency.

Collaboration:

- Medical Data Transfer company, Brno, CZ

Very and ultra-fast oscillations (up to 2000Hz) in the human brain – target epilepsy markers.

Surgical treatment is often the only therapy used in drug-resistant epileptic patients. The preparatory stage for the surgical treatment allows us to obtain a unique deep brain electroencephalographic data using top acquisition technologies, in the development of which we participated. High-quality and extensive data (200 channels, up to 25kHz sampling) allow for highly specific analysis with a targeted diagnosis of the onset of epileptic seizures.

Recent study published in the prestigious neurological journal as the first in the world has shown that in the deep human brain structures, an electrical activity above 1000

Hz (ultra-fast ripples – UHF) can be measured by standard clinical electrodes. This activity is located in areas that are the origin of epileptic activity. There is a high correlation between the reduction of epileptic seizures and surgical removal of tissue with ultra-high frequency oscillations. The precise location of epileptic foci will allow targeted resection of epileptic tissue and introduction of new technologies such as selective deep brain stimulation or micro-ablation.

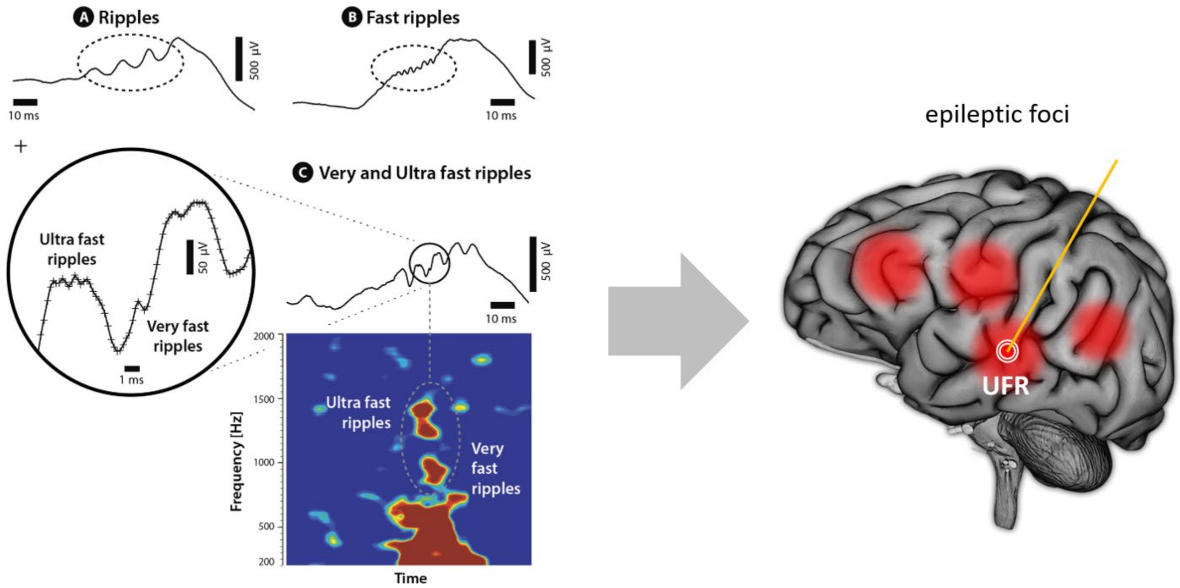


Figure 4. An example of different types of oscillations in the human brain. Ultra-fast ripples (UFR) show oscillation above 1000 Hz. UFRs occur only in the pathological region that is the origin of an epileptic seizure.

Research is supported by publications:

- Brázdil M, Pail M, Halámek J, Plešinger F, Cimbálník J, Roman R, Klimeš P, Daniel P, Chrastina J, Brichtová E, Rektor I, Worrell GA, Jurák P. *Very High-Frequency Oscillations: Novel Biomarkers of the Epileptogenic Zone*. Annals of Neurology, 82, 299-310 (2017).
- Cimbalnik J, Brinkmann B, Kremen V, Jurak P, Berry B, Gompel JV, Stead M, Worrell G. *Physiological and pathological high frequency oscillations in focal epilepsy*. Annals of Clinical And Translational Neurology, 5, 1062-1076 (2018).
- Rektor I, Doležalová I, Chrastina J, Jurák P, Halámek J, Baláž M, Brázdil M. *High-Frequency Oscillations in the Human Anterior Nucleus of the Thalamus*, Brain Stimulation, 9, 629-631 (2016).

Collaboration:

- International Clinical Research Center, St. Anne's University Hospital, Brno, CZ.
- Mayo Clinic, MN, USA.

The whole body impedance cardiography

A new possibility for hemodynamic diagnostics is provided by the recently developed multichannel, whole-body impedance monitor, MBM. The MBM 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 excellent signal quality.

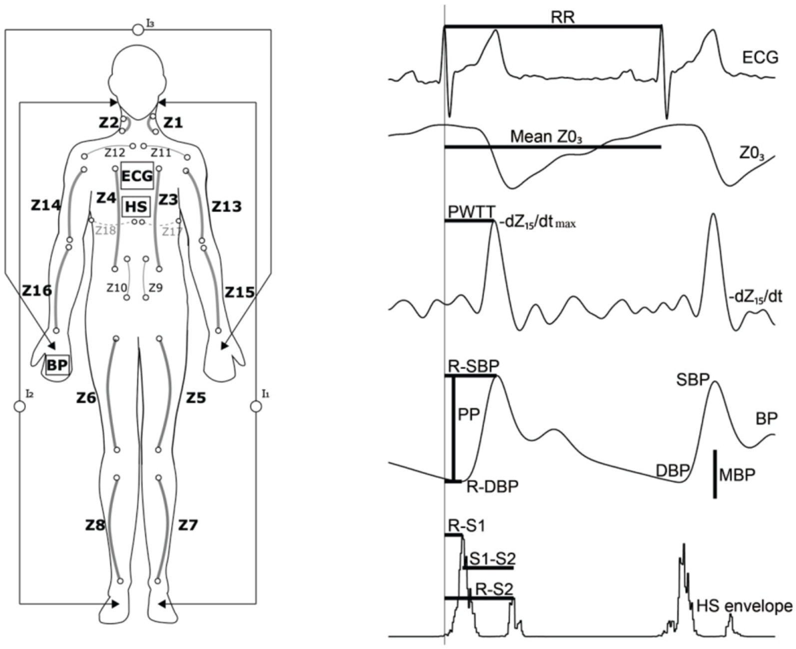


Figure 5. Measurement locations and measured signals of Multichannel Bioimpedance Monitor (MBM), https://www.medisig.com

Research is supported by publications:

- Soukup L, Hruskova J, Jurak P, Halamek J, Zavodna E, Viscor I, Matejkova M, Vondra V. *Comparison of noninvasive pulse transit time determined from Doppler aortic flow and multichannel bioimpedance plethysmography*, Medical & Biological Engineering & Computing, 57, 1151-1158 (2019)
- Langer P, Jurák P, Vondra V, Halámek J, Mešťaník M, Tonhajzerová I, Viščor I, Soukup L, Matejkova M, Závodná E, Leinveber P. *Respiratory-Induced Hemodynamic Changes Measured by Whole-Body Multichannel Impedance Plethysmography*, Physiological Research, 67, 571-581 (2018)
- Vondra V, Jurak P, Viscor I, Halamek J, Leinveber P, Matejkova M, Soukup L. *A multichannel bioimpedance monitor for full-body blood flow monitoring*, Biomedical Engineering, 61, 107-118 (2016)
- Zvonicek V, Jurak P, Halamek J, Kruzliak P, Vondra V, Leinveber P, Cundrle I, Pavlík M, Suk P, Sramek V. *The impact of sedation on pulse pressure variation*, Australian Critical Care, 28, 203-207 (2015)

Collaboration:

- International Clinical Research Center, St. Anne's University Hospital, Brno, CZ
- Mayo Clinic, MN, USA

Machine learning and new signal processing methods

It's exciting to look for normal activities and pathological behavior in the heart and brain signals supported by large data sets.

Deep-learning in epilepsy

Logically, deep learning methods are used for data processing from the deep brain structures. The nature of our neuro research is based on these goals and pillars:

- Support for the implementation of new technologies in invasive epilepsy treatment (local brain stimulation, ablation) requires precise localization of pathology – in millimeters.
- Extensive data is available directly from the deep brain structures, but the high information value is faced with a lot of artefacts and noise.
- Deep learning methods will make it possible to analyse this unique but large data sets and accurately identify pathological sources and exclude artificial information.
- To make the results clinically relevant, the results must be verified in international multicentre studies.

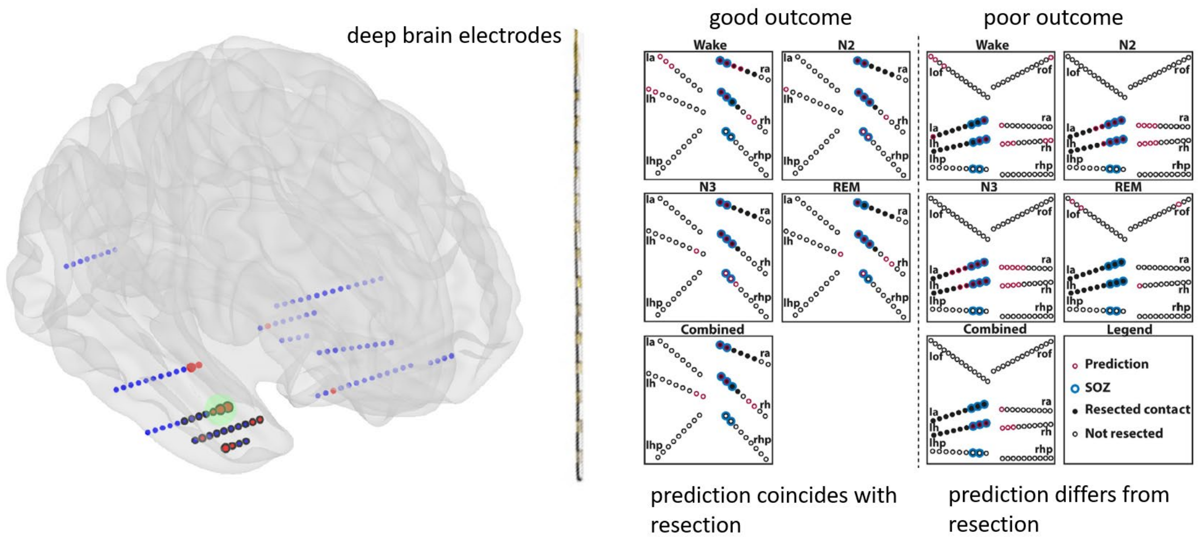


Figure 6. Epileptic foci localization (SOZ) by deep learning methods, validation with surgical treatment outcome.

