Description of the main research directions investigated by the institute

Research activities at the Institute of Photonics and Electronics of the CAS are performed in **six research units**, which consist of **five research teams** and **one specialised laboratory**. The research teams are:

- **Optical Biosensors,**
- **Fiber Lasers and Non-Linear Optics**,
- **Synthesis and Characterization of Nanomaterials,**
- **Bioelectrodynamics**, and
- **Nano Optics.**

The research teams pursue fundamental and applied research in different areas of (bio)photonics, optoelectronics, and electronics. In addition to these research teams, there is one specialised laboratory:

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

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

The main research direction pursued by the research units of the Institute are described below. A detailed account of the activities of the research teams of the Institute can be found in Section 3.4. The Laboratory of the National Time and Frequency Standard, due to its focus on providing specialised 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 direction has been focused on optical biosensors based on surface plasmons and evanescent waves and their applications for the research of biomolecules and their interactions and for the detection of chemical and biological species. This is highly **multidisciplinary research,** which was pursued by a multidisciplinary team combining research efforts in a variety of areas. In the area of **photonic sensing platforms,** the Optical Biosensors team conducted theoretical and experimental research of photonic and plasmonic nanostructures and the development of optical platforms for optical sensors based on plasmonic sensing (including affinity biosensing and surface-enhanced Raman scattering) and evanescent wave sensing. Research activities in the field of **microfluidics** were focused on the theoretical and experimental investigation of mass transport in microfluidic systems, and on the development of strategies for improving the transport of target molecules to the active areas of (nano)biosensors. In the field of **functional coatings,** the team pursued the development of methods for the attachment of functional biomolecules to the planar surface of sensors and to metal nanoparticles. The team also pursued the development of **applications of optical biosensors** in medicine and food safety, especially with respect to research into biomolecular interactions related to diseases such as myelodysplastic syndromes and Alzheimer's disease, and the detection of foodborne pathogens.

The main output of the Optical Biosensors research programme during the evaluation period includes **43 publications and 2 patents**. The multidisciplinary nature of the research and its impact on multiple disciplines was evidenced by the wide range of

journals in which the results were published. The Optical Biosensors team published a large number of research papers in the field of analytical chemistry: 14 papers were published in journals which, according to Web of Science, belong in the first decile, such as *Biosensors and Bioelectronics, Sensors and Actuators B-Chemical*, *Annual Review of Analytical Chemistry, Analytical Chemistry,* and *Lab on a Chip.* Multiple articles have been published in journals in the field of optics and chemistry; these include 5 papers in first quartile optics journals, such as *ACS Photonics, Optics Express,* and *Optics Letters*, and 3 papers in first quartile chemistry journals, such as *Chemical Communications, Chemistry - A European Journal,* and *Frontiers in Chemistry.* Several articles have also been published in quality journals in applied physics (*Applied Physics Letters*) and electrical engineering (*Proceedings of the IEEE*). The publications created by the Optical Biosensors team received about **1,500 citations every year** (according to WOS, this also includes pre-evaluation publications), highlighting the impact of this research programme and its strong international visibility. In addition to research, the team also continued to develop plasmonic biosensor platforms, and during the period of evaluation, provided **6 new platforms** to universities and research institutes in the U.S., Germany and the Czech Republic.

Research Direction #2: Fiber Lasers and Non-Linear Optics

This research programme has mainly been focused on efficient active optical fibres, novel photonic components, high-power fibre lasers and their applications. In this multidisciplinary research area, the team has combined its expertise in material research, fibre drawing, laser physics and numerical modelling. **Efficient active optical fibres for fibre lasers** were addressed by **material research of rare-earth doped nanocomposites**, and theoretical and experimental research of optimum fibre shaping, twisting, and layout. In the field of **fibre lasers**, phase noise, stability, laser-line frequency self-sweeping, and mode-locking mechanisms were investigated. Theoretical studies of **photonic devices** were focused on sub-wavelength grating waveguide devices, such as narrow-band spectral filters made on silicon-on-insulator platforms and on novel plasmonic structures. The team also developed novel **fibre and diffractive optical components for fibre lasers**. The theoretical and experimental research involved pump-signal combiners with minor signal loss, polarisation sensitive diffraction gratings nanofabricated on fibre facets, and broadband metal-dielectric diffraction gratings.

During the evaluation period, the Fiber Lasers and Non-Linear Optics research programme has resulted in **58 publications in scientific journals, 1 book chapter, and 8 patents/utility models**. The majority of these publications were published in high-quality journals in the field of optics (*Optics Express*, *Optics Letters*, *IEEE Journal of Selected Topics in Quantum Electronics*, *Journal of Lightwave Technology*, *Photonics Journal*, *Laser Physics Letters*, and *Journal of Biophotonics*), physics (*Physical Review A*), analytical chemistry (*Sensors and Actuators B*), and material science (*Journal of Alloys and Compounds* and *Journal of the American Ceramic Society*). One of the collaborative projects with industry coordinated by the team has been awarded the Prize of Technology Agency of the Czech Republic.

