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

Institute	Institute of Photonics and Electronics of the CAS, v. v. i.

Research activities at the Institute of Photonics and Electronics are carried out in **five research units**, which consist of **four research teams** and **one specialized laboratory**. The research teams are:

- **Optical Biosensors** (senior team),
- **Fiber Lasers and Non-Linear Optics** (senior team),
- **Bioelectrodynamics** (junior team), and
- **Synthesis and Characterization of Nanomaterials** (senior team).

These teams pursue fundamental and applied research in different areas of photonics, optoelectronics, and electronics. In addition to these research teams, there is one specialized laboratory:

- **Laboratory of the National Time and Frequency Standard**.

This laboratory focuses on highly accurate measurements of time and frequency.

The main research direction pursued by research units of the Institute are described below. A detailed account of activities of research teams of the Institute can be found in Section 3.5. The Laboratory of the National Time and Frequency Standard, due to its focus on providing specialized services, has not been included among the evaluated research teams, and an account of its activities is provided at the end of this section.

Research Direction #1: Optical biosensors

This research program has been focused on optical affinity biosensors that combine very sensitive optical techniques with special biomolecules, which are able to recognize and capture other molecules. The result of this combination of optics and biology is a technology that enables the study of (bio)molecules and their interactions as well as a determination of their concentrations. Specifically, the research has been focused on optical biosensors employing special electromagnetic waves: surface plasmons and guided modes of optical waveguides. This is a highly multidisciplinary research theme and requires a concerted effort in multiple areas across physics, chemistry, and biology. The truly multidisciplinary approach makes the research program at the Institute unique on an international level, and allows the team to conduct research into optical biosensors in its full complexity. In particular, the program encompassed research effort in the following areas: (1) plasmonic and photonic (nano)structures and phenomena, (2) optical platforms, (3) microfluidic effects and devices, (4) functional biomolecular coatings, (5) biosensor-based methodologies for biomolecular interaction analysis, and (6) biosensor-based methodologies for detection of chemical and biological species.

During the evaluation period, this research program has generated 66 publications and 5 patents (including 2 US patents). It should be noted that many of these publications were in high-quality journals in the field of biosensors (*Analytical Chemistry, Analytical Chimica Acta, Biosensors and Bioelectronics*), nanoscience and nanotechnology (*ACS Nano*, *Small*), photonics (*Optics Letters, Optics Express, Plasmonics*), microfluidics (*Lab on a Chip*), and molecular biology (*Nucleic Acids Research*). This breadth and the fact that publications generated by the program produce about 1,000 citations/year (according to WOS, includes also publications before the evaluation period) clearly reflect the broad impact of this research program. The international recognition of the program is also evidenced by more than 25 invited lectures at international conferences. Although the main focus of this research is clearly on cutting-edge research, the team was also active in instrumental development: the unique biosensor platforms developed at the Institute are in use at universities and research institutions situated in the USA, Europe, and Asia. During the evaluation period, the program has received two awards - the Minister of Education, Youth and Sports Award for Outstanding Research and the Premium Academiae of the Czech Academy of Sciences.

Research Direction #2: Fiber lasers and non-linear optics

This research program has been focused mainly on high-power fiber lasers and their applications. The properties of these high-power fiber lasers have been tailored to meet specific needs of material processing and medical applications. Moreover, when combined together with nonlinear optics into parametric nonlinear optical generators, these fiber lasers can serve as a platform for high resolution laser spectroscopy. The program encompassed research in the following areas: (1) efficient optical fibers for fiber lasers, (2) high power ytterbium-, erbium-, thulium- and holmium-doped fiber lasers, (3) nonlinear optical parametric generators, (4) and numerical methods for the investigation of light propagation in fibers and planar lightwave circuits. The Team has collaborated closely in these areas with high-tech companies.

During this evaluation period, this research program has generated 48 publications, 1 monograph, 2 book chapters, and 2 patents/utility models. Many of these publications were published in high-quality journals in the field of optics (*Optics Express*, *Optics Letters*, *IEEE J. of Quantum Electronics*, *J. of Lightwave Technology*, *Laser Physics Letters*), physics (*Physical Review A and B*), analytical chemistry (*Analytical and Bioanalytical Chemistry*, *Sensors and Actuators B*), and material science (*J. of Physical Chemistry C*, *J. of Alloys and Compounds*). The results originating from one of the joint projects with industrial partners was awarded by the Technology Agency of the Czech Republic in 2013.

Research Direction #3: Bioelectrodynamics

The *Bioelectrodynamics research team* is a newly established junior research team, which performs multidisciplinary research on the interface of electrical engineering, biophysics, bioelectronics, and physical chemistry. The activity of the team has been focused on the electromagnetic properties of biomaterials: from the level of single molecules to that of cells. The research activities of this program covered both experimental and theoretical aspects that involve the characterization of the electromagnetic properties of biomaterials. Experimentally, this work has involved the development of experimental equipment and sensor tools using nanofabrication technologies. This work has been combined with the modeling of biophysical processes that generate electromagnetic fields at the cellular level (primarily supra-molecular).

Specifically, research in this program has been focused on (1) the electrodynamic properties of biological protein nanostructures with a focus on cellular fibers (microtubules), and (2) endogenous radiofrequency and optical electromagnetic biosignals generated on the cellular level. This research program has produced 22 papers in highly reputable journals such as *Applied Physics Letters, PLoS ONE, Cell Communication and Signaling*, and *Integrative Biology*. Both research branches as well as their combination are unique on the international level: the results obtained by the Team have provided new insights into ultra-fast cellular signaling, cancer therapy methods, and novel label-free non-invasive diagnostic methods in medicine and biotechnology based on endogenous photonic biosignals.

Research Direction #4: Synthesis and Characterization of Nanomaterials

The research activities have been directed towards a wide range of topics related to the preparation and characterization of electronic and photonic materials. The team's research efforts have concentrated on six principal topics: (1) the investigation of electrical contacts on compound semiconductors, with an emphasis on Schottky contacts and their application in hydrogen sensors; (2) studies of the optical properties of semiconductors and special glasses; (3) nanodiagnostics of semiconductor and photonic materials using scanning ion and electron beams, with a focus on interactions of ions with solid surfaces and on ballistic electron emission spectroscopy and microscopy (BEEM/BEES); (4) the electrochemical preparation of porous III-V semiconductors and their application in lattice-mismatched epitaxial growth; (5) the characterization of thermoelectric materials; and (6) the preparation of high-purity epitaxial layers of III-V semiconductors with rare-earth elements admixtures and their application in radiation detectors. These activities required multidisciplinary research approach in the fields of electrical engineering, materials science, physics, and chemistry.

During the evaluation period, the research team has generated 74 publications, many of which appeared in highly-ranked journals in the field of materials science (*Carbon*, *Journal of Alloys and Compounds*), applied physics (*Applied Physics Letters, Physical Review B*), and chemistry (*Sensors and Actuators B: Chemical*). Although the main focus was on fundamental research, the team was also active in instrumental development. The team designed an apparatus for the rapid optical determination and mapping of Fe concentration in GaN, designed and assembled a unique high-resolution photoluminescence spectrometer, and significantly enhanced the long-term stability of the in-house BEEM/BEES system.

Laboratory of the National Time and Frequency Standard

The specialized *Laboratory of the National Time and Frequency Standard* is entrusted with the management of the National Time and Frequency Standard. The main activities of the laboratory are the physical realization of the unit of time (the SI second) and furthermore, the generation of the national time scale. The laboratory performs demanding calibrations of primary and secondary time and frequency standards (atomic clocks), ultra-stable frequency sources, and equipment for comparing time scales. The laboratory also conducts research in the field of very accurate measurements of time intervals, event times, and time transfer (time scale comparisons) using satellite navigation systems, optical fibers, and full optical networks.

During the evaluation period, the laboratory developed systems for the comparison of time scales using the signals of satellite navigation systems such as GPS and/or GLONASS, GALILEO, and EGNOS. This equipment has been subsequently used all around the world; many national metrology laboratories of time and frequency have used it in order to contribute to the calculation of the Coordinated Universal Time UTC in collaboration with The International Bureau of Weights and Measures (BIPM: Bureau international des poids et mesures). This equipment is also used by several important research institutions, e.g., NASA in its Lunar Reconnaissance Orbiter project, and ESA for the establishment of the GALILEO system time. This equipment has also been used for the independent timeline calibration within the OPERA project (measuring the speed of neutrinos). In addition, the laboratory has developed techniques and instrumentation for optical time transfer. These methods have been tested on newly arranged optical links to partner laboratories in order to increase the number of atomic clocks contributing to the stability of the national time scale and calculation of UTC.

Research Report of the team in the period 2010–2014

Institute	Institute of Photonics and Electronics of the CAS, v. v. i.

Scientific team	Optical Biosensors

a) Research team

The Optical Biosensors research team entered the period of evaluation (2010-2014) as a (major) part of the Department of Optical Sensors, which has pursued research into chemical sensors and biosensors. The restructuring of the research units of the Institute at the end of 2012 brought to the team two researchers from other departments: P. Horák and J. Kaňka and his research activities in the area of optical fiber sensors. This restructuring, along with a continued orientation on optical biosensors have given rise to the Optical Biosensors research team, which has fully focused on research into optical biosensors, especially on optical affinity biosensors based on surface plasmons.

In order to pursue research into optical biosensors in its full complexity, the team has been built as a true multidisciplinary research unit and has strived to attract and incorporate experts from all relevant key areas of optical biosensors research. Currently, the expertise of the team spans across multiple fields, including photonics, biophysics, biochemistry, analytical chemistry, fluid mechanics, electrical engineering, software engineering, and fine mechanics.