Research is supported by publications:

- Nejedly P, Kremen V, Sladky V, Cimbalnik J, Klimes P, Plesinger F, *Viscor I*, Pail M, Halamek J, Brinkmann BH, Brazdil M, Jurak P, Worrell G. *Exploiting Graphoelements and Convolutional Neural Networks with Long Short Term Memory for Classification of the Human Electroencephalogram*. Scientific Reports, 9, 11383 (2019)
- Nejedly P, Cimbalnik J, Klimes P, Plesinger F, Halamek J, Kremen V, Viscor I, Brinkmann BH, Pail M, Brazdil M, Worrell G, Jurak P. *Intracerebral EEG Artifact Identification Using Convolutional Neural Networks*. Neuroinformatics, 17, 225-234 (2019)

- Nejedly P, Kremen V, Sladky V, Nasseri M, Guragain H, Klimes P, Cimbalnik J, Varatharajah Y, Brinkmann BH, Worrell G. *Deep-learning for seizure forecasting in canines with epilepsy*. Journal of Neural Engineering. 16, 2019
- Cimbalnik J, Klimes P, Sladky V, Nejedly P, Jurak P, Pail M, Roman R, Daniel P, Guragain H, Brinkmann B, Brazdil M, Worrell G. *Multi-feature localization of epileptic foci from interictal, intracranial EEG*. Clinical Neurophysiology, 130, 1945-1953 (2019)

Collaboration:

- International Clinical Research Center, St. Anne's University Hospital, Brno, CZ
- Mayo Clinic, MN, USA
- Montreal Neurological Institute and Hospital, Quebec, Canada

New methods and AI in cardiology

Artificial intelligence, specifically machine learning, helps us design methods that can reveal heart activity buried in noisy data as the Holter ECG. Conventional machine learning methods such as shallow neural networks or random forests can significantly improve the performance of algorithms analyzing ECG. But much substantial step is made when deep-learning methods as convolutional and recurrent networks are used. Our research in the field of AI aims to find ways to magnify deep-learning analysis from signal preprocessing to network architecture.

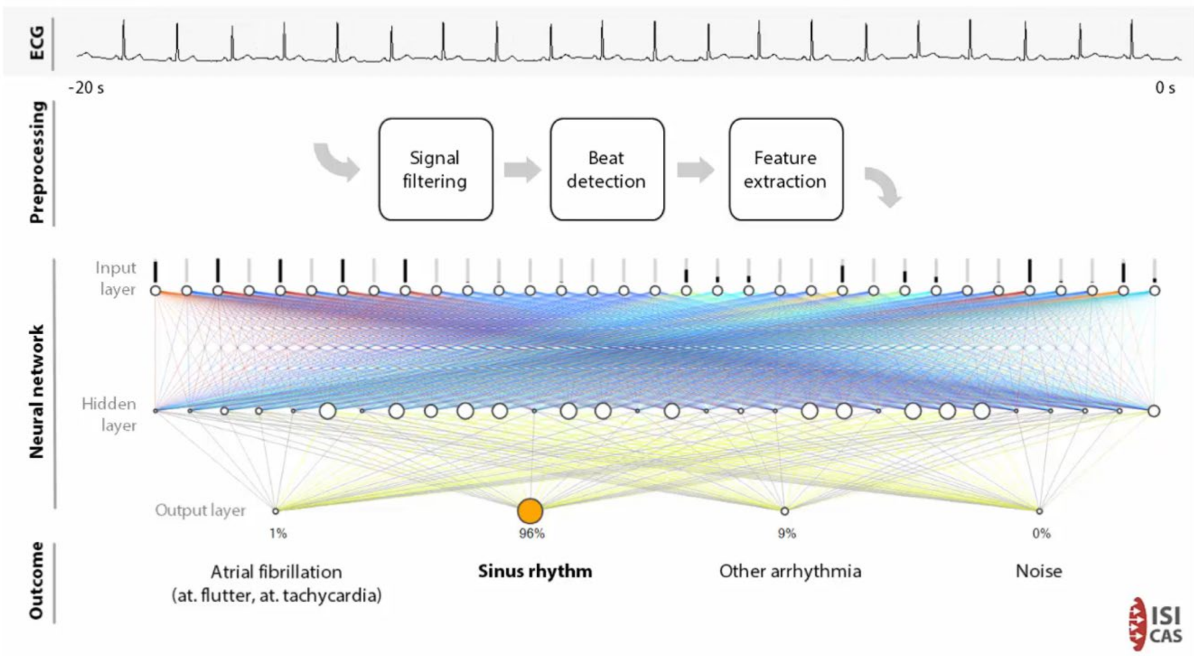


Figure 7. An example of shallow neural network. It analyzes incoming ECG signals (a simplification of real network prepared for a private telemedicine company). A new version related to deep-learning AI is a goal of the ongoing project.

Research is supported by publications:

- Plesinger F, Nejedly P, Viscor I, Halamek J, Jurak P. *Parallel use of a convolutional neural network and bagged tree ensemble for the classification of Holter ECG*, Physiological Measurement, 39, 094002 (2018)
- Plesinger F, Viscor I, Halamek J, Jurco J, Jurak P. *Heart sounds analysis using probability assessment*, Physiological Measurement, 38, 1685-1700 (2017)
- Plesinger F, Klimes P, Halamek J, Jurak P. *Taming of the monitors: reducing false alarms in intensive care units*, Physiological Measurement, 37, 1313-1325 (2016)

Basic research on the human brain normal and pathological behavior

Unique data from deep brain structures allow basic research into physiological and pathological activity in the brain. The research includes invasive data from epileptic patients - up to 200 contacts in the brain and patients suffering from Parkinson's disease and dystonia – 2x4 contacts in the left and right subthalamic nuclei.

The main areas of research include:

- A distinction of pathological and physiological areas.
- Inclusion of individual regions in decision-making processes.
- Functional organization and mutual communication between structures.

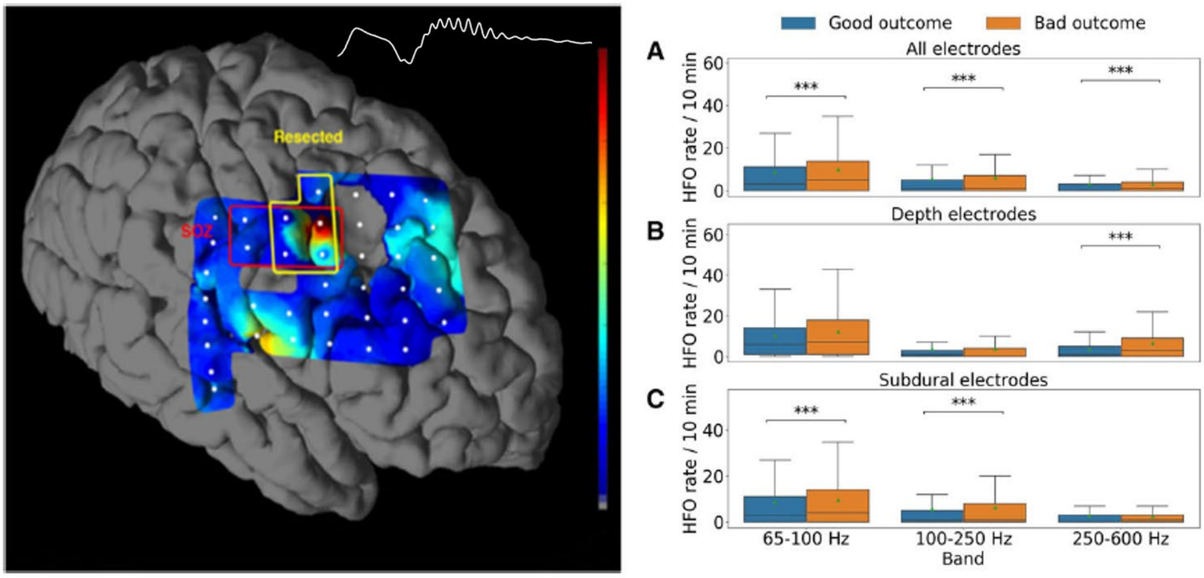


Figure 8. Physiological and pathological high frequency oscillations in focal epilepsy

Research is supported by publications:

- Klimes P, Cimbalnik J, Brazdil M, Hall J, Dubeau F, Gotman J, Frauscher B. *NREM Sleep Is the State of Vigilance That Best Identifies the Epileptogenic Zone in the Interictal Electroencephalogram*, Epilepsia, 60, 2404-2415 (2019)
- Brázdil M, Doležalová I, Koritáková E, Chládek J, Roman R, Pail M, Jurák P, Shaw DJ, Chrastina J. *EEG Reactivity Predicts Individual Efficacy of Vagal Nerve Stimulation in Intractable Epileptics*, Frontiers in Neurology, 10, 392 (2019)
- Klimes P, Jurak P, Halamek J, Roman R, Chladek J, Brazdil M. *Changes in connectivity and local synchrony after cognitive stimulation - Intracerebral EEG study*, Biomedical Signal Processing and Control, 45, 136-145 (2018)
- Bočková M, Chládek J, Jurák P, Halámek J, Rapcsak SZ, Baláž M, Chrastina J, Rektor I. *Oscillatory reactivity to effortful cognitive processing in the subthalamic nucleus and internal pallidum: a depth electrode EEG study*, Journal of Neural Transmission, 124, 841-852 (2017)
- Klimes P, Duque JJ, Brinkmann B, Van Gompel J, Stead M, St. Louis EK, Halamek J, Jurak P, Worrell G. *The functional organization of human epileptic hippocampus*, Journal of Neurophysiology, 115, 3140-3145 (2016)
- Štillová K, Jurák P, Chládek J, Chrastina J, Halámek J, Bočková M, Goldemundová S, Říha I, Rektor I. *The Role of Anterior Nuclei of the Thalamus: A Subcortical Gate in Memory Processing: An Intracerebral Recording Study*, PLOS One, 10, e140778 (2015)
- Brázdil M, Cimbálník J, Roman R, Shaw DJ, Stead MM, Daniel P, Jurák P, Halámek J. *Impact of cognitive stimulation on ripples within human epileptic and non-epileptic hippocampus*, BMC Neuroscience, 16, 47 (2015)
- Bočková M, Chládek J, Jurák P, Halámek J, Štillová K, Baláž M, Chrastina J, Rektor I. *Complex Motor-Cognitive Factors Processed in the Anterior Nucleus of the Thalamus: An Intracerebral Recording Study*, Brain Topography, 28, 269-278 (2015)

Collaboration:

- International Clinical Research Center, St. Anne's University Hospital, Brno, CZ
- Mayo Clinic, MN, USA
- Montreal Neurological Institute and Hospital, Quebec, Canada

Worldwide used software - SignalPlant

SignalPlant is open-platform, 64 bits, parallel multi treat computing software. It is equipped with several advanced functions for visualization and processing of large data sets. It creates a global platform for data analysis in cardiology and neurology. This software has a huge potential and is used worldwide (48 countries) in various research sectors and corporations. SignalPlant was utterly developed and implemented within the MediSig activities.