Research Direction #3: Synthesis and Characterization of Nanomaterials

The research activities here have been directed towards three key areas: (1) the preparation and characterisation of nanomaterials and nanostructures and their applications, (2) charge transport in semiconductor heterojunctions, and (3) the optical characterisation of semiconductors, glasses, and ceramics. The research into semiconductor nanostructures concentrated on one-dimensional ZnO structures and covered several topics, including nucleation and growth, the investigation of electrical and optical properties, and applications in highly sensitive UV photodetectors and Schottky gas sensors outperforming conventional resistive sensors. The team demonstrated that by locally modifying the surface of semiconductor substrates using focused ion beams, the nucleation and growth of one-dimensional nanostructures can be precisely controlled, which opens up space for the position-controlled deposition of semiconductor nanostructures on lattice-mismatched substrates, even when the morphology of the substrate is nonuniform. The investigation of charge transport in semiconductor heterojunctions was focused on Schottky contacts on semiconductor substrates and on one-dimensional nanoscale heterojunctions. The team developed a method for the in-situ electrical characterisation of individual semiconductor nanostructures using a nanomanipulator in the scanning electron microscope. The unique in-situ measurements have shown great promise for the characterisation of the arrays of nanostructures, where individual nanostructures possess different electrical properties and help identify the phenomena that are responsible for these differences. The team has expanded collaborations with domestic and foreign laboratories in the field of the characterisation of optical properties of semiconductors, ceramics, and glasses, especially those that had been doped with rare-earth ions.

During the evaluation period, the research team has published **1 book chapter and 28 journal papers**, many of which appeared in high-quality journals in the field of nanotechnology (*ACS Applied Materials and Interfaces*), electronics (*Solid State Electronics*), materials science (*Journal of Alloys and Compounds* and *Journal of Non-Crystalline Solids*), and applied physics and chemistry (*Superlattices and Microstructures* and *Journal of Physics and Chemistry of Solids*).

Research Direction #4: Bioelectrodynamics

The Bioelectrodynamics research team, established in 2013, performs multidisciplinary research at the interface of electrical engineering, biophysics, bioelectronics, and physical chemistry. The activity of the team has been focused on the passive and active electromagnetic properties of biomaterials: from the level of single molecules to that of cells. The research activities of this programme covered both experimental and theoretical aspects of (1 - passive properties) the response of biomolecular systems and organisms to an external electromagnetic field, as well as (2 - active properties) an electromagnetic field endogenously generated by processes in biomolecular systems. In particular, the team explored novel approaches, based on **molecular computational modelling** and experimental microfabricated **electromagnetic chips**, to understand the dielectric and vibrational response of biomolecular systems in the microwave band. Furthermore, it was demonstrated that intense short nanosecond electric pulses can modulate the structure and function of biomatter at the nanoscale. In several works, the team explored statistical signal properties of **biological autoluminescence,** and showed that the detection of this light phenomenon can be leveraged for a real time monitoring tool of oxidative processes in biological systems both for biomedical and biotechnological applications.

This research programme has resulted in the publication of **28 papers in reputable journals,** such as *Advanced Materials*, *Sensor and Actuators B: Chemical*, *Physical Review E*, *Scientific Reports*, *Journal of Photochemistry* and *Photobiology B*. Both of these research branches, as well as the combination of the two, are unique on the international level: the results obtained by the team have provided new insights into the electromagnetic control of bionanomaterial self-assembly, potential cancer therapy methods, and novel label-free non-invasive diagnostic methods in medicine and biotechnology based on biological autoluminescence.

Research Direction #5: Nano Optics

The newly established Nano Optics research team has been focused on ultrasensitive and super-resolution microscopy with applications in biophysics and cell biology. In particular, the Nano Optics research team pursued optical imaging of weakly scattering species, including single proteins, and applied new developments to decipher the mechanics and function of complex biological systems with a resolution of one molecule at a time. The main research areas included: 1) ultra-fast optical imaging to capture the heterogeneity of fast protein dynamics and transient protein interactions; 2) 3D holography with nanoscopic resolution to understand the complex volume of biological systems; 3) structure-resolving super-resolution microscopy to capture conformational changes taking place within a diffraction-limited volume; and 4) single-photon ultrasensitive microscopy to reach beyond the noise limitation of coherent states of light.

During the evaluation period, the research programme has generated **3 publications** and **1 patent application,** and opened several new research directions defining its future activities. During the evaluation period, the research programme was awarded with the J. E. Purkyně Fellowship of the Czech Academy of Sciences.