At the end of the evaluation period (December 31, 2014), the team consisted of the Head of the team (J. Homola, age - 49), 1 senior scientist (J. Kaňka, age - 70), 1 research scientist (H. Vaisocherová (Lísalová), age - 36), 3 post-doctoral fellows (N. S. Lynn, age - 38, M. L. Ermini - 31, H. Šípová - 31), 1 research assistant (P. Horák, age - 45), 6 Ph.D. students, and 12 technicians consisting of 2 nanostructure and thin film fabrication specialists, 4 chemical and biological lab technicians, 2 mechanical and optical system designers, 2 electrical engineers, and 2 software and data processing engineers. It should be noted that 5 of the technicians were also providing services to other research units of the Institute. Of the total of these 25 employment contracts, two expired on December 31, 2014 and were not extended. There were also 2 MS students involved in the research activities of the team.

b) Description of research and main achievements

During the period of evaluation, research efforts of the Optical Biosensors research team were focused on the following key areas: (1) plasmonic and photonic (nano)structures and phenomena, (2) optical platforms, (3) microfluidic effects and devices, (4) functional biomolecular coatings, (5) biosensor-based methodologies for biomolecular interaction analysis, and (6) biosensor-based methodologies for detection of chemical and biological species. The research approaches and representative results accomplished in these areas are presented below. References to selected results are provided at the end of this section; a complete list of the team's publications is provided in Section 3.8.

(1) Research into **plasmonic and photonic (nano)structures and phenomena** for novel optical biosensors encompassed several topics, including electromagnetic simulations of photonic/plasmonic phenomena on a variety of metal-dielectric (nano)structures (planar as well as cylindrical systems), the design and fabrication of selected (nano)structures, and the experimental investigation of their properties. The simulation methods used to investigate selected (nano)structures included the finite-difference time-domain (FDTD) method, the boundary element method (BEM), rigorous coupled wave analysis (RCWA; collaboration with the Faculty of Nuclear Science and Physical Engineering), and generalized multi-particle Mie theory. Selected plasmonic nanostructures were fabricated using three different techniques: electron beam lithography (EBL), hole-mask colloidal lithography (HCL), and laser interference lithography (LIL). The morphological characterization of prepared (nano)structures was performed using atomic force microscopy (AFM) and scanning electron microscopy (SEM). Optical properties of the selected plasmonic nanostructures were investigated by measuring the transmission and/or reflection spectra of the prepared (nano)structures.

In collaboration with researchers from the University of Graz (Austria), the team has proposed and demonstrated an approach allowing the characterization of the sensitivity of localized surface plasmons (LSP) to local changes in the refractive index on the nanometer scale. Specifically, this method was based on the use of a polymer mask covering a variety of well-defined areas of metallic nanoparticles, and furthermore, has confirmed experimental observations by simulations carried out using the finite-difference time-domain method [1]. In addition, the team has conducted a theoretical analysis of lattice resonances supported by arrays of gold nanoparticles and derived analytical expressions for their figure of merit (FOM) for refractive index sensing. This study revealed that affinity biosensors based on lattice resonances can provide performances on the same level as biosensors based on localized surface plasmons [2].The team has also investigated micro- and nano-structured optical fibers as a potential platform for new chemical sensors and biosensors. This work was performed in collaboration with researchers at the Stevens Institute of Technology (USA), who carried out the experimental portions of the work. The team has conducted computational modeling of the functionalized fibers, aimed at an interpretation of the experimental results and optimization of fiber-optic sensor platforms. Specifically, the team analyzed the dependence of the sensitivity on the order of the coupled cladding modes in a long-period fiber grating for the monitoring of LbL growth of PVPON/PMAA polyelectrolyte thin films, and numerically demonstrated that high order modes possesses the highest measurement sensitivity [3] Furthermore, a numerical-analytical model has been developed to investigate the effect of the coverage density of metallic nanoparticles on the interplay between Raman gain and attenuation of guided mode in SERS-active optical fibers. As a function of nanoparticle coverage density, covered fiber length, and excited mode, the predicted Raman intensity was in an excellent agreement with published experimental results for the contrasting types of SERS-active fibers [4]. The team has also strived to expand its nanofabrication capabilities and has developed a novel approach for the fabrication of ordered plasmonic arrays based on four-beam interference lithography. This approach has been demonstrated to enable the rapid fabrication of large macroscopic areas of perfectly periodic and defect-free two-dimensional plasmonic arrays of nanoparticles as small as 100 nm [5].

(2) The team has also pursued the development of **novel optical platforms for affinity biosensors.** This effort encompassed the design of optical platforms, the fabrication of laboratory prototypes, the development of software and data processing approaches, and the characterization of each platform as well as a determination of its performance characteristics.

The Optical Biosensors research team has developed a high-performance optical platform for high-throughput screening based on surface plasmon resonance imaging in polarization contrast and internal referencing. This platform has been demonstrated to enable the simultaneous measurement in more than 100 sensing channels [6]. The method of imaging in polarization contrast has also been exploited in a new optical sensor based on localized surface plasmons excited on an array of gold nanorods [7]. The fact that this optical platform may be used for biosensing using both propagating and localized surface plasmons has enabled the team to perform a comparison of their biosensing capabilities [7]. Although there is an ongoing debate in plasmonic community about the relative merits of biosensors based on different types of surface plasmons, such comparisons are still rather rare. The team has also developed a laboratory prototype of a compact SPR sensor based on a novel patented approach to the spectroscopy of surface plasmons [8, 9], and furthermore, the team has demonstrated that this platform provides performance that is comparable to that offered by the best laboratory-based systems [10].

(3) Activities of the team in the area of **microfluidic effects and devices** have been focused on the design and fabrication of microfluidic systems for the enhancement of analyte transfer rates to the sensing surface (regarding devices based on both propagating and localized plasmons).

One of the difficulties arising in the use of biosensors for biomolecular analysis is the effect of axial dispersion in tubing sections between the sample source and the sensing chamber. To combat this effect, the team recently developed a dispersionless microfluidic switching systems based on vinyl-gaskets and miniaturized three-way valves [11]. When activated, this system provides a complete change of fluids within the sensing chamber within a short period of time (3s), and allows for accurate measurements of analyte binding and disassociation rates free of dispersion effects. The team also has studied the effect of the size and shape of the sensing chamber on the sensor sensitivity [12]. Through the lithographic fabrication of gaskets made from the thick-film photoresist Su8, the team was capable of modifying the sensor chamber geometry with high accuracy; furthermore, through numerical and analytical modeling the team was able to enhance the sensor response by a factor of 4 through modification of the sensor chamber height [12]. These fabrication capabilities have been further extended for use in the SPR imaging sensors; a multiplexed analysis system using passive mixing structures has been demonstrated to improve the mass transfer of analyte to a microarray-based sensor [13].

(4) In the area of the development of **functional biomolecular coatings** for affinity biosensors, the team has pursued the development of strategies for delivering functional biomolecules (in a form of a functional coating) to three different types of plasmonic

structures: continuous metal films, metallic nanoparticles on a solid substrate, and metallic nanoparticles in solution. In the process of functionalization, the surface of material to be functionalized (predominantly gold) is initially covered with a linker layer that contains functional groups to which biorecognition elements are subsequently attached. The team has explored the potential of a variety of linker layers, including both self-assembled monolayers of alkanethiols as well as a variety of polymer brushes, such as hydroxy-functional poly(2-hydroxyethyl methacrylate) (pHEMA) and carboxy-functional poly(carboxybetaine acrylamide) (pCBAA). Likewise, the team has also investigated several procedures for the attachment of biorecognition elements (covalent coupling or coupling via functional molecules). The development of functional coatings based on polymer brushes was carried out in close collaboration with the Institute of Macromolecular Chemistry CAS (IMC), Prague, and the University of Washington, Seattle (USA). Characterization of these functional coatings with regard to their biorecognition and fouling properties was performed using the SPR method, while their physical properties were characterized using spectral ellipsometry and atomic force microscopy (AFM).

The Optical Biosensors research team has also functionalized gold nanoparticles and used them to enhance the response of a conventional SPR biosensor [14]. The nanoparticles were functionalized with both streptavidin and bovine serum albumin (BSA); the streptavidin provided a high affinity for the biotinylated secondary antibody used in the second step of a detection assay, while the BSA helped minimize the nonspecific interaction of the functionalized nanoparticles with blood plasma. The functionalized nanoparticles have been validated in an experiment in which physiologically-relevant concentrations of carcinoembryonic antigen (a cancer biomarker) were detected in human blood plasma [14]. Recently, in collaboration with IMC researchers, the team carried out a comparative study in which three different functionalization approaches (OEG-based carboxy-functional alkanethiolate self-assembled monolayer and two polymer brushes: pHEMA and pCBAA) were evaluated with respect to their fouling resistance from complex samples (blood plasma and milk). The results of the study suggested that pCBAA provided the best combination of biorecognition ability and fouling resistance [15].