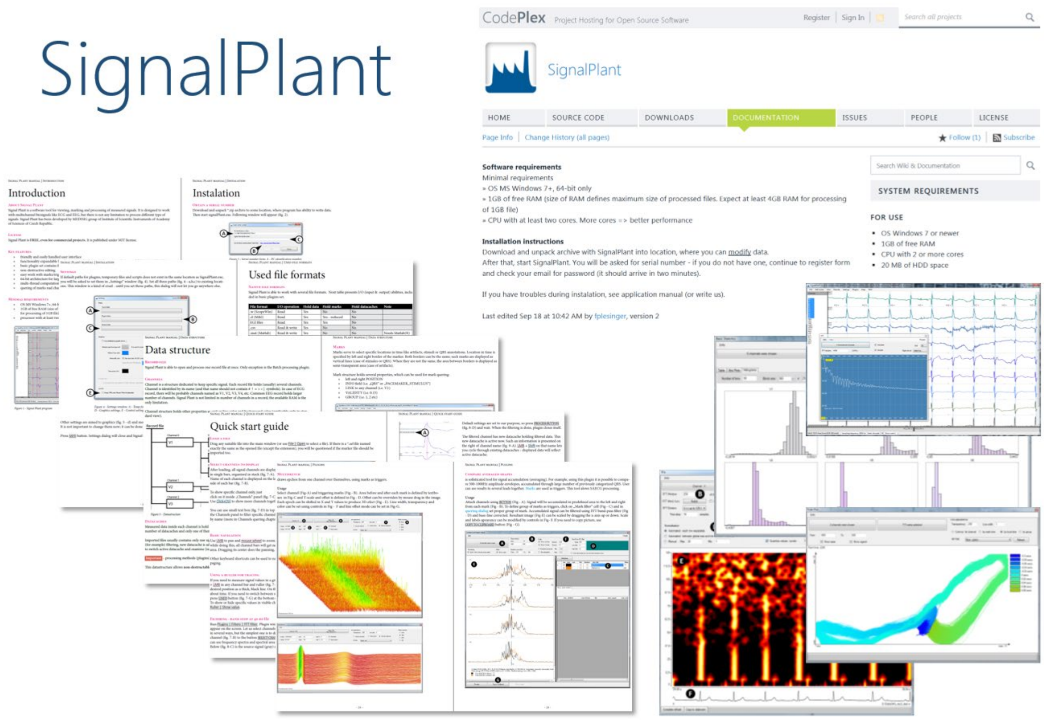


Figure 9. Signal plant open-platform, https://www.medisig.com/signalplant/

Research is supported by publications:

- Nejedly P, Plesinger F, Halamek J, Jurak P. *CudaFilters: A SignalPlant library for GPU-accelerated FFT and FIR filtering*, Software-Practice & Experience, 48, 3-9 (2018)
- Plesinger F, Jurco J, Halamek J, Jurak P. *SignalPlant: an open signal processing software platform*, Physiological Measurement, 37, 38-48 (2016)

Research activity and characterisation of the main scientific results

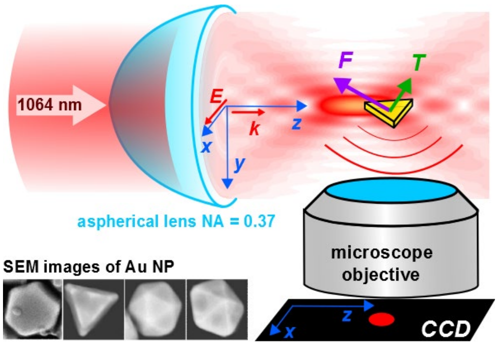
I. Basic Research

These exploratory activities are driven by our enthusiasm to reveal the hidden secrets of nature, understand them, and finally employ our understanding in applications useful for the mankind. Our approach usually combines theoretical or numerical studies with experimental investigations in the laboratories.

Understanding of interaction of complex objects with structured laser beams

*Motivation: A mechanistic example of such interaction - **optical micromanipulation** - employs tightly focused laser beams to confine, move and examine microscale and nanoscale objects such as colloidal particles, plant or animal cells or individual macromolecules. Majority of optical trapping experiments and theoretical studies have dealt with isotropic objects of spherical shape, whose behavior is governed by the optical force acting upon the object's center of mass. Optical manipulation with non-spherical particles, however, leads also to optically induced torques that rotate the particle around its axis and make the dynamics of one or more simultaneously trapped objects more complex and exciting. We tried to address the following questions:*

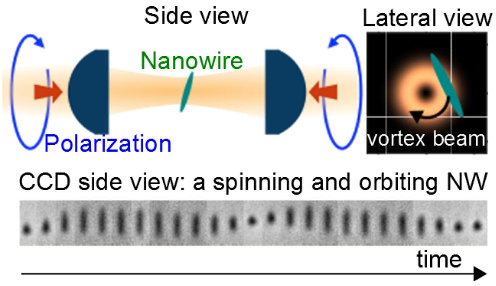
- *Is it feasible to illuminate microobjects immersed in liquids and driven by stochastic forces with a structured laser beam and force them to self-arrange into functional microstructures by light?*
- *Can laser illumination of heterogeneous suspensions of particles lead to spatial separation of their components based on shape, size, or composition?*



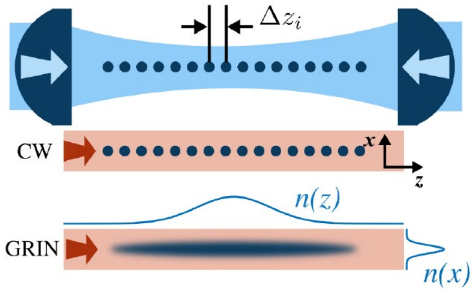
While performing experiments with larger, nominally spherical Au nanoparticles (NPs; diameter ~100 nm), we observed their 3D trapping even in relatively wide laser beams obtained by focusing optics with a low numerical aperture of 0.37. This behavior contrasted with the theoretically predicted impossibility of stable confinement of such nanoparticles. Electron micrographs (obtained by our colleagues from the Electron Microscopy group) revealed the presence of decahedrons, icosahedrons, and hexagonal and triangular prisms, but no spheres in the sample (see the figure). We proved that plasmonic resonance phenomena, which strongly depend on the particle shape, significantly affect the trapping stability and we obtained encouraging agreement between the trap stiffness measured and calculated for triangular-prism NPs[1,2]. These findings support our conclusion that the 3D trapping of larger Au NPs is enabled by their non-sphericity, which also leads to their preferred stable orientation in the trap, even though they are smaller than the trapping wavelength.

---

[1] Brzobohatý O, Šiler M, Trojek J, Chvátal L, Karásek V, Paták A, Pokorná Z, Mika F, Zemánek P. *Three-Dimensional Optical Trapping of a Plasmonic Nanoparticle using Low Numerical Aperture Optical Tweezers*. Sci. Rep., 5, 8106 (2015).
[2] Brzobohatý O, Šiler M, Trojek J, Chvátal L, Karásek V, Zemánek P. *Non-spherical gold nanoparticles trapped in optical tweezers: shape matters*. Opt. Express, 23, 8179-8189 (2015).



We developed a theory for calculating optical forces and torques acting on Si nanowires (NWs) and implemented codes for stochastic simulations of behavior of a single NW or more optically and hydrodynamically coupled NWs. We demonstrated that the model described well the experimental NW behavior observed in a single beam optical trap by our colleagues from CNR-IPCF in Messina[3]. Moreover, the model correctly predicted the behavior of optically bound NWs in counter-propagating beams of various polarizations[4] and agreed with our own experimental observations in counter-propagating Gaussian or Laguerre-Gaussian (vortex) laser beams[5] of circular (see the figure) or linear polarizations. In the same geometry of counter-propagating, elliptically polarized beams, we also studied behavior of larger non-spherical polystyrene particles[6] or chiral particles[7], their self-arrangement due to optical and hydrodynamic interactions, and we succeeded in explanation of their principal orientation and dynamical behavior[8]. Under certain circumstances, an asymmetric structure could keep sustained periodic motion driven by a non-conservative part of the optical forces.



When a denser colloidal suspension is illuminated with counter-propagating beams, optically bound matter can form from tens up to thousands of constituent particles. The inter-particle distances in this assembly can be tuned by the width of the trapping beams[9] and the whole structure can serve as a tunable colloidal optical waveguide (COW) for other wavelengths[10]. Spectral transmission properties of such COWs then depend on the inter-particle distances and can be well described using the analogy to a gradient index lens (GRIN; see the figure).

In collaboration with our colleagues in CIBION in Buenos Aires, we studied pushing of plasmonic nanoparticles along the beam propagation direction and their deposition on a solid substrate. In particular, we theoretically analyzed the particles' trajectories

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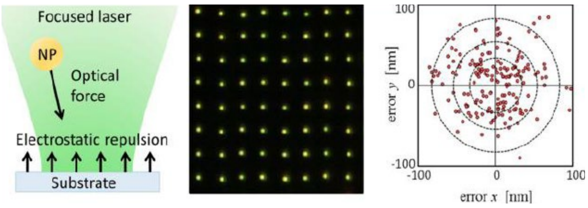
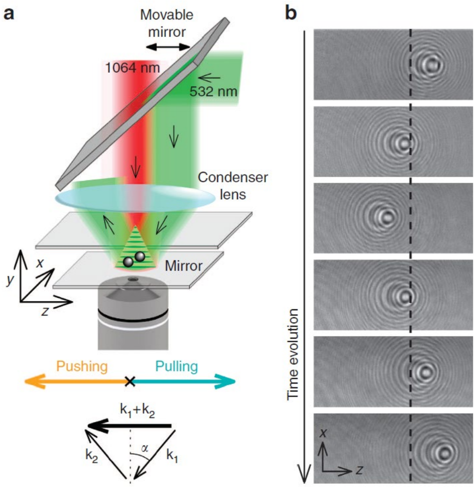
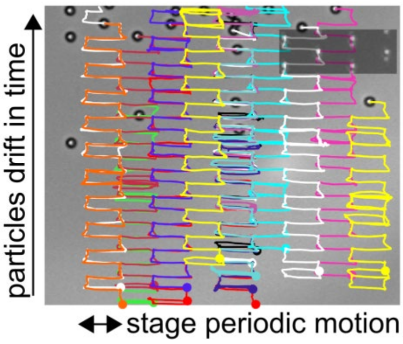
[3] Irrera A, Magazzu A, Artoni P, Simpson SH, Hanna S, Jones PH, Priolo F, Gucciardi P Giuseppe, Maragò OM. *Photonic Torque Microscopy of the Nonconservative Force Field for Optically Trapped Silicon Nanowires*. Nano Lett., 16, 4181-4188 (2016).
[4] Simpson SH, Zemánek P, Maragò OM, Jones PH, Hanna S. *Optical Binding of Nanowires*. Nano Lett., 17, 3485-3492 (2017)
[5] Donato MG, Brzobohatý O, Simpson SH, Irrera A, Leonardi AA, Faro MJLo, Svak V, Maragò OM, Zemánek P. *Optical Trapping, Optical Binding, and Rotational Dynamics of Silicon Nanowires in Counter-Propagating Beams*. Nano Lett., 19, 342-352 (2019).
[6] Brzobohatý O, Arzola AV, Šiler M, Chvátal L, Jákl P, Simpson SH, Zemánek P. *Complex rotational dynamics of multiple spheroidal particles in a circularly polarized, dual beam trap*. Opt. Express, 23, 7273-7287 (2015).
[7] Brzobohatý O, Hernandez RJosue, Simpson SH, Mazzulla A, Cipparrone G, Zemánek P. *Chiral particles in the dual-beam optical trap*. Opt. Express, 24, 26382-26391 (2016)
[8] Simpson SH, Chvátal L, Zemánek P. *Synchronization of colloidal rotors through angular optical binding*. Phys. Rev. A, 93, 023842 (2016)
[9] Brzobohatý O, Chvátal L, Zemánek P. *Optomechanical properties of optically self-arranged colloidal waveguides*. Opt. Lett., 44, 707-710 (2019)
[10] Brzobohatý O, Chvátal L, Jonáš A, Šiler M, Kaňka J, Ježek J, Zemánek P. *Tunable Soft-Matter Optofluidic Waveguides Assembled by Light*. ACS Phot., 6, 403-410 (2019)



and helped to explain the experimental observations in laser printing of nanostructures from Au and Ag nanoparticles obtained at CIBION[11].



Somewhat counterintuitively, illuminated particles can also be pulled against the flux of incoming photons; this phenomenon is sometimes referred to as the “tractor beam” (left part of the figure), inspired by the Star Trek series. Using this illumination geometry, we observed experimentally and explained theoretically why two and more optically bound particles move faster comparing to a single illuminated particle[12] and why they change the direction of motion (right part of the figure).



In collaboration with our colleagues from UNAM in Mexico City, we demonstrated a new approach to transporting many particles in any direction in a plane using an array of asymmetric optical potential wells[13]. This array is formed by a system of properly shaped, focused laser beams and serves as a “ratchet”, i.e., it prefers transport of particles along a selected direction. The figure illustrates an example of particles’ drift in the direction perpendicular to the direction of periodic motion of the stage. Rectangular figure inset then reveals the corresponding positions of overlapping asymmetric potential wells created by individual laser beam foci. The above described demonstration represents an example of a non-equilibrium experimental system that could serve as an artificial Brownian motor. Our team helped to design and build the experimental system and to develop the idea.