Laboratory of the National Time and Frequency Standard

The specialised Laboratory of the National Time and Frequency Standard is entrusted with the management of the National Time and Frequency Standard. The research in the Laboratory has been aimed at time transfer, i.e. time scale comparisons at a very long distance using signals from new and/or modernised global or regional satellite navigation systems, such as GPS, GLONAS, Galileo, BeiDou, and IRNSS, with sub-nanosecond accuracy. In cooperation with the International Bureau of Weights and Measures (BIPM), the Laboratory prepared a new standard for exchanging results from the measurements against these signals and participated in the pilot analysis of time transfer using these signals for improving the calculation of Universal Coordinated Time (UTC). Concerning shorter distances, the Laboratory focused on two-way time transfer and time distribution via dedicated optical fibres and all-optical telecommunication networks.

Research activity and characterisation of the main scientific results

During the period of evaluation, the Optical Biosensors team pursued research in the following key areas: (1) photonic and plasmonic nanostructures and sensing platforms, (2) mass transport and microfluidic devices, (3) functional biomolecular coatings, and (4) biosensor-based investigation of biomolecular interactions and detection of chemical and biological species. The research approaches used in these areas and representative results are presented below. References to the selected results are provided at the end of this section (the names of authors from the Optical Biosensors team are highlighted in bold).

(1) The team's research into **photonic and plasmonic nanostructures and sensing platforms** focused on the sensing approaches based on plasmonic sensing (including affinity biosensing and surface-enhanced Raman scattering) and evanescent wave sensing and the photonic and plasmonic nanostructures and optical platforms used thereof.
In the area of biosensors based on surface plasmons, the team has pursued both the theoretical and experimental investigation of plasmonic phenomena on a variety of (nano)structures [1]. In order to perform electromagnetic simulations of diverse metal and metal-dielectric (nano)structures, a portfolio of electromagnetic simulation methods has been established; these methods included, in particular, the finite-difference time-domain method, boundary element method, rigorous coupled wave analysis (in collaboration with the Faculty of Nuclear Science and Physical Engineering), and generalised multi-particle Mie theory. The team has established a nanofabrication facility to prepare a variety of plasmonic (nano)structures by various methods using commercial systems (electron beam lithography, focused ion beam nanofabrication, and direct-write laser lithography) and in-house-developed set ups (multiple-beam interference lithography [2], hole-mask colloidal lithography, and nanoparticle colloidal synthesis). The characterisation of the fabricated plasmonic (nano)structures was performed using characterisation methods available in the team's laboratories, such as optical and electron scanning microscopy, atomic force microscopy, optical profilometry, and VIS-NIR optical spectroscopy. The developed (nano)structures (e.g. arrays of gold nanorods, nanodiscs, nanowires and microwires, gold-coated gratings with constant and linearly increasing period) were used in conjunction with in-house-developed optical platforms for plasmonic affinity biosensing and an (adopted) commercial platform for Raman scattering spectroscopy.

The team has demonstrated a new type of biosensor based on spectroscopy of electromagnetic modes guided by a monolayer of sparsely distributed plasmonic nanoparticles, and showed that performance of such a biosensor can exceed that of biosensors employing localised surface plasmons on metal nanoparticles or propagating surface plasmons on continuous metal films (traditionally referred to as surface plasmon resonance (SPR) biosensors) [3]. The team has also studied an outstanding issue in plasmonic biosensing – how electrostatic charges (that are contained in abundance in biomolecules used in plasmonic biosensors) affect the response of a plasmonic biosensor. Experimental studies revealed that a negative static charge in a biomolecular layer on the surface of an SPR sensor may significantly alter the SPR response. Two mechanisms responsible for such behaviour were identified: the formation of an electrical double layer (ionic mechanism), and changes in the electron density at the surface of a metal (electronic mechanism) [4]. Various

plasmonic nanostructures were also evaluated for SERS-based sensing [5], [6]. In collaboration with researchers at the University of Washington (USA), the team explored the possibility of creating SERS substrates exhibiting strong signal enhancement and long-range behaviour that is not available from common SERS substrates, and have demonstrated both theoretically and experimentally that a gold nanohole array can support modes exhibiting the long-range characteristics and can therefore be used in applications where the analyte is located farther from the SERS-active surface [5]. The team has also investigated the use of plasmonic nanoparticles for the measurement of distances at the nanoscale using the so-called plasmonic ruler. The team collaborated with researchers at the Nanyang Technological University (Singapore) and contributed their expertise in the modelling of plasmonic nanostructures and the interpretation of plasmonic signals to studies aiming to advance the understanding of behaviour of lipids on solid surfaces. In particular, these studies included measurements of the deformation of lipid vesicles on solid supports with respect to their potential fusion and formation of supported lipid bilayer [7] and the formation of lipid bilayers on different oxide surfaces [8]. In collaboration with researchers at the Stevens Institute of Technology (USA), the team explored the use of plasmonic nanostructures (gold nanorings - AuNR) for the plasmon-enhanced generation of singlet oxygen for photodynamic therapy. The team used the boundary element method to establish the relationship between the increased generation of singlet oxygen and the transition rates of the photosensitiser molecule (PS) modified by coupling with a nearby AuNR. The study has shown that, in order to achieve the significant singlet oxygen generation enhancement, the system needs to be irradiated at the plasmonic wavelength of AuNR; at the same time, PS coupling with AuNR plasmons at the PS emission wavelength should be avoided by using PS with a larger red shift of the emission wavelength [9].