(5) The team has also pursued applications that utilize the developed biosensors for **biomolecular interaction analysis**. Although SPR biosensors have been proven to be an important and trusted tool for biomolecular interaction analysis, the currently available SPR biosensors are limited to the observation and quantification of interactions under rather idealized conditions (two isolated biomolecules, one immobilized on the sensor surface, one contained in buffer). In order to expand the SPR technology to more realistic systems (biomolecules contained in their native environment with a large variety of other biomolecules present), the team has proposed a novel approach for the observation of biomolecular interactions based on the use of a distinctive microfluidic system. This microfluidic system allows for rapid switching between a complex sample of interest and a washing solution that serves to remove non-specifically adsorbed proteins [16]. In addition, the SPR method was applied for the quantification of complex enzymatic interactions. The development of an assay for the determination of RNase H activity was used in a study on new DNA modifications and their potential as candidates for antisense oligonucleotides (AOs). In collaboration with both the Faculty of Mathematics and Physics

and the Institute of Organic Chemistry and Biochemistry, the team investigated different oligothymidylates with respect to their hybridization properties with oligoriboadenylates and their ability to induce RNA cleavage by RNase. Several chemical modifications were found to have high potential to be effective antisense oligonucleotides working by the RNase H mechanism [17].

(6) During the period of evaluation the team has also developed a variety of **optical biosensors and methodologies for detection of chemical and biological agents**. In order to expand the applicability of plasmonic biosensors to complex biological samples, the team has developed a new approach to referencing based on an identically functionalized surface in both the detection and reference channels. This approach has been demonstrated to provide superior accuracy and biological variability compared to previously existing referencing approaches [18].

Analytes targeted during the period of evaluation included analytes related to food safety (detection of veterinary drug residues, foodborne pathogens and toxins) and medical diagnostics (detection of molecular biomarkers of cancers, Alzheimer's disease, myelodysplastic syndromes, and organ damage). In particular, the detection of disease biomarkers in real-world biological samples has received a great deal of attention. The team has developed an SPR biosensor and detection methodology based on a sandwich assay and the use of functionalized gold nanoparticles for the detection of carcinoembryonic antigen (CEA): a biomarker related to gastrointestinal, breast, and lung carcinoma. This technology allowed for the detection of CEA in blood plasma at concentrations down to 0.1 ng/mL, well below normal physiological levels that are typically at low ~ ng/mL [12]. The team has also developed a biosensor for nucleic acid biomarkers and, in collaboration with the researchers at the Institute of Systems Biology, Seattle (USA), has applied it for the detection of specific microRNA: a biomarker of drug-induced liver damage. This approach allowed for the detection of miRNA in less than 30 minutes, with an absolute amount of miRNA necessary for the positive detection in the range of 100s of attomoles. The methodology was applied to the analysis of miRNA from mouse liver tissues; results were found to agree well with those provided by the conventional laboratory method (quantitative polymerase chain reaction) [19].

c) Collaborations

The Optical Biosensors research team collaborated with a large number of research groups worldwide. The list of the main collaborations that were supported by research grants and/or resulted in important joint publications is provided below.

Austrian Institute of Technology, Vienna (Austria): optical platforms for plasmonically-enhanced fluorescence-based biosensing.

Ewha Womans University, Seoul (Korea): bottom-up fabricated nanostructures for plasmonic affinity biosensing and Surface-Enhanced Raman spectroscopy (SERS).

Faculty of Mathematics and Physics of Charles University in Prague: applications of plasmonic biosensors for molecular interaction analysis.

Faculty of Nuclear Science and Physical Engineering of Czech Technical University, Prague: advanced simulation methods for electromagnetic analysis of plasmonic nanostructures.

Karl-Franzens University Graz (Austria): plasmonic nanostructures and plasmonic affinity biosensing.

Imperial College, London (United Kingdom): optical biosensors for biological agents based on plasmonically-enhanced spectroscopies.

Institute of Hematology and Blood Transfusion, Prague: development of plasmonic biosensors for early diagnostics of myelodysplastic syndromes.

Institute of Macromolecular Chemistry CAS, Prague: advanced functional coatings for plasmonic biosensors for the analysis of complex samples.

Institute of Organic Chemistry and Biochemistry CAS, Prague: applications of plasmonic biosensors for characterization of potential drug candidate molecules.

Prague Psychiatric Center, Prague: investigation of molecular interactions involved in the pathogenesis of Alzheimer's disease, development of plasmonic biosensors for early diagnostics of Alzheimer's disease.

Stevens Institute of Technology, Hoboken (USA): fiber optic chemical sensors and biosensors.

University of Washington, Seattle (USA): advanced low-fouling functional coatings for plasmonic biosensors for the analysis of complex samples, top-down plasmonic nanostructures for optical affinity biosensing and SERS.

In addition, the team has participated in international networking actions, such as *COST Action MP0803: Plasmonic components and devices*, and E*SF Network New Approaches to Biochemical Sensing with Plasmonic Nanobiophotonics*.

The team has also collaborated with industry. In particular, these industrial partners have included:

Phenogenomics, Bothell (USA): commercialization of SPRCD optical biosensor technology developed by the Optical Biosensors research team.

VIDIA spol. s.r.o., Vestec: applications of SPR biosensors for the optimization of immunological methods, development of plasmonic biosensors for early diagnostics.

d) Impact

Biosensors are a disruptive technology with applications in numerous important sectors and potentially have significant societal and economic impacts. The value of the total biosensor market was over $11 billion in 2013 and is expected to exceed $22 billion by 2020. Label-free optical biosensors – and plasmonic biosensors in particular – have already been adopted in biomolecular interaction analysis and drug discovery markets; however, their potential for applications in bioanalytical market has not been harnessed yet.

Although the main focus of the Optical Biosensors research team is on cutting-edge research, the team has developed several high-performance SPR biosensor platforms suitable for molecular interaction analysis. More than 20 SPR biosensor platforms developed by the team are in use at universities and research institutions situated in the USA, Europe, and Asia (7 added during the period of evaluation). Moreover, to facilitate the transition of plasmonic biosensors to high-gain markets such as healthcare, environmental monitoring, food safety, and security, the team has focused on the development of compact, high-performance yet potentially low-cost plasmonic biosensors. The team has strived both to create and manage intellectual property (5 patents granted and 2 applications filed during the period of evaluation), and to form partnerships with industry and potential end users. During the reported period the team worked closely with a US-based company (Phenogenomics, Inc.) who had licensed one of the biosensor technologies developed by the team; however, commercial biosensing devices based on the team's inventions have yet to come.

e) Educational activities

The main educational activities undertaken by the team include both the supervision of MS and PhD students (through joint accreditation with Czech universities) as well as course instruction. The Optical Biosensors research team has a tradition of strong involvement of PhD students in its research activities. Over the period of evaluation (2010-2014), 6-8 PhD students pursued their PhD research at the Institute under the supervision of J. Homola. These students came from both the Faculty of Mathematics and Physics of Charles University in Prague and the Faculty of Nuclear Science and Physical Engineering of Czech Technical University. J. Homola has also regularly instructed courses on optical sensors at both of these universities, and likewise, has served on multiple degree committees. J. Homola has also contributed to education at an international level by instructing short courses on optical biosensors at the University of Padova, Italy, and the University of Oulu, Finland. As a member of the Executive Board he has also contributed to the Advanced Study Course on Optical Chemical Sensors (ASCOS): a biannual event providing an education and networking platform to young post-docs, PhD students, and outstanding M.Sc. students. A full account of the educational activities of the team is provided in Section 3.10.

f) Public outreach

The Optical Biosensors research team considers public outreach an important part of its mission and therefore actively participates in a number of activities aimed at increasing public awareness and understanding of optical biosensors research. The main regular event is Open Doors Days, which is organized by the Institute every year. During Open Doors Days, over 250 visitors (high school and university students and the general public) are provided an opportunity to visit selected labs and talk with the Optical Biosensors research team members. In addition, team members have given lectures on optical biosensors to students and the general public at a variety of occasions. The research of the team has been frequently featured in the Czech media. This has included articles in the Czech newspapers and interviews of members of the team on both radio and TV. The full account of the public outreach activities of the team is provided in Section 3.10.

g) Services to community

The senior scientists of the Optical Biosensors research team (J. Homola and J. Kaňka) are active members of the scientific community. Their contributions to the community include a broad range of activities, from serving on advisory committees and editorial boards to organizing conferences. Selected contributions are highlighted below; the full account is provided in the Section 3.10.

J. Homola is a member of the Expert Commission for Technical Sciences and Engineering of the Research and Development Council of the Czech Republic and serves on the editorial boards of the journal Sensors and Actuators B, Journal of Sensors, and Analytical and Bioanalytical Chemistry. He has also served as chair of several international scientific meetings, such as the 10th European Conference on Optical Chemical Sensors and Biosensors, the SPIE Conference on Optics & Optoelectronics, and the NSF Workshop on US-Czech Frontiers in Photonics. J. Homola has also served as a member of the program committee for many additional conferences. He is Fellow of SPIE and a Senior Member of IEEE. J. Kaňka has been serving as a regular member of program committees of SPIE Defense, Security and Sensing, SPIE Photonics Europe, and SPIE Optics and Optoelectronics Conferences.

h) Resources

The Optical Biosensors research team has extensive laboratory facilities and state of the art equipment. The main laboratories consist of (*i*) a Nanofabrication Lab (equipped for the fabrication of nanostructures by electron beam lithography and focused ion beam systems), (*ii*) a Nanostructure Characterization Lab (equipped with a spectral ellipsometer, mechanical and optical profilometers, and an atomic force microscope), (*iii*) a Diffractive Optics Lab (containing equipment for the preparation of diffractive structures using interference lithography and their replication using soft lithography), (*iv*) a Guided-Wave Optics Lab (equipped with optical sources, detectors, and micropositioning equipment), (*v*) a Thin Film Lab (with two systems for the deposition of thin films by evaporation in vacuum), (*vi*) a Biochemical Lab (with equipment for the functionalization of sensors), (*vii*) a Spectroscopy Lab (with spectroscopic systems for UV-VIS and NIR and a Raman spectroscopic system), and (*viii*) a Sensor Characterization Lab (with 4 spectroscopic SPR systems, 1 SPR imaging system and a commercial SPR system BIACORE 3000). The Head of the team regularly solicits input for acquisition of new equipment to translate the needs of the team to applications for capital equipment to the Czech Academy of Sciences and applications for competitive research grants.