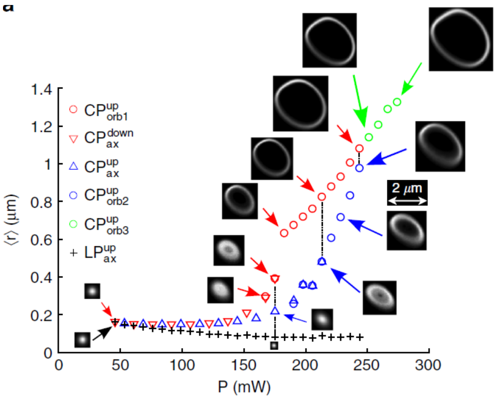
Light beams offer many exciting ways how to deliver objects in space. We summarized all the currently known methods into an extensive review with 583 references[14]. This review article represents an invaluable source of information for its broad readership including both experts and non-experts in the field of optical manipulation.

[11] Gargiulo J, Violi IL, Cerrota S, Chvátal L, Cortès E, Perassi EM, Diaz F, Zemánek P, Stefani FD. *Accuracy and Mechanistic Details of Optical Printing of Single Au and Ag Nanoparticles*. ACS Nano, 11, 9678–9688 (2017).
[12] Damková J, Chvátal L, Ježek J, Oulehla J, Brzobohatý O, Zemánek P. *Enhancement of the `tractor-beam' pulling force on an optically bound structure*. Light: Sci. Appl., 7, 17135 (2018).
[13] Arzola AV, Villasante-Barahona M, Volke-Sepúlveda K, Jákl P, Zemánek P. *Omnidirectional Transport in Fully Reconfigurable Two Dimensional Optical Ratchets*. Phys. Rev. Lett., 118, 138002, (2017).
[14] Zemánek P, Volpe G, Jonáš A, Brzobohatý O. *Perspective on light-induced transport of particles: from optical forces to phoretic motion*. Adv. Opt. Photon., 11, 577–678 (2019).

Development of classical and quantum optomechanics with optically levitated objects in vacuum

<u>Motivation:</u> Majority of optical trapping experiments have been performed in liquids where the trapped particle represents an overdamped thermally driven stochastic system. What kind of new phenomena can be observed when particle inertia plays a role, damping is very low and the object mainly interacts with the surrounding environment via photons of the scattered light? How much can one slow (cool) down the particle's center-of-mass motion by light, similar to atom cooling? Could one reach the quantum ground state of such a mechanical oscillator?

During the evaluated period, we built several experimental setups of different designs, with which we addressed various aspects of vacuum levitation. We applied the mechanism of feedback cooling and succeeded in cooling the center-of-mass motion of a silica nanoparticle of 160 nm diameter down to 3 mK[15] and observed optical binding between multiple optically levitated objects[15].



In 2016, S. Simpson participated in theoretical analyses of an experimental demonstration of so-called spin force[16]. This force was predicted about 80 year ago and was expected to be experimentally undetectable. However, it turned out the spin force could be detected locally, using circularly polarized evanescent light and an AFM cantilever. Two years later, we found a more elegant way how to demonstrate this force using a microparticle that was optically levitated in two counter-propagating circularly polarized Gaussian beams in an evaporated vacuum chamber. The spin force acted upon the levitated particle in the azimuthal direction and with increased power, it led to a qualitative change in the particle behavior – instead of chaotic motion around the beam center (lower power in the figure), the particle started orbiting at micrometer distances from the beam center[17] (bright nearly circular trajectories in the figure).

Study of nonlinear stochastic dynamics of optically trapped systems

<u>Motivation:</u> An underdamped optically levitated particle represents a unique system of a stochastic oscillator, for which one can relatively easily tune all the key parameters – mass, damping, spatial profile of the restoring force and effective temperature of the stochastic thermal excitation. Since the restoring force is determined by the spatial profile of the trapping laser beam, one can shape it to quite complex profiles with a strong nonlinear part. Can one observe some new phenomena in such strongly nonlinear stochastic systems and do the observations correspond to theoretical predictions?

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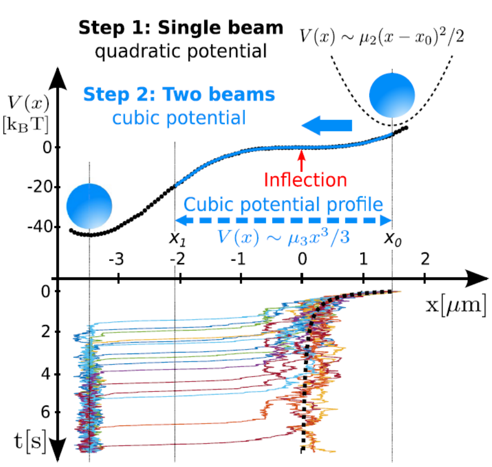
[15] unpublished results

[16] Antognozzi M, Bermingham CR, Harniman RL, <u>Simpson SH</u>, Senior J, Hayward R, Hoerber H, Dennis MR, Bekshaev AY, Bliokh KY, Nori F. *Direct measurements of the extraordinary optical momentum and transverse spin-dependent force using a nano-cantilever*. Nat. Phys,12, 731-735 (2016)

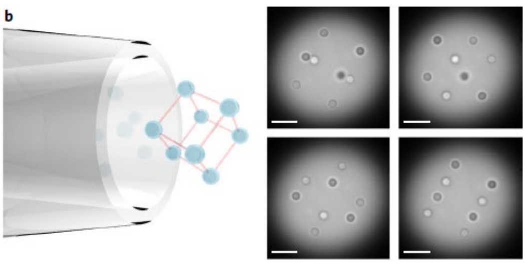
[17] <u>Svak V, Brzobohatý O, Šiler M, Jákl P, Kaňka J, Zemánek P, Simpson SH</u>. *Transverse spin forces and non-equilibrium particle dynamics in a circularly polarized vacuum optical trap*. Nature Commun., 9, 5453 (2018)



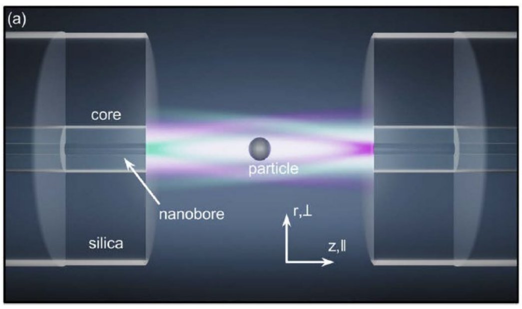
As the first step in our exploration of nonlinear stochastic systems, we focused on potentials following cubic polynomial dependence under overdamped conditions. Together with our colleagues from Palacky University in Olomouc, we analyzed stochastic noise-to-signal transition in overdamped Brownian motion and demonstrated that such a system might transform environmental noise to a useful mechanical effect[18]. We proposed and implemented a model experimental system[19], experimentally demonstrated the noise-to-signal transitions[20], derived properties for the first-passage times and verified our predictions experimentally[21], and finally proposed a new theoretical approach for describing highly unstable systems, which we again verified experimentally[22]. Our team contributed to the development of the idea, designed and implemented the experiment, ran simulations and analyzed the data.

Exploiting fiber optics in optical micromanipulation

Motivation: Could we miniaturize the end part of the trapping and observation paths in optical systems to the output face of an optical fiber?



We participated in the deployment of maneuverable arrays of optical traps through hair-thin flexible optical fibers using the principles of computer holography. With their small diameter, the fibers are capable of penetrating through complex media, including living tissues, without causing any major damage[23].



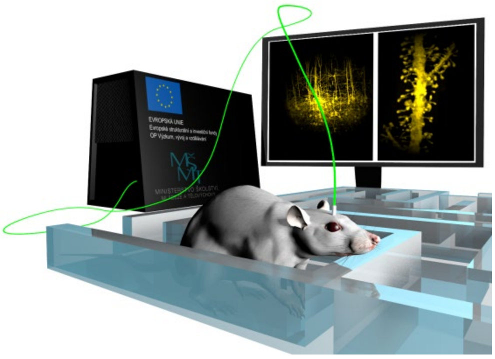
We demonstrated a novel type of dual-fiber optical trap that involves the use of nanobore fibers, having a nano-channel located in the center of their fiber cores. This nano-element leads to a profound redistribution of the optical intensity and to higher field gradients, yielding a trapping potential with greatly improved tuning properties compared to the standard step-index

[18] Filip R, Zemánek P. *Noise-to-signal transition of a Brownian particle in the cubic potential: I. general theory*. Journal of Optics, 18, 065401 (2016).
[19] Zemánek P, Šiler M, Brzobohatý O, Jákl P, Filip R. *Noise-to-signal transition of a Brownian particle in the cubic potential: II. optical trapping geometry*. Journal of Optics, 18, 065402 (2016).
[20] Šiler M, Jákl P, Brzobohatý O, Ryabov A, Filip R, Zemánek P. *Thermally induced micro-motion by inflection in optical potential.* Sci. Rep., 7, 1697 (2017).
[21] Ryabov A, Zemánek P, Filip R. *Thermally-induced passage and current of particles in highly unstable optical potential.* Phys. Rev.E, 16, 042108 (2016).
[22] Šiler M, Ornigotti L, Brzobohatý O, Jákl P, Ryabov A, Holubec V, Zemánek P, Filip R. *Diffusing up the Hill: Dynamics and Equipartition in Highly Unstable Systems*. Phys. Rev. Lett., 121, 23601 (2018).
[23] Leite IT, Turtaev S, Jiang X, Šiler M, Cuschieri A, Russell PSt. J, Čižmár T. *Three-dimensional holographic optical manipulation through a high-numerical-aperture soft-glass multimode fibre*. Nature Photon., 12, 33–39 (2018).

fiber types[24].

Development of fiber-based imaging methods for neuroscience and medicine

Motivation: Existing optical imaging methods, including the endoscopic ones, employ rather bulky optical elements and, therefore, may induce profound tissue damage when applied inside living organisms. Could we implement advanced microscopic and spectroscopic techniques via multimode optical fibers with diameters less than a hundred of micrometers and reach submicron spatial resolution? Can we image individual active neurons or red blood cells deep in the brain of a living mouse? Can we attain chemical imaging inside active brain?



Multimode optical fibers have recently been employed as high-resolution ultra-thin endoscopes, capable of imaging biological structures deep inside the tissue of small animal models[25], first in the laboratories of our colleagues at the University of Edinburgh and at Oxford University and, since 2019, also in the ISI labs. We extended this technique to label-free non-linear microscopy with chemical contrast using coherent anti-Stokes Raman scattering (CARS) through a multimode fiber endoscope[26], which opens up new avenues for instant *in situ* diagnosis of potentially malignant tissue.

Liquid crystal optofluidics

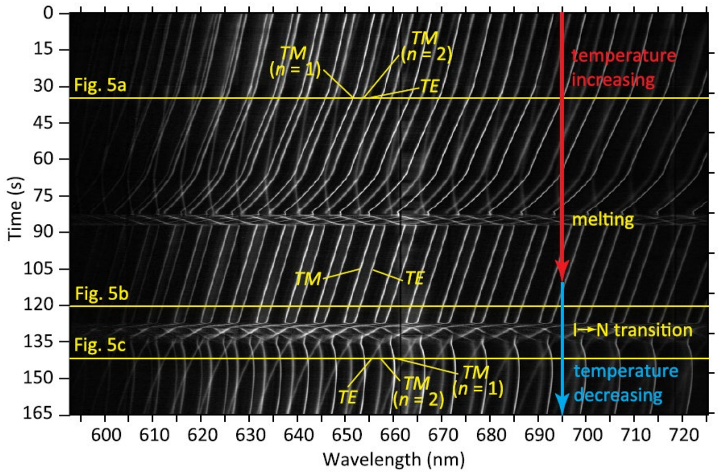
Motivation: Optofluidics denotes the combination of two research fields - optics and microfluidics – aiming at the design and implementation of unique reconfigurable optical and photonic systems inconceivable with conventional solid-state materials. Among the numerous topics studied in this research discipline, we addressed the following question: Is it feasible to create miniature thermally tunable sources of coherent light that can be manipulated and stabilized by optical forces?