In order to support its research activities, the team vigorously pursues research funding opportunities at both the national and international level. During the period of evaluation (2010-2014), the team was supported by 16 competitive research grants received from domestic and foreign sponsors (Czech Science Foundation, Ministry of Education Youths and Sports, Ministry of Health, Czech Academy of Sciences, European Commission, US Army, US Office and Naval Research). The total amount of funding received through these grants was over 3,3 mil. EUR (in case of multi-institutional grant projects, this amount includes only the funds available to the Optical Biosensors research team). The Head of the team has been repeatedly entrusted with coordination of large multiple-year research projects, such as *Surface plasmon resonance biosensors and protein arrays for* *medical diagnostics* (2007-2011) and a center of excellence *Nanobiophotonics for future health care* (2012-2018). A list or research grants of the team can be found in Section 3.1.

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Research Report of the team in the period 2010–2014

Institute	Institute of Photonics and Electronics of the CAS, v. v. i.

Scientific team	Fiber Lasers and Non-linear Optics

a) Research team

The research team Fiber Lasers and Nonlinear Optics is active in

- material research for active optical fibers,
- development of passive optical fibers for high-power and high-pulse energy delivery,
- investigation of high power fiber lasers and their use for material processing, medicine and nonlinear optics.

The main research topics in the evaluation period were:

- efficient rare-earth doped optical fibers for fiber lasers and amplifiers,
- high-power fiber lasers with operational wavelengths above 1900 nm,
- nonlinear optics for generation of mid-infrared radiation,
- optical fiber gas and pH sensors,
- all-optical signal processing.

The research team Fiber Lasers and Nonlinear Optics was formed in 2013 by merging the Guided-wave Photonics Group and the Laboratory of Optical Fibers with the aim of unifying the research effort to fiber laser applications. This reflected in the shift of the research interests of the members of both teams from advanced optical communication systems and fiber optic sensors to high power fiber lasers and their applications.

The research team Fiber Laser and Nonlinear Optics listed 6 senior scientists, 1 scientist, 1 associate scientist, 3 postdoctoral fellows, 7 graduate students and 3 research assistants at the end of the evaluation period.

The age structure of the team is balanced and has improved considerably over the last 5 years. Two senior scientists were older than 60, two senior scientists were in the range 50-60, two senior scientists including the leader of the team were 40-50, the scientist, associate scientist assistant and three postdoctoral fellows were in the range of 30-40, all graduate students were younger than 31 at the end of the evaluation period.

The research team includes laser physicists, sensor physicist, chemists specialized to sol-gel techniques, specialists on numerical computations, device engineer, and technologists of fiber preform fabrication, fiber drawing, periodic poling of nonlinear crystals, long period grating inscription.

b) Description of research and main achievements

The main research directions of our team were more efficient rare-earth-doped optical fibers, high-power fiber lasers and amplifiers, mid-infrared light sources and methods of numerical simulations.

In the field of active fibers, our research effort was devoted to nanoparticle doped optical fibers with the aim of improving the solubility of rare earth (RE) ions and to increase the lifetime value of the active transition. Long lifetime is essential for lasers with a low threshold and for amplifiers with a high quantum conversion efficiency while the high doping concentration allows building of short fiber lasers and amplifiers that are suitable for generation or amplification of ultrashort pulses and for single frequency lasers.

We have pioneered gamma-phase alumina nanoparticle-doped fibers since 2007 [1]. We have achieved excellent homogenity of ion distribution and low background losses in gamma-phase alumina nanoparticle-doped fibers compared to the fiber prepared by conventional solution doping [2]. A lifetime of the upper state of active laser transition (3F4-3H6) of thulium ions in our alumina nanoparticle-doped silica glass fibers prepared in 2014 is about 40% above the state of the art commercial thulium-doped fibers [3], and we have indications that this value can be even greater.

Some paradigmatic effects related to the formation of nanoparticles were studied on zinc-titanates [4, 5]. As it was demonstrated on rare earth doped zinc-titanates, europium-titanates and europium-yttrium-titanates the distortion of crystal lattice can strongly improve the luminescence in the visible spectral range [5]. Europium-doped optical fibers are also useful as radiation fiber sensors that are o great scientific interest to our colleagues from the Institute of Physics of the Czech Academy of Sciences. Nanoparticles and glass samples in these papers were prepared by our team by sol-gel method while the co-authors from other institutions contributed by particular experimental measurements.

Our fiber lasers research was focused on rare-earth doped fiber lasers operating in a spectral range of 800nm to 2100nm. We investigated fiber lasers based on commercial as well as on our own ytterbium-, erbium-, thulium- and holmium-doped core-pumped and double-clad optical fibers. Special attention was dedicated to the self-pulsing instabilities of the fiber lasers and their relation to the self-induced laser line sweeping. Self-pulsing instabilities can lead to damage of components inside the laser and could have detrimental consequences in laser applications, therefore their research is of high importance for fiber laser community. We were the first team that described the effect of fiber laser wavelength self-sweeping in literature. This effect can be explained by spatial-hole burning in the active medium caused by a standing-wave in the laser cavity. We reported also for the first time the laser-wavelength self-sweeping in erbium-doped fiber lasers [6, 7].

The output power of fiber lasers have increased by several orders of magnitude during the last fifteen years and it is close to the physical limits now. Possible methods how to further scale up the fiber laser power are the coherent and spectral beam combing. We investigated both methods in thulium-doped fiber lasers. We provided the first demonstration of coherently combined thulium-doped fiber laser at moderate powers. By coherently combining two fiber lasers we achieved an output power of 20 W with combining efficiency close to 1. We observed suppression of self-pulsing in the coherently combined laser as an important side effect which we attributed to the Vernier effect on laser modes [8].

We demonstrated various applications of our optical fibers and fiber lasers. We developed the CW mid-infrared coherent source tunable in a spectral range of 3100-3650nm based on the difference frequency generation in periodically-poled KTA crystal. The phase synchronism was maintained by simultaneously tuning the high-power ytterbium- and erbium-doped fiber lasers without changing the crystal temperature or position [9].

We developed the spectrally flat ASE source with −10 dB bandwidth of 645 nm that was based on our own thulium–holmium-doped optical fiber [10]. We believe that it is the widest bandwidth achieved in rare-earth- doped fiber based ASE sources to date. In order to achieve such a large bandwidth, we used the fact that the forward and the backward ASE in the optical fiber are spectrally shifted due to the reabsorption. We combined the forward and the backward ASEs generated in our fiber using wideband fused fiber couplers. The source is now used in a spectroscopy application.

Beside the intensive experimental work, our team was active in the theoretical analysis of various optical structures and in the development of numerical simulation methods. We have developed a special Adaptive Spatial Resolution approach to the Fourier Modal Method that allows the modelling of very thin layers (e.g. metals). This approach was implemented independently by our team and a team from the Czech Technical University in Prague. This made it possible to mutually compare results and thus significantly enhance their reliability. The results were summarized in a paper to which both teams contributed equally [11]. Prof. Ctyroky of our team also developed a full 3D modelling algorithm that addressed issues such as boundary conditions and mode orthogonality. It is based, somewhat unconventionally, on sine and cosine Fourier expansions, which allow for the direct full utilization of the symmetry of the analyzed structure, thus reducing the required computational efforts [12]. Our own implementation of the numerical model was also used in a paper on asymmetric transmission of surface plasmon-polaritons [13] and on the controlling of surface plasmon-polaritons by external magnetic fields [14].

We also concluded our previous research on advanced telecommunication systems at the beginning of the evaluation period through a series of works on all-optical wavelength converters [15, 16]. We have shown the possibility of energy efficient all-optical processing in relatively simple optical devices up to very high transmission rates.

Determination of the pH in microscopic bio-samples has been of a great interest for many years of botanists of the Institute of Experimental Botany of the Czech Academy of Sciences since the knowledge of pH values or pH gradients can contribute to explanation of complex processes in animal or plant organisms. In such cases the analyzed samples have often small volumes in a range from microliters down to volumes of individual cells with diameters of 20-50 μm. The elaborated technology of fiber tapering and the long-time research in fiber sensors made us possible to deal with point detection of samples of a microliter volume. Fiber-optic tips with a tip diameter reduced to a few micrometers were prepared and sensitized with fluorescence pH indicators. Fiber probes were tested in vivo and in vitro samples of exudates excreted by leaves of cultivated oat plants of a volume of about 6 microliters [17]. Later we achieved a resolution of about 0.04 pH in tissue samples of Arabidopsis thaliana.

c) Collaborations

Our team appreciated the international collaboration with other research teams. In the field of fiber fabrication technology we had a fruitful collaboration with Sciences Chimiques de Rennes, Université de Rennes, Université Claude Bernard in Lyon, Laboratoire des Matériaux Inorganiques in Université Blaise Pascal in Clermont-Ferrand in France. We had also a joint project with the Optical Research Centre in Southampton, UK on heavy metal oxide glass fibers.