In collaboration with our colleagues from Koc University in Istanbul, we showed that active optofluidic microcavities formed by six different types of dye-doped emulsion

[24] Plidschun M, Weidlich S, Šiler M, Weber K, Čižmár T, Schmidt MA. *Nanobore fiber focus trap with enhanced tuning capabilities*. Opt. Express, 27, 36221–36230 (2019).

[25] Flaes DEB, Stopka J, Turtaev S, de Boer JF, Tyc T, Čižmár T. *Robustness of Light-Transport Processes to Bending Deformations in Graded-Index Multimode Waveguides*. Phys. Rev. Lett., 120, 233901:1-5 (2018).
Turtaev S, Leite IT, Altwegg-Boussac T, Pakan JMP, Rochefort NL, Čižmár T. *High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging*. Light: Sci. Appl., 7, 92 (2018).
Vasquez-Lopez SA, Turcotte R, Koren V, Ploschner M, Padamsey Z, Booth M, Čižmár T, Emptage NJ. *Subcellular spatial resolution achieved for deep-brain imaging in vivo using a minimally invasive multimode fiber*. Light: Sci. Appl., 7, 110 (2018).

[26] Trägårdh J, Pikálek T, Šerý M, Meyer T, Popp J, Čižmár T. *Label-free CARS microscopy through a multimode fiber endoscope.* Opt. Express, 27, 30055–30066 (2019).
Pikálek T, Trägårdh J, Simpson S, Čižmár T. *Wavelength dependent characterization of a multimode fibre endoscope.* Opt. Express, 27, 28239–28253 (2019)



droplets of nematic liquid crystals suspended in an aqueous host liquid and confined in optical tweezers could emit lasing spectral lines corresponding to the whispery gallery modes (WGMs). Moreover, we demonstrated that such WGM emission spectra could be largely tuned in both directions by periodic changing of the droplet temperature[27]. Our team developed the sample preparation protocols, contributed to the design and implementation of the experimental setup, carried out part of the experiments, and analyzed the data.

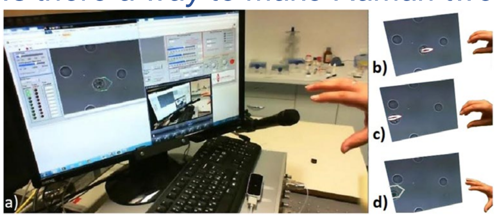
II. Applied research

Activities in this area cover our targeted research focused on finding methods or techniques to solve a particular practical problem to the level of obtaining a proof of the concept under relevant laboratory conditions.

Characterization of living microorganisms by Raman tweezers and Raman microspectroscopy

*Motivation: Microorganisms are a diverse group of micrometer-sized living objects including viruses, bacteria, fungi, archaea, and protists, which assume various important roles in the ecosystem and typically live suspended in water. Due to their small size, they can move either passively by Brownian motion or actively using chemically powered molecular motors, with typical speeds of tens of μm/s. Raman tweezers - a combination of **optical tweezers** for fixing the microorganisms in space and **Raman microspectroscopy** for analyzing their chemical composition - **offers a unique approach for contactless and harmless, real-time characterization of living microorganisms in their natural habitat.** We have sought applications of this method to various microorganisms, trying to answer the following questions:*

Is there a way to make Raman tweezers user-friendly or even autonomous?



In collaboration with our colleagues from the Institute of Experimental Physics of the Slovak Academy of Sciences in Košice, we developed a user-friendly interface that allows controlling the position of a single or multiple optical traps by naked-hand and finger gestures, eventually using also eye tracking, and running control messages by voice[28]. Our team provided fundamental ideas, built the Raman tweezers setup and implemented its control

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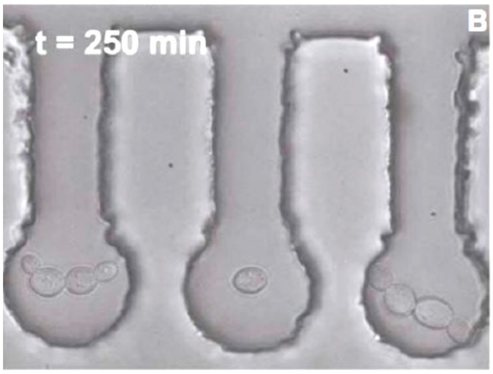
[27] Jonáš A, Pilát Z, Ježek J, Bernatová S, Fořt T, Zemánek P, Aas M, Kiraz A. *Thermal tuning of spectral emission from optically trapped liquid-crystal droplet resonators*. J. Opt. Soc. Am. B, 34, 1855-1864 (2017).
[28] Tomori Z, Keša P, Nikorovič M, Kaňka J, Jákl P, Šerý M, Bernatová S, Valušová E, Antalík M, Zemánek P. *Holographic Raman tweezers controlled by Multimodal Natural User Interface*. J. Opt., 18, 015602:1-9 (2016).

software. Independently we also developed a system for automatic sorting of cells based on the online analysis of their Raman spectra.

Is the confinement in optical tweezers harmful for the trapped microorganisms?



Using optical tweezers with the wavelength of 1064 nm, we trapped individual *Saccharomyces cerevisiae* (yeast) cells for 15 min and, subsequently, observed their stress response in specially designed microfluidic chambers over time periods of several hours by time-lapse video-microscopy. We characterized the dynamics of cell growth by calculating the population doubling period and cell areas for increasing trapping power at a constant trapping time. Our approach represents an attractive, versatile microfluidic platform for quantitative optical trapping experiments with living cells. We demonstrated its application potential by assessing the limits for safe, non-invasive optical trapping of *Saccharomyces cerevisiae* with infrared laser light[29].

How many different types of bacteria can we identify and what is the precision of this identification?

In collaboration with our colleagues from the St. Anne's University Hospital (SAUH) in Brno, we applied machine learning algorithms to process the Raman spectra of 16 different strains of staphylococci and achieved identification success rate of more than 99% for individual spectra and even 100% for selected strains[30]. Using the same approach, we were able to differentiate between biofilm-positive (i.e., forming complex structured layers on the surfaces of, e.g., medical catheters or implants) and biofilm-negative strains of *Staphylococcus epidermidis* and *Candida parapsilosis* with an accuracy 98.9% for *C. parapsilosis* and 96.1% for *S. epidermidis*, respectively[31]. These findings have important implications for the early detection and successful treatment of microbial infections in the clinical environment. Our team designed and performed the experiments and analyzed the recorded data.

Could we detect antibiotic treatment of a particular bacterium?

In collaboration with SAUH, we combined Raman tweezers and a microfluidic system composed of multiple micro-chambers, in which we analyzed individual bacterial cells of *E. coli* with and without antibiotic treatment with cefotaxime. Using principal components analysis, we were able to distinguish between the cefotaxime-treated and control cells[32]. Our team designed and performed the experiments and analyzed the data.

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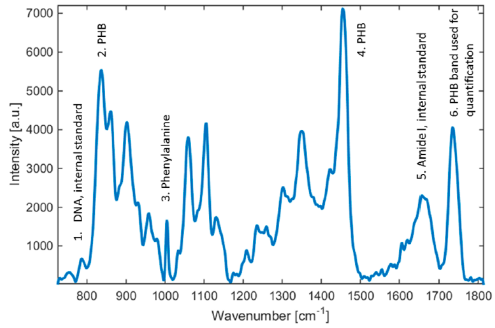
[29] Pilát Z, Jonáš A, Ježek J, Zemánek P. *Effects of Infrared Optical Trapping on Saccharomyces cerevisiae in a Microfluidic System*. Sensors, 17, 2640 (2017).

[30] Rebrošová K, Šiler M, Samek O, Růžička F, Bernatová S, Holá V, Ježek J, Zemánek P, Sokolová J, Petráš P. *Rapid identification of staphylococci by Raman spectroscopy*. Sci. Rep., 7, 14846 (2017)

[31] Rebrošová K, Šiler M, Samek O, Růžička F, Bernatová S, Ježek J, Zemánek P, Holá V. *Differentiation between Staphylococcus aureus and Staphylococcus epidermidis strains using Raman spectroscopy*. Future Microbiology, 12, 10 (2017).
Hrubanová K, Krzyžánek V, Nebesářová J, Růžička F, Pilát Z, Samek O. *Monitoring Candida parapsilosis and Staphylococcus epidermidis Biofilms by a Combination of Scanning Electron Microscopy and Raman Spectroscopy*. Sensors, 18, 4089 (2018).

[32] Pilát Z, Bernatová S, Ježek J, Kirchhoff J, Tannert A, Neugebauer U, Samek O, Zemánek P. *Microfluidic Cultivation and Laser Tweezers Raman Spectroscopy of E-coli under Antibiotic Stress*. Sensors, 18 1623 (2018)

Can we monitor the production, concentration and composition of biopolymer capsules inside living microorganisms?

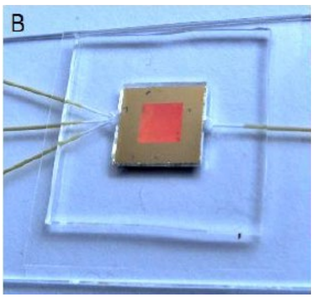


In collaboration with our colleagues from the Faculty of Chemistry of the Brno University of Technology and the Electron microscopy team of the ISI, we used Raman microspectroscopy to analyze the role of poly(3-hydroxybutyrate) in *Cupriavidus necator* H16 bacterial strain. We identified this polymer helps the cells to survive freezing, heating, oxidative damage or hypertonic environments[33]. Our team was responsible for the Raman spectroscopic experiments.

Design and implementation of optofluidic chips

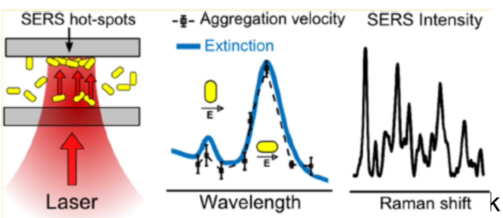
Motivation: Biotechnological research and development is constantly searching for cheap analytical and preparative procedures that can save large amounts of expensive chemicals and enable time efficient parallel sample processing. In this context, microfluidic systems, known as lab-on-a-chips, hold great promise especially when they are combined with optical diagnostic methods. Their recent generation using emulsion droplets formed from immiscible liquids represents an exciting step forward, provided that such individual droplets can be optically investigated.

Can we enhance the sensing capacity by surface-enhanced Raman spectroscopy (SERS) implemented in a microfluidic chip?



In collaboration with our colleagues from the Special technology team at the ISI and from Loschmidt Laboratories at Masaryk University in Brno, we manufactured structured gold surfaces with topologic features that allow localized plasmon oscillations to give rise to the SERS effect, in which the Raman spectral lines are intensified by the interaction of the plasmonic field with the electrons in the molecular bonds. The SERS substrate was enclosed in a microfluidic system, which allowed transport and precise mixing of the analyzed fluids. To illustrate its practical use, we employed the device for quantitative detection of a persistent environmental pollutant 1,2,3-trichloropropane in water in sub-millimolar concentrations,[34] which were not detectable by classical Raman spectroscopy. Our team designed the chips, and performed and analyzed the experiments.

Could one create a SERS substrate at a desired place inside a microfluidic system?



In collaboration with our colleagues from CNR-IPCF in Messina, we tested a method of optical printing (by laser radiation pressure) of SERS-active substrates directly in microfluidic chips for

k P, Benešová P, Kučera D, Márová I, Ježek J, Bernatová S, Zemánek P. *Quantitative Raman Spectroscopy Analysis of Polyhydroxyalkanoates Produced by Cupriavidus necator H16*. Sensors, 16, 1808 (2016) plus 4 other publications

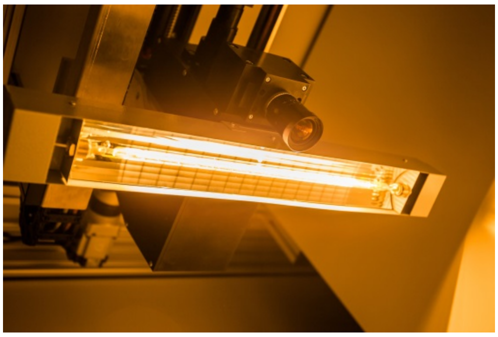
[34] Pilát Z, Kizovský M, Ježek J, Krátký S, Sobota J, Šiler M, Samek O, Buryška T, Vaňáček P, Damborský J, Prokop Z, Zemánek P. *Detection of chloroalkanes by surface-enhanced Raman spectroscopy in microfluidic chips*. Sensors, 18, 3212 (2018).

ultrasensitive SERS-based detection of proteins down to pM concentration[35]. Such structures can serve as the detection part in microfluidic bioassays or lab-on-a-chip devices. Our team contributed with the experimental design, data acquisition and processing, and preparing and running the experiment.

Could one directly quantify the merits of droplet-based microfluidics?
In collaboration with our colleagues from the Loschmidt Laboratories at Masaryk University in Brno, we presented a novel approach for substrate delivery into microfluidic droplets and applied it to high-throughput functional characterization of enzymes that convert hydrophobic substrates. This droplet-on-demand microfluidic system reduces the reaction volume 65 000-times and increases the analysis speed almost 100-fold compared to the classical test tube assay[36]. Our team contributed with the optical imaging and detection part of the experimental system.

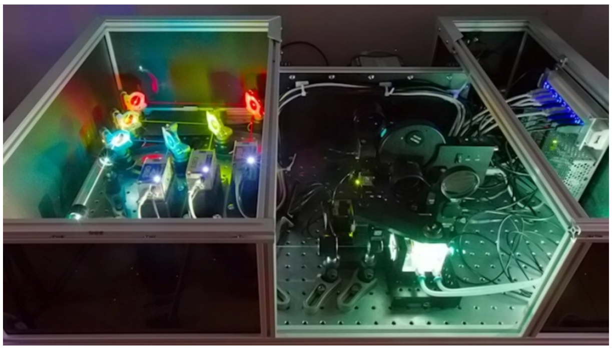
III. Custom-initiated activities for external academic and industrial partners

These activities link together our long-term expertize in the areas of fine mechanics, optics, laser physics, electronics, software control of systems, and building of laboratory opto-mechanical experimental systems. This extensive know-how enables us to design and build on-demand semi-laboratory experimental systems for external academic and industrial partners. Examples of such systems are listed below.



We developed two types of **hyperspectral imaging cameras** for repeatable non-contact investigation of plant physiology in the spectral regions 350-900 nm or 900-1700 nm. The systems are commercially offered in the product catalog of Photon Systems Instruments (PSI) company and are implemented into PSI automatic diagnostic stations within the contract of know-how transfer.

For a commercial partner, we designed and manufactured an advanced type of **light-sheet microscope** providing 3D fluorescent imaging of extended biological samples at 488 nm, 561 nm a 647 nm. The system's adjustment and acquisition is PC controlled and compatible with OpenSPIM/Fiji software.

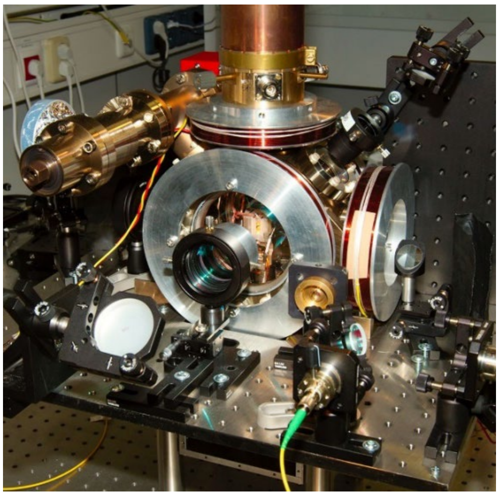


[35] Bernatová S, Donato MGrazia, Ježek J, Pilát Z, Samek O, Magazzu A, Maragò OM, Zemánek P, Gucciardi PG. *Wavelength-Dependent Optical Force Aggregation of Gold Nanorods for SERS in a Microfluidic Chip*. J. Phys. Chem. C, 123, 5608-5615 (2019).
[36] Buryška T, Vašina M, Gielen F, Vaňáček P, van Vliet L, Ježek J, Pilát Z, Zemánek P, Damborský J, Hollfelder F, Prokop Z. *Controlled Oil/Water Partitioning of Hydrophobic Substrates Extending the Bioanalytical Applications of Droplet-Based Microfluidics*. Anal. Chem., 91, 10008-10015 (2019).

Research activity and characterisation of the main scientific results

The DCO relies on project (grant) funding that covers not only the expenses for instrumentation and consumables but nearly all personal costs of the whole team. It means that our research effort is closely associated with our research projects.

Ion clock – quantum metrology



The **flagship research topic** of DCO has become quantum metrology and research in the field of quantum optics. It has started within a large project 'Centre of Excellence for Classical and Quantum Interactions in Nanoworld' where we joined forces with the Department of Microphotonics of ISI and Palacký University in Olomouc. Our goal has been to put together a complex experimental setup that can be operated as an optical clock based on Calcium ions trapped in an electromagnetic trap. At the same time, it serves as an 'infrastructure' for quantum optical experiments where we collaborate closely with the Department of Optics, Faculty of Science of the Palacký University in Olomouc. Between 2015 and 2019, we succeeded in putting this very complex setup into operation and performed several experiments.

The topic of **laser cooling of trapped calcium ions** consists of an extreme high vacuum chamber with pressure below 10^{-10} Pa. The critical part of the setup is a linear Paul trap developed in cooperation with University of Innsbruck, Austria. The trap operates with unprecedented low heating rate and electrical noise. The vacuum chamber is of our design as well as a unique tuneable radiofrequency transformer that is driving the Paul trap with very low loss and a broad range of frequencies. The efficiency of laser cooling and control of the quantum and motional state of the trapped ion depends on the accuracy of setting the optical frequency of the excitation lasers (five lasers). The DCO has come up with a unique approach, where all interrogation lasers are phase-locked on an optical frequency comb disciplined by an active Hydrogen maser, both operated in our lab. Thanks to this, it is possible to control the quantum experiments with high reproducibility, not usual in this field. For such a complex setup, it is necessary to control several operating parameters. Specialised electronics and data server developed by the DCO allows remote management and archiving of all signals in real-time. The implementation of specialised quantum experiments needs to have full control of the quantum state of the ion. The excitation of the energy state of the ion to the forbidden transition requires a specialised laser source with sub-Hz linewidth. The DCO developed this super-coherent laser source working at 1,540 nm (the telecom band) which is transferred to 729 nm – the calcium ion forbidden transition wavelength – over transfer beat technique based on the DCO optical frequency combs. The joint team of the DCO and Palacký University in Olomouc has successfully achieved the following results:

1) The novel method of the residual pressure of the vacuum chamber measurements based on observation of chemical reactions of trapped ions with Hydrogen - Obšil, P.; Lešundák, A.; Pham, M.-T.; Lakhmanskiy, K.; Podhora, L.; Oral, M.; Číp, O.; Slodička, L.*, A room-temperature ion trapping apparatus with hydrogen partial pressure below 10(-11) mbar*, Review of Scientific Instruments, 90, 083201 (2019).
2) The nonclassical behaviour of light emitted from large Coulomb crystals formed by cooled ions was demonstrated historically for the first time - Obšil, P.; Lachman, L.; Pham, M.-T.; Lešundák, A. et al., *Nonclassical Light from Large Ensembles of Trapped Ions*, Physical Review Letters, 120, 253602 (2018).
3) We have proven the existence of interference maxima of light quanta generated by chain Coulomb crystals - Obšil, P.; Lešundák, A.; Pham, M.-T.; Araneda, G.; Čížek, M.; Číp, O.; Filip, R.; Slodička, L., *Multipath interference from large trapped-ion-chains*, New Journal of Physics, 21, 093039 (2019).
4) The first experimental operation of optical atomic clocks based on trapped and laser-cooled Ca ions in December 2019 with time stability better than 10^{-15}.

Thanks to these successes, we got involved in a prestigious H2020 – EMPIR European project oriented to the theoretical study and experimental tests of a new generation of optical atomic clocks based on Coulomb crystals of laser-cooled ions – **Coulomb Crystals for Clocks**. The DCO is responsible for simulations and experimental verification of ion trajectories in the electrical trap and frequency systematic shifts of forbidden transition compensation at a ‘magic frequency’ of the ion electrical trap’s radiofrequency drive.

Transfers of highly stable optical frequencies

Transfers of highly stable optical frequencies over fibre-optic links are closely related to the previous topic. Whoever operates optical clocks has to share their stable optical frequency with the rest of society and other laboratories in future. We try to exploit our geographic position at the very heart of Europe to contribute significantly to a Europe-wide network for the optical frequency transfer. The DCO was the first in Central Europe that developed and utilised the technique of phase-coherent transmissions of super stable frequencies over optical communication networks. This technique suppresses unwanted noise and interference induced by the Doppler effect from the environment through which long communication lines pass. Our significant contribution to this technology is in the deployment of our unique methods of digital signal processing and adaptive control. Dedicated instrument modules, such as digital voltage-controlled synthesizers, extremely sensitive phase detectors and demodulators based on programmable FPGA arrays and digital signal processors, are among the advanced systems in this field. With the help of CESNET, Czech research infrastructure, and a provider of data connectivity for the Czech Academia we achieved the following successes:

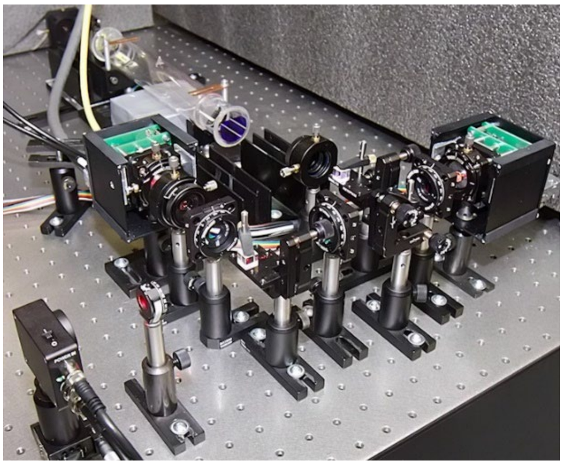
1) 2015 – we put into operation a 306 km long experimental fibre link between the DCO Brno and the CESNET headquarter in Prague to remotely calibrate the CESNET Acetylene optical frequency standard.

2) 2018 – we opened a 401 km long link between the DCO in Brno and the nuclear power plant in Temelín to synchronise an optical sensory network to monitor the shape deviations of the reactor's containment building in the power plant.
3) 2019 – the pilot operation of a 280 km long link between the DCO in Brno and the BEV Wien, Austria (national metrology institute).
4) The intellectual property of the phase-coherent control unit for long-haul fibre links is protected by **Czech utility model no. 32014**.