We had numerous joint publications on fiber lasers with colleagues from Laboratoire de Physique de la Matière Condensée, Université de Nice-Sophia-Antipolis France, with Dr. Martin Becker from the Institute of Photonic Technology, Jena, Germany and with Dr. Anirban Dhar from Central Glass and Ceramics Research Institute, Kolkata, India. Theoretical and numerical analyses were performed in coordination with the team of Prof. Nosich in NASU, Charkov, Ukraine, Department of Physics and Astronomy, University of California, Irvine, USA, Centro de Física Teórica e Computacional and Departamento de Física, Faculdade de Ciências, Universidade de Lisboa, Portugal Centro de Física, Universidade do Minho, Braga, Portugal, Dipartimento di Fisica "E.R. Caianiello", Università di Salerno, Fisciano, Italy. The validity of numerical methods was tested with other teams in Europe, e.g., Laboratoire Interdisciplinaire Carnot de Bourgogne of Université de Bourgogne, Dijon and Laboratoire Charles Fabry de l'Institut d'Optique, CNRS-Université Paris Sud, France. Fiber sensors were investigated with our colleagues from the Department of General and Physical Chemistry and Szentágothai János Research Center of the University of Pécs in Hungary.

We also participated in the international project MNT-ERA-NET scan4surf - Inline Process Metrology for Laser Structuring Systems where we were responsible for the development of a high-power wide-band ASE source that was part of the OCT system. This project was led by the Fraunhofer Institute of Manufacturing Technology and Advanced Materials, Germany in cooperation with the company Precitec, GmbH.

The team actively participated in the COST actions MP1204 TERA-MIR Radiation: Materials, Generation, Detection and Applications, and TD1001: Novel and Reliable Optical Fiber Sensor Systems for Future Security and Safety Applications (OFSeSa).

On a national level, we collaborated with the Faculty of Nuclear Sciences and Physical Engineering of the Czech Technical University in the research on fiber lasers and on numerical methods, with the Institute of Experimental Botany of the Czech Academy of Science in the development of intracellular pH-meters, with the Institute of Physics of the Czech Academy of Sciences on the numerical simulations of photonic structures and in the field of radiation sensors. We collaborated with Faculty of Electrical Engineering of the Czech Technical University in the field of all-optical processing.

We are proud of collaboration with Czech high-tech companies e.g. SQS Vláknová optika a.s., Safibra s.r.o, Optokon a.s. in the field of fiber components and optical applications.

d) Impact

Senior members of the research team have established informal scientific networks on numerical modelling, fiber lasers, and nanoparticle-doped optical fibers. These naturally formed core competencies manifest themselves through the joint scientific papers listed elsewhere. The impact of our research to the global scientific community can be seen from numerous invited lectures e.g.:

- V. Matějec, J. Mrazek, T. Martan, I. Kasik, M. Pospisilova: "Sol-gel materials for optical microsensors", ROMPHYSCHEM-14, June 2-4,2010,
- J. Čtyroký and I. Richter: "Recent developments in Fourier modal methods for modeling of guided-wave devices", Information Photonics 2011, May 18-20, 2011, Ottawa, Canada,
- J. Čtyroký et al.: "Modal methods for 3D modelling of advanced photonic structures", ICTON 2012, July 2-5, 2012, University of Warwick, UK;,
- P. Honzatko: „Latest achievements in fiber lasers,"XVIII Czech-Polish-Slovak Optical Conference, September 6, 2012,
- P. Peterka, I. Kašík, P. Honzátko, B. Dussardier and W. Blanc, "Thulium-doped silica fibers with enhanced 3H4 level lifetime for fiber lasers and amplifiers", In Proc. 3rd International Conference on Photonics 2012 (ICP2012), IEEE conf. publications, 1-3 October 2012, Penang, Malaysia,
- J. Mrazek, I. Kasik, O. Podrazky, V. Matejec: "Colloidal routes to nanocrystalline materials for fiber lasers," 10th Conference on Colloid Chemistry" 2012, Budapest, Hungary,
- J. Čtyroký: "Photonic waveguide structures with loss and gain", META 2013, March 18-22, 2013, Sharjah, UAE,
- J. Čtyroký: "Computational analysis of subwavelength grating waveguide structures", 17th European Conference on Integrated Optics ECIO – MOC 2014, June 24-27, 2014, Nice, France,
- P. Peterka, P. Honzatko, and I. Kasik: "Thulium-doped optical fibers and components for fiber lasers in 2 μm spectral range", SPIE 9441:94410B, 2014,
- I. Kašík, J. Mrázek, O. Podrazký, P. Peterka, J. Cajzl, J. Aubrecht, J. Proboštová, J. K. Sahu, X . Feng, A. Dhar: „RE-doped special optical fibers," Conference on Optical Fibers and Their Applications (OFTA), January 2014,Bialystok, Poland,

participation in conference scientific committees (cf. Appendix 3.10, part 2), visits of excellent scientists in our labs (e.g., Prof. Jayanta Sahu of ORC, Southampton, UK, Acad. Prof. Evgenii Mikhailovich Dianov of FORC, Moscow, Russia, Robert A. Lieberman, SPIE president-elect, Prof. Otto S. Wolfbeis, rector of Univ. Regensburg, Prof. Marcel Poulain, Universite de Rennes, Prof. Sergei A. Babin, IAE Novosibirsk, Russia, to name a few). Our research was also appreciated in a local R&D community, as evidenced by the Visegrad Group Academies Young Researcher Award to O. Podrazký for achievements in the field of Material Research of Optical Fibers for Sensors and Lasers or Award of the Technology Agency of the Czech Republic to our team for the project Optical Packet Switch.

We contributed to understanding of the instabilities in fiber lasers. Self-pulsing instabilities are frequently encountered problem in high-power fiber lasers. The laser systems are often stabilized by trial and error. We linked self-pulsing instability to the laser line sweeping and evaluated a reflectivity of the transient gratings in the self-swept fiber lasers. We observed laser line self-sweeping in ytterbium- and erbium-doped fiber lasers for the first time.

We have pioneered a technique of doping the fiber core by gamma-phase alumina nanoparticles in order to achieve excellent homogeneity of ion distribution, low background losses and elevated excited state lifetime.

Our team carried out extensive activities in applied research which has some economic impact. Several Czech companies collaborated with our team in the frame of joint projects funded by the Czech government. The high-tech company SQS Vláknová optika a.s. collaborated with us on the development of optical fiber components and fiber sensors. The company considerably increased its portfolio of optical fiber products over the last 5 years, partly thanks to this collaboration. Optokon a.s. company collaborated with us on the development of a tunable mid-infrared radiation source and it is interested in exploiting of our technology of nonlinear crystal poling. Safibra s.r.o. company collaborated with our team on the development of broadband light sources.

We collaborated closely with the Czech academic network operator CESNET while we were involved in the research of the advanced communication systems during the beginning of the evaluation period. CESNET provides Internet infrastructure to academic institutions in the Czech Republic. We participated in the development and testing of the repeater-less optical line from Prague to Brno. We were involved in the development of multichannel gain-clamped amplifiers, multichannel Raman amplifiers with time-domain switched pulse pumping and in the investigation of power transients in multichannel networks that are caused by the channel drop-off.

We participated in the 7FP European project scan4surf (project of the European Micro and Nano Technology program MNT-ERA.net) that was aimed at the development of a measurement system for an industrial laser that processes precision parts such as moulding parts for the automotive or aerospace industry. These moulds are very expensive and inaccurate focusing of the high-power laser could irreparably damage them. The measurement system provides distance inspection and precise focusing of the laser processing head in-line, i.e. during the laser structuring process. The coordinators of the project were the German company Precitec (the world-leading manufacturer of laser cutting and welding heads) and the Fraunhofer Institute for Manufacturing Technology. Our team developed a high-power, active-fiber based source of radiation with tailored spectral characteristics for the measuring sensor. The prototype was presented in 2013 in Munich at Laser-World of Photonics, the largest laser trade fair in Europe.

e) Educational activities

Every year up to 6 of our researchers gave lectures at Czech universities in the total amount of more than 200h. The team members are leading the half-year course of Fiber lasers and amplifiers that is to our knowledge the only comprehensive university course dealing with the topic of fiber lasers in the Czech Republic.

During the evaluation period 8 pregraduate students and 2 graduate students prepared their thesis under the supervision of members of our team. Currently 7 graduate students and 1 pre-graduate student are supervised by members of our team.

5 young researchers were sent to international summer schools for scientific training and networking during the evaluation period.

A list of all activities can be found in Appendix 3.10.

f) Public outreach

The team is renowned nationally for its successful and appreciated communication of fiber lasers and waveguide photonics to general public. Systematic PR activities of the team were focused on the professional society and the general public. In the field of professionally oriented activities our team regularly participated in national conferences and exhibitions such as OK (Optical communications conference), Laser (Laser technology conference), Amper (International Trade Fair) and presented results of the research in a comprehensible form.

In the field of general public activities the team annually gave various demonstrations during the Week of Science and Technology, the largest science festival in the Czech Republic. About 500 visitors enjoyed our lab-tours during this week. Our researchers gave numerous lessons and made practical demonstrations on ultrafast communications and optical fibers in the framework of the project Open Science supported by the Czech Academy of Sciences. These activities are targeted to talented students of secondary schools and their teachers. Members of our team repeatedly participated in TV and radio broadcasting dedicated to optical fibers and optical communications on various occasions. Short TV releases were shot in our labs as well. Two booklets describing the research activities and the technological facilities of our team were printed and distributed. A list of all activities can be found in Appendix 3.10.

g) Services to community

Members of our team have participated in the evaluation panels of the Czech Science Foundation, the sub-program board of the Technological Agency of the Czech Republic and the academic boards and the scientific committees of Czech universities. They served in both the organizing and the technical committees of international and national conferences and workshops. They served regularly as reviewers of Optics Express, Optics Letters, IEEE Journal of Lightwave Technology, IEEE Photonics Technology Letters, Optics Communications and many other scientific journals. A more detailed list of all activities can be found in Appendix 3.10.

h) Resources

The team Fiber Lasers and Nonlinear Optics has a facility for special fiber preform preparation and fiber drawing that is amongst ca. 8 academic research facilities in the EU and it is unique in the Czech Republic. The facility is equipped with Special Gas MCVD, two 6.5m long fiber drawing towers, preform and fiber analysers from Photon Kinetics and a fiber strength tester.

Our modern chemical laboratories fully meet the international safety standards which allow us to operate with dangerous materials and to realize sophisticated experiments. The laboratories are air-conditioned, equipped with powerful fume chambers and with gas distribution piping which allows the work in controlled atmosphere. Beside common laboratory equipment the instrumentation includes a spin-coater Laurent and a dip-coater IDLab which are used to the thin film deposition. The set of tube furnaces and chamber furnace allows the thermal processing of bulk samples up to 1700 °C. Thin films can be processed in a rapid thermal annealing furnace from AccuThermo company.

Our pressurized and air conditioned laboratory of high power fiber lasers is equipped with a fiber splicing and processing device Vytran GPX-3400, laser diode drivers able to supply up to 60A, pump laser diodes operating at 793nm, 915nm, 976nm, 980nm and a complete set of devices for the characterization of fiber lasers including a FTIR spectral analyser for the spectral range of 700-8000nm, power meters for measurements of powers from pW levels up to 100 W, an autocorrelator for measurements of optical pulses from 10fs to 120ps, and a pyroelectric camera for evaluation the quality of laser beam.

Our air conditioned laboratories for fiber components are equipped with CO2 lasers for tapering the fibers and for inscription of long period gratings, tomography for measurements of the stress in optical fibers and a set of devices for the characterization of active fibers which includes spectral absorption and emission measurements, laser transition lifetime measurements and evaluations of the fiber efficiency in standard laser configuration.

We have a facility for nonlinear optical crystal poling and the experience with poling of KTP and KTA crystals. An agreement was signed with the Czech company Optokon a.s. at the end of the evaluation period on the exploitation of this facility. We hope that we will be able to finance further development of the technology of nonlinear optical crystal poling thanks to this agreement.

Our senior researchers regularly score in funding competitions of the Czech Science Foundation, the Ministry of Education, Youth and Sports, and the Technology Agency of the Czech Republic. We have been involved in the solution of 22 basic research projects and 4 applied research projects that were supported by 2750 thousand Euro.

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Research Report of the team in the period 2010–2014

Institute	Institute of Photonics and Electronics of the CAS, v. v. i.

Scientific team	Bioelectrodynamics

a) Research team

The Bioelectrodynamics junior research team was founded in 2013 as a result of generation transition within the previous team structure. During the evaluation period (2010 – 2014), five of the seven team members retired and the leadership was handed over to Dr. Cifra, the then sole postdoctoral fellow in the team. Dr. Pokorný, the previous team leader, was awarded the emeritus status. The team then returned back to the size of seven members, each below the age of 35. Two doctoral students of the team successfully defended their thesis and became postdoctoral fellows. Three other doctoral students began their studies during the evaluation period. One technician joined the team.

The change of the structure of the team had an impact on the research topics fostered. Electromagnetic activity of cells, the cornerstone of previous research directions, was gradually reduced in favour of new perspective topics discussed in the following.

b) Description of research and main achievements

In our research team, we analyze electromagnetic properties of biological, protein nanostructures with the focus on cellular fibers – microtubules. Furthermore, we focus on the endogenous radiofrequency and optical electromagnetic biosignals. Our activities cover experimental and theoretical work, together with the development of experimental equipment. Using nanotechnology fabrication methods we develop micro/nanosensors for our measurement systems of very weak electromagnetic molecular and cellular signals. Experimental work is followed by the modelling of biophysical processes which generate electromagnetic fields at a cellular, primarily supra-molecular, level.

The main achievement during the period of 2010 – 2014 was the development of the model of electrodynamic effects connected with polar vibration normal modes of supramolecules, namely microtubules. Microtubules are electrically polar protein nanostructures present naturally in almost every biological cell and are fundamental to cell division, intracellular transport and signalling. Our microtubule research has a straightforward scientific impact in the field as well as across scientific disciplines. Vibrational and electrostatic properties of protein structures, including microtubules, had previously only been researched separately. However we have combined these properties and introduced for the first time a model of electrodynamic properties of microtubules.

Since the first version of our microtubule model (Cifra et al. 2010) and its follow up modifications (Havelka et al. 2011, Kučera and Havelka, 2012), it has evolved into a versatile tool which can be used for computational predictions of high-frequency electric fields of vibrating microtubules and also can be generalized for any biomolecular structure. In our paper (Havelka et al. 2014a), we showed *in silico* that multimode excitation of electrically polar collective vibration modes of microtubules forms an ultrafast electromechanical pulse propagating along the microtubule. The biophysical mechanism we described lends support to the argument that microtubules may comprise a substrate for novel ultra-fast electrical signaling in neurons or other living cells. Our results indicate that such pulses can mediate ultra-fast intracellular signaling, and furthermore, can be utilized in bio-inspired electronic devices. At the end of the evaluation period, we launched a project dedicated to experimental verification of the predictions made by the use of our model.

In another important result (Havelka et al. 2014b) based on our microtubule model, we have computationally predicted that ultra-short electrical or mechanical pulses may cause vibrations of the mitotic spindle, the main functional machinery involved in the separation of duplicated genetic information between two daughter cells during cell division. We have found that microtubules which constitute the mitotic spindle, driven to vibrate induce a strong rapidly changing electric field in the region where chromosome separation takes place. For certain values of parameters, the intensity of the electric field and its gradient reach values which may exert a not negligible force on chromosomes which are aligned in the spindle midzone. Such high intensity of the generated field may consequently disrupt the cell division in progress. Our results provide the computational foundations for future analysis of possible mechanisms describing the effects of ultra-short electrical and mechanical pulses on dividing cells. We believe that this is an important contribution to the discussion on the application of ultra-short pulses as a prospective means of future therapy of cancer.

Another line of research which was launched in 2013 concerns ultra-weak photon emission from biological samples. Ultra-weak photon emission originates from electron excited species which are produced chemically during the endogenous oxidative metabolism and oxidative stress in biological samples. Since ultra-weak photon emission carries information about the metabolic processes in cells, we examine its relationship with the parameters of biochemical analysis of cellular processes. Together with the development of robust methods of its measurement, we also examine the possibilities of using this issue in medical diagnostics and other biochemical measurements, e.g. in the food industry. Besides initial experimental results, this research has led to a critical assessment of a feasibility of cell-cell communication through their endogenous light signals (Kučera and Cifra 2013). These results are important for the rationalization of further research in optical cell-cell interactions. We also analyzed and summarized definition, mechanisms, properties, detection and applications (Cifra and Pospisil 2014) and statistical properties (Cifra et al. 2015 – work was carried out in 2013-2014) of biological ultra-weak photon emission. This research topic has been developed in collaboration with the Department of Biophysics, Palacký University Olomouc. The contribution of our partner resides in biochemical analysis of the samples under study while our role is to develop novel experimental instrumentation and to quantitatively study the underlying mechanisms.

The previous line of research, which was developed by members of the team who retired during the evaluation period, was crowned with a hypothesis about the role of electromagnetic activity of biological cells (Pokorný et al. 2013). Although there is currently a vast amount of research into the molecular function of living systems, a conceptual physico-chemical understanding is far from complete. A framework of postulates has been postulated in (Pokorný et al. 2013) suggesting that electromagnetic activity in biological systems plays a role both in the normal organization of biological processes as well as a pathway in cancer transformation. Discussion in the paper suggests paths for the future research that may lead to novel non-invasive electromagnetic diagnostic and therapeutic methods. J. Pokorný made a major contribution to this work as can be seen in his earlier publication profile. Two other co-authors from other institutions helped to write and proof read the paper.

The research of the electromagnetic properties of microtubules and the possibilities of influencing them via electromagnetic fields contributes to the development of new therapeutic approaches for tumour treatment. In the field of electrical engineering, our research of high-frequency electric fields generated by microtubules is important since it estimates limits of prospective artificial bio-electronic devices based or inspired by microtubule electrodynamic properties. A significant part of our research lies within the emerging field of high-frequency bio-electronics where only early preliminary experimental results have been published up to date. The main importance of this branch of science and technology resides in the potential of its future applications in the development of novel electronic components, sensors, information storage and processing units, materials for fuel cells, etc. Controlled self-organization of model supra-molecules we use is a property which may overcome the technological limitations of the current manufacturing process of nanoelectronic elements. Knowledge of the basic principles governing the electrical properties of the building blocks of these prospective components is vitally important for any future development in this area and it will have a direct economic impact.