To integrate our effort with the **fibre-optic network being gradually built in the EU**, we became involved in the Horizon H2020 – EMPIR project '**TiFOON – Advanced time/frequency comparison and dissemination through optical telecommunication networks**' and participated in the H2020 – INFRA-INNOV as well as the subsequent INFRA-DEV initiatives '**CLOck NETwork Services: Strategy and innovation for clock services over optical-fibre networks**' where we became an integral part. The strategic plan here is to build an EU-wide infrastructure that would serve the scientific community (primarily for metrologists) as well as the wider public as a tool for more precise frequencies and time synchronisation.

Highly coherent lasers

Highly coherent laser sources have been historically one of the key research topics within the DCO. Apart from primary metrology, we focussed our attention on lasers for dimensional metrology (interferometry), for laboratory applications, industrial practice, and telecom applications.



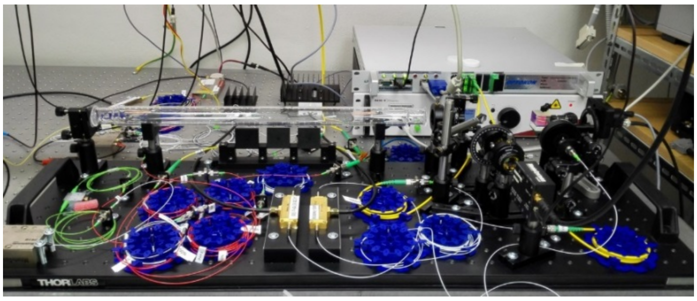
Traditional **interferometric measuring techniques** rely on He-Ne lasers, but the quest for a **replacement** in the form of a semiconductor laser or died pumped, solid-state laser (DPSSL) laser is on. There are new demands for such lasers, not only stability and coherence. Advanced interferometric techniques require extensive and continuous tuning range, high-frequency modulation, two-frequency operation, higher power, low noise, and even controlled coherence. We tried to address these demands with our designs.

We developed a complex solution of the **stabilised laser system with a 633 nm DBR diode**, where the stabilisation technique relies on spectroscopy in iodine either through linear spectroscopy for less demanding applications or sub-Doppler spectroscopy for laboratory metrology. It has been one of our tasks within the international project '**Metrology for movement and positioning in six degrees of freedom**' funded from EMRP programme of EU. The developed versatile laser is an advanced substitution of traditional red He-Ne laser at 633 nm. The DCO team achieved the following results:

1) The laser system has been tested in collaboration with NPL, Teddington, UK and has proven itself as equal to He-Ne laser in stability and coherence with much more to offer in tuning range, power and modulation capability - Řeřucha, Š.; Yacoot; A.; Pham, M.-T.; Čížek, M., et al., *Laser source for dimensional metrology: investigation*

of an iodine stabilized system based on narrow linewidth 633 nm DBR diode, Measurement Science and Technology, 28, 045204 (2017).

2) The DCO introduced a unique and complex system of two 633nm DBR lasers. Both stabilised through spectroscopy in iodine in one absorption cell and with frequency as well as phase stabilisation of the beat frequency. Especially the phase lock has proven to be crucial for the laser to operate properly in heterodyne interferometry scheme. The laser system has been tested on nanocomparator measuring system in PTB Braunschweig, Germany in 2017.



The wavelength range, where telecom lasers operate, has grown in metrology significance quite a lot. Stability and coherence of telecom lasers are crucial for the development of any broadband and high-capacity communication network. With the prospect of a network of the phase-coherent distribution of highly stable optical frequencies over fibre links, the need for precise, **metrology-grade laser sources** is growing further. We proposed, designed, and experimentally verified a set of **stabilised lasers** based on reference absorption cells filled with acetylene. The DCO also exploited phase noise suppression techniques based on the fibre-spool interferometer, which is necessary for linewidth narrowing of interrogation lasers for such optical frequency standards. The DCO team achieved the following:

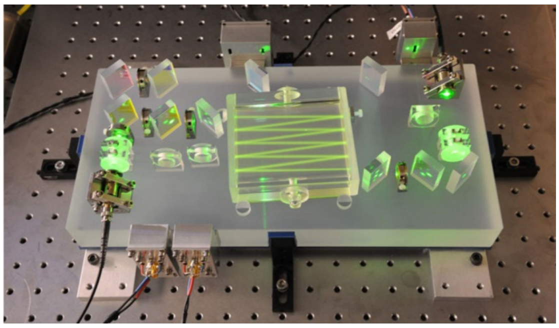
1) A reliable turn-key setup of the acetylene based optical frequency standard at 1,540.6 nm for the Czech national academic network operator CESNET, which is used for optical networks characterisation, remote measurements, and research of novel methods and techniques to control for instabilities in optical links and their suppression.
2) The experimental setup for suppression of the phase noise of an extended cavity laser for high-resolution spectroscopy in telecom band - Šmíd, R.; Čížek, M.; Mikel, B.; Číp, O., *Frequency Noise Suppression of a Single Mode Laser with an Unbalanced Fiber Interferometer for Subnanometer Interferometry*, Sensors, 15, 1342-1355 (2015).

Laser sources for interferometry should be ‘as coherent, as possible’. As a result of collaboration with a leading Czech optical manufacturer, Meopta-Optika, we were asked to develop a **light source for Twyman-Green interferometer** designed for inspecting complex optical setups. We came up with a unique solution combining three light sources, narrow-linewidth He-Ne laser, broad linewidth superluminescent diode (SLED), and in between SLED spectrally filtered by a fibre grating. Optical switches select them. The DCO team achieved these results:

1) A prototype of the light source with variable coherence has been used for surface measurements at company Meopta-Optika (leading, large Czech optical company), where there is a sequence of several reflecting optical surfaces producing interference patterns with multiple interference fringe patterns superimposed into one. The approach utilising **laser with variable coherence length** together with the

possibility to adjust the length of the reference arm proved to be a useful solution eliminating this problem.

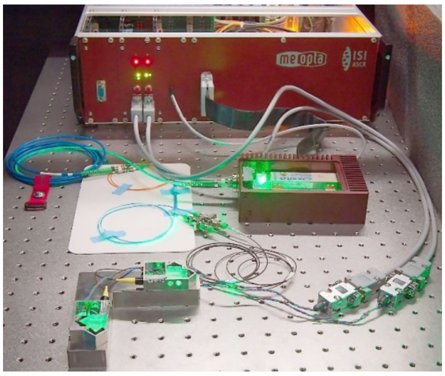
2) The intellectual property of the optical radiation source assembly is protected by **Czech utility model no. 31749**.



The DCO ISI, thanks to a vast pool of accumulated know-how and technological background, has established itself as a leading provider of **optical frequency references – absorption cells** for the metrology community of the whole world. These references are essential for any frequency stabilised laser source, **primarily standards of optical frequencies in fundamental metrology**. We do continuously develop and improve our technology and respond to new trends and demands. The most challenging designs of absorption cells were those for future space missions. Thanks to our close cooperation with the Laboratoire national de métrologie et d'essais – Système de Référence Temps-Espace, France (LNE-SYRTE), the German Aerospace Centre, Institute of Space Systems, Germany (DLR), as well as frequency references for telecom applications, remote calibrations, and precise time & frequency transfers over optical links, the DCO team achieved these results:

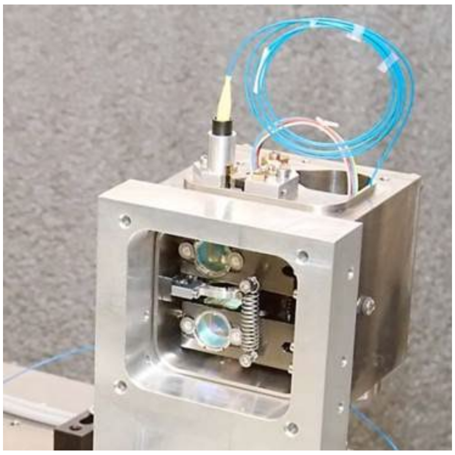
1) The DCO came up with **compact multi-pass cell designs**, and one of them showed its space-born capability in a **test flight** when it was launched in a rocket on May 13, 2018, from the Esrange Space Centre, Kiruna, Sweden, as a part of TEXUS 54 mission.
2) The DCO supervised international comparison of a set of iodine absorption cells together with renowned national metrology institutes LNE-SYRTE Paris, France and INRIM, Turin, Italy, with getting the excellent result of the purity check of the DCO iodine cells - Hrabina, J; Zucco, M.; Philippe, Ch.; Pham, M.-T.; Holá, et al., *Iodine Absorption Cells Purity Testing*, Sensors, 17, 17010102 (2017).

Interferometry



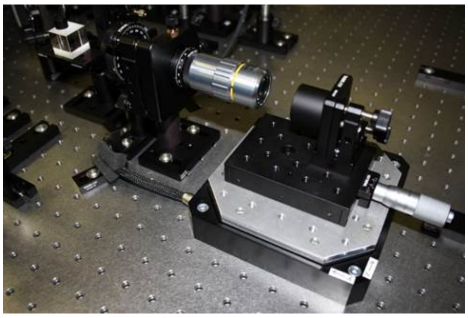
There is a legacy from the previous period (2010-2014) – development of an **interferometric system for nanometrology**, which also became part of our contribution to the large international project 'Metrology for movement and positioning in six degrees of freedom' funded by the EU programme EMPRP. The result was a **complex and modular interferometric system** developed in collaboration with the Czech company Meopta-Optika, that contributed to the development of specialised precision optics. The whole setup includes electronics, detection, optics, laser source, and also the unit to evaluate the refractive index of air. It offers a single axis configuration with corner-cube retroreflectors, coordinate configuration with reflectors in the form of planar mirrors, as well as a differential, single-axis arrangement.

The configuration of the coordinate 2D measuring setup was a redesigned module to fulfil the **precise and high-resolution wafer positioning** demands by the stage of an e-beam writer (lithograph). The system described here introduces a unique three-axis (x, y and yaw) configuration. Several prototypes have been built as a design for **electron-beam lithography systems by the German company Raith Nanofabrication, GmbH**. The need to add the ability to measure angle deviations (yaw angle) reflects the need for an upgrade of their e-beam writers up to larger scale wafers and longer positioning ranges (which leads to greater more significant errors, that have to be contained). These e-beam writers are entering the market at the moment.



Similarly, we got involved in the development of a system for a new e-beam writer with the Czech company TESCAN. The **TESCAN e-beam writer** aspires to enter the market (and become a competitor to instruments produced by Raith Nanofabrication) as a medium-sized instrument with a compact design (a chamber with the size of an electron microscope) and features of a larger instrument. We developed an **interferometric system for sample positioning**, which introduces several novel approaches and techniques. The system can eliminate all deformations of the vacuum chamber thanks to its differential setup referenced, a central element (e-beam nozzle) that includes all components, such as infrared stabilized laser source, infrared optics and detection, electronics, software, adaptive signal processing, as well as unique detection system deriving the quadrature signal through modulation of the laser source.

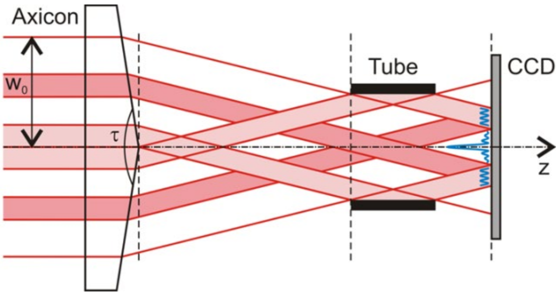
In the field of **interferometry**, we also focus on more advanced techniques employing sophisticated **beam shaping and digital holography methods**. Most of this research was done within a framework of collaborative projects with the company Meopta-Optika, our leading optical manufacturer. Meopta-Optika is involved in designing optical instrumentation of their own for their measurement and quality control purposes. Our joint research effort represents an oriented exploration that intends to give Meopta-Optika guidance which way the development of new optical instrumentation might lead.