Ultra-weak photon emission represents a new class of endogenous biosignals for which the detection is completely non-invasive, does not require an injection of contrast chemicals into the diagnosed tissues or cells or any external stimulation, e.g. irradiation. These biosignals can therefore be used for gentle medical diagnostics in the future. Ultra-weak photon emission is already being implemented in biological and biomedical areas with the need of non-invasive activity measurement of free radicals which play an important role in aging and cardiovascular as well as degenerative diseases.

c) Collaborations

The team has developed an intensive collaboration with the Faculty of Electrical Engineering, Czech Technical University in Prague. Department of Electromagnetic Field was a partner in the joint project (*Czech Science Foundation, project no. P102/11/0649 - 2011–2013 - Measurement and analysis of signals from nanostructures*). Further cooperation is being formed with the Department of Circuit Theory in the signal analysis research questions. The collaboration with the Czech Technical University in Prague has led to several joint publications and a synergy of knowledge exchange.

Intense collaboration of our team is also taking place with the Department of Biophysics, Palacký University Olomouc, in the form of a joint project (*Czech Science Foundation, project no. GA13-29294S 2013–2015 - Photonic Biosignals: Measurement and Characterization*). While the team from Olomouc provides qualitative insights into mechanisms underlying chemical generation of electron excited species during cellular metabolism, our team brings these insights onto a quantitative level exploiting our mathematical modelling background and engineering skills to precisely master and control measurement systems.

Department of Cytoskeleton Biology (lead by Pavel Dráber) at the Institute of Molecular Genetics, Czech Academy of Sciences, is an important collaborator as it provides know-how, insights and pure tubulin protein samples for the experimental part of our research. A similar role, but on the cellular level of our experimental work, exists a collaboration with Jiří Hašek, head of the Department of Cell Reproduction at the Institute of Microbiology, Czech Academy of Sciences.

The team intensively collaborates with Marco A. Deriu from the University of Applied Sciences and Arts of Southern Switzerland (SUPSI), Manno, Switzerland. Dr. Deriu contributes to our models of supramolecular electrodynamics by performing normal mode analysis of studied objects as he did in our common publication (Havelka et al. 2014b).

The Tian research group at the University of Chicago, James Franck Institute / Dept. of Chemistry, is a new and prestigious research partner of our team since it possesses a unique synthesis system for the fabrication of semiconductor nanowire interfaces and devices with application in bioelectronic sensors. Dr. Cifra has been a visiting researcher in Tian's research group since august 2014 and introduced projects which combine the expertise of both research teams.

SmartBrain Ltd, a venture company which seeks and funds innovative high-risk high-gain projects, is major business partner of our team, and has already financed three small exploratory projects and this collaboration is still ongoing.

In 2014 we adopted a game plan to develop new strategic partnerships with potential partners in Europe through BMBS COST Action BM1309 *European network for innovative uses of electromagnetic fields in biomedical applications*. Two members of our team act as members of the Management Committee in this action.

d) Impact

We believe that our research responds to the current call for non-invasive low-cost therapeutic and diagnostic methods in biotechnology and medicine. Broader commercial exploitation and application of these techniques is still in the early development phase and therefore economic and societal impact is expected on a longer time scale of about 5-10 years. However, we are aware of procedures in the commercialization process and we are continuously identifying outputs of our research which could have a broader economic and social impact. We are initiating applications to protect intellectual property, which can enable further commercialization of the techniques developed by our team. Great support is available from the Technology center of The Czech Academy of Sciences for technology transfer from and to the Czech Republic. Potential investors for commercialization using a spin-off company as a tool would be either SmartBrain, Ltd. who we cooperate with, or the patents will be directly sold to larger pharmaceutical/cosmetic product companies such as Johnson & Johnson (ultra-weak photon emission detection based methods for evaluation of skin care products) or to companies producing spectroscopy equipment for chemical analysis such as Agilent (microvolume dielectric spectroscopy techniques of biomolecules).

e) Educational activities

Participation in higher education at domestic universities through supervision of the students is the most important educational activity of the team members. During 2010-2014, team members supervised and co-supervised several successfully defended doctoral, bachelor and master thesis: František Jelínek (1 master thesis), Michal Cifra (1 doctoral student – defended, 3 doctoral students ongoing, 8 master theses, 3 bachelor theses), Jiří Pokorný (1 doctoral student, defended, 1 master thesis) and Ondřej Kučera (1 bachelor thesis). A number of these theses were awarded several prizes for outstanding thesis, two of them even won the Werner von Siemens Excellence Award, which is granted annually by the Siemens Corporation in The Czech Republic. The research team regularly accepts educational visits organized for students from the Czech Technical University in Prague and students continuously seek thesis topics from a selection offered by the research team.

f) Public outreach

Members of the team have also devoted their time to activities aimed at increasing public awareness and understanding of their research.

Within the edition *The science around us* published by the Czech Academy of Sciences, we published the work *Electromagnetic fields of living cells* (2014)*,* a pop-science booklet written by Ondřej Kučera describing our research to the general public. This booklet is freely available online and is also being disseminated in a printed version during public and educational events which team members organize or participate in.

Members of the team presented our research in several pop-science and general public events, including talks within *TEDx* conference (2012) or the *Week of Science and Technology* (2014), the largest pop-science festival in The Czech Republic. We also presented our research at an invited talk during the *AMPER* electronics trade show (2014), the largest (more than 40 000 visitors) and the most significant trade fair of Electrotechnics, Electronics, Automation, Communication, Lighting and Security Technologies in the region.

g) Services to community

Team members serve the scientific community as a regular reviewers of manuscripts and reviewers of national and international project proposals (Technology Agency of the Czech Republic, European Commission - FP7 program Future and Emerging Technologies - project review panel committee member). Team members are also members of IEEE, SPIE, OSA, Material Research Society, Biophysical Society, Protein society and International Union of Radio Science (URSI). M. Cifra is also an elected member of the Czech National Committee of URSI.

h) Resources

The team has built completely new laboratory facilities during the evaluation period. These include biological/biochemical lab, laboratory of high-frequency and microwave electronics. Additionally, a dark room has been added to accommodate unique inhouse built equipment used for the measurement of the ultra-weak photon emission from biological samples. Three table-top dark chambers with sensitive photomultiplier detectors, temperature stabilization and in- house built detection electronics were created in the evaluation period. The team members also use equipment shared within the institute, especially electron lithograph, atomic force microscope and various spectroscopic facilities to complement the team's laboratory instrumental capabilities. In the evaluation period, our research ream received external funding for research from public and private sources totalling to an amount of 426 kEUR.

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Research Report of the team in the period 2010–2014

Institute	Institute of Photonics and Electronics of the CAS, v. v. i.

Scientific team	Synthesis and Characterization of Nanomaterials

a) Research team

In the evaluated period the Synthesis and Characterization of Nanomaterials Research Team went through a major transformation both in terms of scientific topics and in terms of personal composition and instrumental equipment. This transformation was based on the recommendations of the final evaluation report from 2011, in which the previous period 2005-2009 was evaluated. The major identified issues included an uneven age structure, where only a limited number of scientific workers had a long-term perspective, and a highly fragmented research focus.

In 2010-2011 the section of Materials comprised two teams focusing on the technology and diagnostics of materials for electronics and optoelectronics. These two teams were unified in 2012 and formed grounds for the establishment of the team Synthesis and Characterization of Nanomaterials in 2013. The transformation of the research structure has led to a substantial reduction in the size of the team mostly by retirement and most importantly, streamlining the research by focusing on three major topics.

b) Description of research and main achievements

The following topics were studied by the team in the evaluation period:

(1) Schottky barriers on compound semiconductors and their application to hydrogen sensors.

(2) Optical properties of semiconductors and special glasses.

(3) Nanodiagnostics of semiconductor and photonic materials using scanning ion and electron beams.
- Interaction of ions with solid surfaces, including focused ion beams and secondary ion mass spectroscopy (SIMS).
- Ballistic electron emission spectroscopy and microscopy (BEEM/BEES).

(4) Preparation of porous III-V semiconductors and their application in epitaxial growth (phased out in 2013).

(5) Thermoelectrics (phased out in 2012).

(6) Rare-earth elements in III-V semiconductors prepared by liquid phase epitaxy and their application in radiation detectors (phased out in 2011).

The main achievements in the listed research directions follow.

Schottky contacts are one of the key structures in semiconductor devices. We developed a novel method for the preparation of high-quality Schottky contacts on different semiconductor materials by the deposition of colloidal graphite at room temperature and described the charge transport mechanisms [1-3]. The Schottky diodes showed a high rectification ratio, a large Schottky barrier height, and were stable at elevated temperatures [2]. The graphite contacts have great potential to replace conventional contacts prepared by metal evaporation. We further demonstrated that when the graphite/semiconductor interface is decorated with catalytic metal nanoparticles, extremely sensitive hydrogen sensors can be fabricated [4-6]. The preparation and characterization of Schottky contacts in semiconductor nanostructures has received much less attention than their bulk counterparts. We demonstrated for the first time that graphite creates a Schottky contact to upright standing arrays of ZnO nanorods and described the charge transport mechanism through the graphite/ZnO nanorod junction [7]. An excerpt from the article [3] was selected for the section Highlights in the European Journal/Condensed Matter in Europhysics News 45/2, page 9, (2014).