The main problem optical manufacturing faces now is the grinding and polishing of aspheric lenses, as well as measuring their shape. Optical surfaces, when polished to optical quality, are easily inspected by (stitching) interferometry. However, to

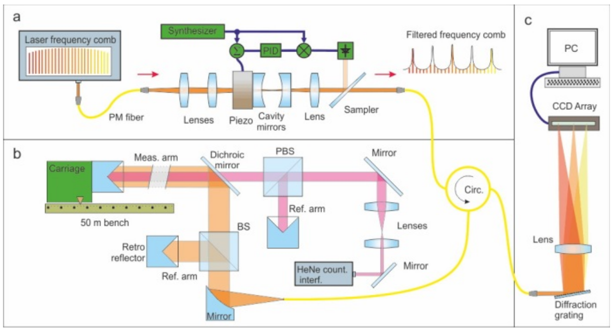
measure the shape of a rough surface is a problem not yet fully solved. We proposed a **technique based on an advanced beam-shaping** method using a spatial light modulator to measure the shape of a diffuse surface in a scanning regime that can achieve submicron resolution. It means that this sensor might be able to be combined with an interferometer to measure boundary surfaces where spatial decoherence starts to fade. Overlapping of our sensor resolution with the range within a single interference fringe of an interferometer offers a unique sensing technique with unprecedented measuring range, robustness (on rough surfaces), and precision on optical surfaces.

- Šarbort, M.; Holá; M.; Pavelka, J.; Schovánek, P.; Řeřucha, Š.; Oulehla, J.; Fořt, T.; Lazar, J., *Comparison of three focus sensors for optical topography measurement of rough surfaces*, Optics Express, 27, 33459-33473 (2019).



Similarly, we tried to solve the problem of **measuring hollow cylindrical surfaces**. The motivation came from measuring the shape and straightness of firearm barrels. We came up with a unique technique using a beam-shaping from Gaussian into a Bessel beam through an axicon and spatial light modulator. The resulting setup uses a grazing incidence reflection of the beam from the inner walls of the to be measured cylinder and interference with a reference wave. The grazing incidence approach made the technique insensitive to less than mirror-quality surfaces, and the final image can give the information about the whole topography of the surface with the precision of a fraction of a micrometre.

- Šarbort, M.; Řeřucha, Š.; Holá, M.; Buchta, Z.; Lazar, J., Self-referenced interferometer for cylindrical surfaces, Applied Optics, 54, 9930-9938 (2015).



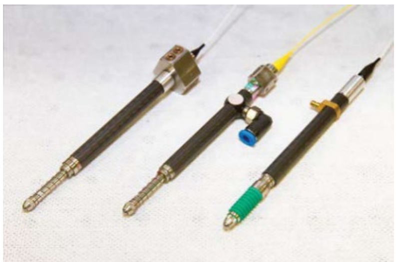
Interferometric techniques are quite essential tools for **measuring long-distances from hundreds of meters up to tens of kilometres**. The accuracy of global navigation systems (e.g. GPS) with the development of novel autonomous vehicles and drones is an issue. These systems are based on the decoding of signals from satellites orbiting the Earth. Thus, laser interferometers have to be used for the calibration and verification of GPS data. As a result, they must be able to determine the measured distance with an absolute scale. The DCO has been invited to participate in the 'Metrology for long distance surveying' project funded by the EU programme EMRP. Within this project, we introduced several novel methods of **interferometry driven by optical frequency combs** with femtosecond pulsed lasers. It leads to measurement of interference of many wavelengths at the same time. Therefore, the detection becomes crucial. The DCO developed specialised optical cavities for broadband filtering of the optical spectrum generated by the optical frequency combs. Such a low-dispersive cavity is a crucial component for synchronising with the frequency comb. Thus, we developed a specialised adaptive electronics for a real-time phase lock. The implementation of the detection technique with dispersion grating and linear CCD array photodetector was the next step. Then, the DCO, in cooperation with the Dutch metrology institute VSL Delft, completed measurements of this novel long-distance measuring system and performed a comparison with a traditional incremental laser interferometer. The results confirm the initial prediction of unprecedented low uncertainty of this novel multiwavelength measuring technique (hundreds of nanometers for 50 meters).

- Lešundák, A.; Voigt, D.; Číp, O.; van der Berg, M., *High-accuracy long distance measurements with a mode-filtered frequency comb*, Optics Express, 25, 32570-32580 (2017).

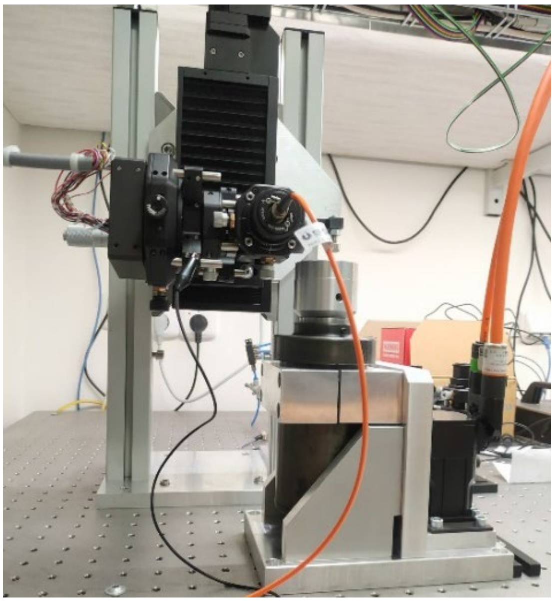
Within the last evaluation period (2009-2014), we introduced a novel method of **contactless short gauge blocks calibration and diagnostics** based on an interferometer with a new principle, combining coherent and white light. We developed it into a fully automated, computer-controlled system. With the help of automatic feeder, it can perform a serial calibration of 126 pieces of gauge blocks in one measuring cycle. In cooperation with the DCO industrial partner Mesing and Czech Metrology Institute, we completed the measuring methodology in following our patented method (**Czech patent 302948**) of gauge blocks calibration. Following comparison with the TESA-NPL three-colour interferometry-based measuring instrument verified the nanometre uncertainty of our quite complex contactless calibration system. The project has been concluded with the installation of this measuring system at the Czech Metrology Institute, where it provides fully automatic mass calibrations.

- Buchta, Z.; Šarbort, M.; Čížek, M.; Hucl, V.; Řeřucha, Š. et al., *System for automatic gauge block length measurement optimized for secondary length metrology*, Precision Engineering, 49, 322-331 (2017).

Optical sensors



As part of a collaborative project, we joined forces with a small innovative Czech company from Brno called Mesing. The project focused on introducing a novel and miniaturised **contact displacement measurement gauge** prototype for further production with state-of-art performance. The concept was the **miniaturisation of laser interferometer optics** into a small form-factor, compatible with that of the 8 mm pencil-gauge (used for LVDT sensors). As a result, the sensor, to a maximum extent, preserves the ultimate precision of the laser interferometer. The miniaturisation involves an originally designed optical arrangement that requires a minimum of optical components, in conjunction with an advanced interferometric phase detection technique. In combination with the optoelectronic interrogator, that incorporates a metrologically traceable laser standard, the experimental investigation revealed the gauge represents an innovative technological leap. The intellectual property of the principle and technical design of the sensor is protected by the **Czech utility model No. 33046**.



Next to measuring techniques involving the principle of light interference, we developed several other methods of optical sensing, again within a framework of a collaborative project with Mesing. Together, we introduced a new kind of **sensor for the inspection of surface quality of rotary elements** such as frames for ball bearings, as well as shafts and other fine machined components. The principle involves the monitoring of inhomogeneous scattering of the light from surface defects occurring on the precision machined components. The integration of specialised planar diffractive optics and advanced digital signal processing in conjunction with digitally controlled positioning of the tested component in a single measuring gage allows rapid diagnostic control with quality evaluation and automatic decision on whether the product follows the required quality or is defective.

- The DCO has protected the intellectual property of the principle and technical design of the sensor by the **Czech utility model No. 33047**.
- This prototype is now in its implementation stage for deployment to the new quality check stations manufactured by Mesing. Our industrial partner envisages their widespread implementation, following the Industry 4.0 strategy, in particular, when considering the growing production of electric and autonomous vehicles.

The result with the probably **most considerable socioeconomic impact** of all was achieved during the period where the **system of fibre Bragg Grating strain sensors** was designed for measurement of large structures. The porotype we developed and put into operation went through a testing measurement inside the **containment of a nuclear power plant in Temelín**, Czech Republic. During this test run, our system competed in a tender with several commercial systems based on electric strain sensors, interferometric sensors, and even optical fibre sensors – our product stood out positively. An implementation project for the installation of our set of sensors into the containment at the Temelín plant will start in 2021 and is currently prepared. The Czech State Office for Nuclear Safety recently approved this technology. There is also a significant interest in our new system from operators of nuclear power plants equipped with similar containments in Ukraine and Russia. Next to the commercial success, it is also a **significant contribution to the safety of nuclear power** in general.

Laser technologies

The study of welding processes by monitoring secondary radiation. We concentrated on the individual components of the radiative spectrum of the laser weld with a focus on the retro-reflected laser light. The system for monitoring of this radiation represents a significant simplification compared to our previous designs. It uses a built-in detector, which is a part of the welding laser head – no additional components are needed.

- Mrňa, L.; Šarbort, M.; Řeřucha, Š.; Jedlička, P., *Autocorrelation analysis of plasma plume light emissions in deep penetration laser welding of steel*, Journal of Laser Applications, 29, 012009 (2017).

The visualisation of gas flow for laser technologies. We used the Schlieren imaging technique to visualise complex kinematics of the gasses acting as a protective atmosphere in laser welding and cutting. The results discovered were part of an informal collaboration with the University of Modena, Italy as well as contract research for a company called Termacut. The Schlieren method has not been tested in laser welding and cutting system before. Thanks to it, we were able to investigate a new phenomenon, a **counter-flow of the hot air from the weld**. In case of laser cutting, we designed a glass block copy of the slot to visualise the gas flow.

- Darwish, M.; Mrňa, L.; Orazi, L.; Reggiani, B., *Numerical modeling and Schlieren visualization of the gas-assisted laser cutting under various operating stagnation pressures*, International Journal of Heat and Mass Transfer. 147, 118965 (2020).

Hybrid welding technique Laser-WIG. We applied this technique in a novel way to actively slow the laser weld cooling during the laser welding process. Our simulations have shown that it is possible to slow down the cooling 30% of its original speed. It has a significant effect on the structure and mechanical properties of the material. This technique was tested in collaboration with the Institute of Physics of Materials, CAS, and in research projects with EBZ Hoffmann (automotive) and Siemens Energy. We have shown that this technique can **improve the weld properties** under high-cycle fatigue stress conditions, and under best conditions, prolong its lifetime twice. Compared to other secondary material pre-heating techniques, this method is basic (from the instrumentation point of view), as well as economical.

- Šebestová, H.; Horník, P.; Mrňa, L.; Doležal, P.; Mikmeková, E., *The Effect of Arc Current on Microstructure and Mechanical Properties of Hybrid LasTIG Welds of High-Strength Low-Alloy Steels*, Metallurgical and Materials Transactions B, 49, 3559-3569 (2018).

The **design and deposition of optical thin films** are one of our specialisations. It contributes to all projects and helps us to design optical systems on demand. In particular, the technology of optical frequency references – absorption cells – rely heavily on specialised, quality AR and HR coatings. Similarly, our setups for interferometry and laser systems operating in ultrashort pulsed regime require complex multilayer coatings. Problems associated with Laser-Induced Damage Threshold (LIDT) are also addressed.

- Oulehla, J.; Pokorný, P.; Hrabina, J.; Holá, M.; Číp, O.; Lazar, J., *Influence of coating technology and thermal annealing on the optical performance of AR coatings in iodine-filled absorption cells*, Optics Express, 27, 9361-9371 (2019).