In collaboration with domestic and foreign laboratories we investigated optical and in particular luminescence properties of special glass materials doped with rare-earth (RE) ions. Chalcogenide and heavy metal oxide glasses are promising materials that are widely studied since they are transparent in the near- and mid-infrared spectral regions and enable drawing of optical fibers. These glasses are also characterized by the high quantum efficiency of the radiative transitions from the doped-in RE^{3+} ions. Low-temperature photoluminescence (PL) spectroscopy was used to systematically investigate radiative efficiencies of RE^{3+} ions in various glass matrices. Low-temperature measurements enabled the simultaneous observation of the luminescence of the host glass with superimposed narrow features due to *4f-4f* transitions in the doped-in RE^{3+} ions. Our laboratory has been the only one to observe these narrow absorption dips superimposed on the broad-band luminescence of the host glass, and to come up with a proper interpretation of these absorption dips as being due to *4f-4f* up-transitions within the doped-in RE^{3+} ions [8]. Low-temperature PL also enabled the determination of the Stark levels splitting of corresponding 4*f* manifolds by identifying the fine structure of relevant emission bands of RE^{3+} ions [9].

We have been studying the interaction of ions with solid surfaces since the late 70's and continued this research in the evaluation period. A fundamental result, which we value the most from that period, is the development of a phenomenological theoretical model [10, 11] describing ion-induced electron emission from solid surfaces at very low impact velocities of the projectiles on the order of ~0.01 or less Bohr velocity. The emission at those velocities was named the subthreshold kinetic electron emission – a phenomenon that has not yet been fully explained. The model provides an analytical formula for the total electron yield as a function of the surface work function, the impact velocity and the atomic number of the projectile and therefore can be directly verified by experiments [12]. The importance of this work consists in the observation that for slow ions, the electron yield depends asymmetrically on the atomic number of the projectile and on that of the target; strongly on the former, weakly on the latter.

One of the team’s achievements preceding the current evaluation period was the design and manufacture of an in-house BEEM/BEES instrument. In the evaluation period we significantly improved the long term stability of our BEEM/BEES system, which enabled us to map the density of states of self-assembled InAs quantum dots in the GaAs/AlGaAs matrix grown by metal-organic vapour phase epitaxy (MOVPE) in correlation to the shape of the quantum dot for the two lowest observed energy levels.

Moreover, a relation between the inhomogeneous stress distribution in non-symmetrical quantum dots and the lowest energy level splitting was found [13].

The preparation of high quality lattice mismatched epitaxial layers is among the most challenging tasks related to semiconductor technology. We culminated our long term effort in the preparation of porous semiconductors by showing that highly uniform porous GaAs substrates with low surface roughness are capable of reducing the density of misfit dislocations, thereby increasing the critical epilayer thickness in the InGaAs/GaAs system [14]. The lack of appropriate semi-insulating (SI) substrates for epitaxial growth of InAs layers has stimulated certain interest in the heteroepitaxy of InAs on SI GaAs substrates. One of the methods to overcome the large lattice mismatch between InAs and GaAs consists in the deposition of a low temperature InAs buffer layer. Based on a detailed experimental analysis, we established for the first time the optimum thickness of the low temperature buffer layer to grow high quality InAs [15].

c) Collaborations

The team has developed an intensive collaboration in the preparation and characterization of semiconductor structures with the MOVPE group of the Institute of Physics ASCR. In the evaluation period our collaboration was focused on:

- the characterization of HVPE grown GaN:Fe supplied by Kyma Technologies, USA within the project *Characterization of Low Defect Density Native Gallium Nitride Materials* funded by the Missile Defence Agency, USA. P. Gladkov discovered the method of non-destructive quantification of Fe doping levels in GaN using optical measurements and had a major contribution to the data interpretation [16, 17].
- the direct measurement of the quantum levels in self-assembled InAs quantum dots (QD) present in a GaAs/AlGaAs matrix grown by metal-organic vapour phase epitaxy. A significantly improved home-built BEEM/BEES system enabled us to map the density of states of QDs (in correlation with their shape) for the two lowest observed energy levels [13].
- the technology of lattice mismatch compensation in epitaxial growth by introducing porosity into the substrate [14] and by the deposition of a low temperature buffer layer [15] within the project *Lattice mismatch compensation in heteroepitaxy on micro and nanoporous A3B5 semiconductors and deposition of metals and semiconductors into micropores* of the Czech Science Foundation.

Another long-term collaboration in the field of preparation and characterization of special glasses was with Petr Kostka, head of the group of Special Glass Materials within the Laboratory of Inorganic Materials, University of Chemistry and Technology Prague and Institute of Rock Structure and Mechanics AS CR. This collaboration was supported by two joint projects in the evaluation period [8, 9].

The team had a broad range of international collaborations (only those with common journal articles are listed) in all principal research directions with:

- James H. Dickerson: assistant director of the Centre of Functional Nanomaterials, Brookhaven National Laboratory, USA. This collaboration was established under the international collaboration project *Electrophoretic deposition through a time-varying electric field for research into new physical properties of nanostructured materials* [6].

- Philomela Komninou: head of the Nanostructured Materials Microscopy Group, Dpt. Physics, University of Thessaloniki. This collaboration was established under the EC mobility project *III-V semiconductor heterostructures/nanostructures towards innovative electronic and photonic applications* and focuses on advanced characterization and modelling of semiconductor interfaces and nanoparticles by transmission electron microscopy [14, 15, 18].
- Viktor Brus: University of California, Santa Barbara, USA. This collaboration is on the modelling of transport properties in semiconductor materials [7, 18].
- Leonid A. Kosyachenko: Dpt. of Optics and Electronics, Chernivtsi National University, Ukraine. This collaboration aims at the description of charge transport in semiconductor structures [3].
- Andrey Lomov: Institute of Physics and Technology, Russian Academy of Sciences, Moscow. This collaboration focuses on the characterization of porous semiconductors by X-ray diffraction.
- Peter Williams: Dpt. of Chemistry and Biochemistry of the Arizona State University. This collaboration was established under the bilateral Czech – US project *Micro-Faraday array detector with high dynamic range for multicollector isotopic SIMS* and focuses on SIMS fundamentals [19] and instrumental development [20, 21].
- a number of laboratories in the optical characterization of special glasses: Marcel Poulain, Verres et Ceramiques of Université de Rennes, Zoya.G. Ivanova, Institute of Solid State Physics Bulgarian Academy of Sciences, Marian Kubliha, Slovak Technical University in Bratislava, Vladimir Labas, Catholic University of Ruzomberok, Mihail Iovu, head of laboratory of photoelectrical properties of semiconductors, Institute of Applied Physics, Academy of Sciences of Moldova.

d) Impact

The team has significantly contributed to the scientific and technological development of the Czech Republic by advancing fundamental knowledge in the field of electronic and photonic materials. Unique laboratories of low-temperature photoluminescence spectroscopy and ballistic electron emission spectroscopy have been highly competitive at an international level.

Schottky-based hydrogen sensors which employ the graphite/metal nanoparticle/semiconductor interface are highly sensitive and selective with short response times. Fast monitoring of minute hydrogen concentrations is essential for early-warning against the leaks of combustive hydrogen gas.

The research of the optical properties of infrared transmitting glasses represents one of the most important branches of today’s chemistry and physics of non-crystalline solids. The findings obtained have deepened our knowledge and broadened the basis of materials for applications in photonics. In the short term view, the infrared transmitting glasses will find applications in signal transmission and amplification, fibre lasers, or in medicine.

Besides the dominant application of GaN in high brightness light-emitting diodes and lasers for which Japanese scientists were awarded the Nobel Prize in Physics in 2014, GaN also has significant applications in the microwave power electronics. The apparatus developed for the express contactless determination and mapping of the Fe-concentration in GaN crystals pushes forward the technology of high electron mobility field effect transistors, which demand an electrical isolation between the transistor conductive channel deposited as a thin film and the lattice matched high thermal conductivity substrate. The semiinsulating state is achieved by Fe doping.

e) Resources

The team was active in competing for research funding; 19 research projects were pursued during the evaluation period, twelve of them had a principal investigator from the team, seven had a co-principal investigator from the team. The funding structure was as follows: The Czech Science Foundation (8), bilateral international projects (5), multilateral international projects COST with additional funding from the Ministry of Education CR (3), and The Academy of Sciences CR (3). The projects cover all the topics listed in the research description section. The total budget was 758 thousand euros. A full list of projects is available in part 3.1.

Three unique commercial as well as home-built instruments are installed in the laboratories of the team.

Low-temperature photoluminescence (PL) spectrometer designed and assembled by the team members enables sensitive and high resolution measurements in broad spectral (300-9000 nm) and temperature (3.5-300 K) ranges. It consists of the Sumitomo optical closed cycle helium optical cryostat coupled to the THR 1000 monochromator (Jobin-Yvon) with a spectral range of 300-1700 nm and to uniquely designed setup consisting of modified FTIR spectrometer Nicolet 5700 with spectral range 700-9000 nm. The spectrometer with its exceptionally broad spectral range allows us to investigate a wide range of phenomena and materials.

The team members designed and built ambient condition Ballistic Electron Emission Microscopy/Spectroscopy (BEEM/BEES) instrument based on the Lyding type Scanning Tunneling Microscope (STM). Additional temperature compensation to the microscope shielding and concentric emplacement of the measuring piezoelectric tubes improve the system time stability of lateral drift to 0.4 angstroms per hour. Moreover, the team members developed the on-fly software registration utility maintaining identical scanning area for more than one week measurement duration.

Since 2013 the team has had a new major facility available – multifunctional nanotechnological instrument, which combines the techniques of scanning electron and ion beam microscopy with electron and ion beam induced deposition and etching and time-of-flight mass spectrometry. This instrument allows us to observe, machine, and manipulate nanostructures; to prepare contacts to them; and to perform their in-situ electrical and chemical analysis.

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