The team has explored a variety of optical configurations and approaches to develop novel platforms with advanced features (throughput, cost-effectiveness) or improved performance characteristics (sensitivity, resolution, limit of detection). The team has a long tradition of developing precision sensor instruments. During the period of evaluation, multiple plasmonic sensor platforms were developed, ranging from compact cost-effective portable systems for field use, to high-performance laboratory instruments. Six table-top SPR systems were built and sold to three domestic research institutions and to research institutions in Germany and the U.S. Special attention was given to research of high-throughput platforms for highly multiplexed and cost-effective detection of biomolecular analytes based on plasmonic imaging [10]. The team has also collaborated with researchers at the National Institute of Optics of CNR (Italy) and contributed their experience in plasmonic biosensing to a joint study exploring the potential of a new SPR sensing approach based on an optical-heterodyne clock detector approach [11].

In the area of evanescent wave sensing, the team worked closely with researchers at the Stevens Institute of Technology (USA) and contributed in particular to electromagnetic simulations of metal-dielectric and dielectric cylindrical waveguides (the experimental part of the joint research was performed by the U.S. partner) [12], [13]. Silica nanoparticles (SNPs) were integrated with an optical fibre long-period grating (LPG) platform and with the process of layer-by-layer assembly to allow for the monitoring of the controlled drug release. The SNPs provided a high surface area for increased drug loading, as well as an increased overlap with the evanescent field of

fibre modes. Simulations performed by the Optical Biosensors team using the finite element method allowed for the exploration of the evanescent field of selected fibre modes in the presence of SNPs and their effect on the sensitivity of the LPG-based sensing platform [12]. Moreover, a new sensing concept based on silica optical fibres with anodised aluminium oxide (AAO) cladding with highly organised nanopore channels vertically aligned to the fibre has been explored. The Optical Biosensors team contributed numerical simulations based on the finite element method that provided a foundation for the theoretical exploration of the AAO-clad silica optical fibre as an evanescent wave sensing platform [13].

2) In the research of **mass transport and microfluidic devices**, the team focused on the investigation of mass transport effects in microfluidic systems of plasmonic biosensors, and on strategies to enhance the transport of target molecules to the active areas of plasmonic biosensors. The team has established both a theoretical and experimental infrastructure for the theoretical analysis, design and fabrication of microfluidic structures and devices. The portfolio of available theoretical methods includes various numerical and analytical tools for flow and mass transport simulations (random walk particle tracking, finite element method within COMSOL, and analytical approaches for the analysis of molecular kinetics). The fabrication of microfluidic structures was carried out using single and multilayer UV lithography with SU8 photoresist (2D mask lithography and 2D direct-write laser lithography). Microfluidic devices were typically produced using the laminar approach, in which functional microfluidic structures were combined with 3D machined manifolds with input and output fluidic ports and vinyl gaskets defining lateral flow patterns and providing a seal against the surface of an optical sensor.

The team has investigated the mass transport effects in microfluidic channels and explored the possibility of improving the mass transport by passive micromixers. In particular, the stirring of the fluid was analysed for staggered herringbone mixers by a model based on the finite element method. The structure geometry was optimised in order to maximise the efficiency of mass transfer of the targeted molecules towards the sensing surface. The enhancement of the sensor with stirred flow to unstirred flow was theoretically predicted [14] and experimentally demonstrated [15] for small biomolecular analytes (oligonucleotides), as well as large analytes (bacteria).

With advances in research of affinity biosensors based on plasmonic nanostructures, the team has been increasingly interested in the effect of mass transport on the detection characteristic of nanoplasmonic biosensors. The team investigated this issue for plasmonic nanostructures consisting of individual nanoparticles [16] and their random and periodic arrays [17], [18]. The team used the finite element method to study analyte transport to individual nanoparticles (of variable shapes) embedded in a fluidic channel, and established a simple analytical model estimating the rate of analyte transport to a single nanoparticle [16]. Subsequently, the team studied mass transport characteristics in sensors consisting of an array of metal nanoparticles. The analyte flux was obtained by a combination of random walk and finite element methods, and was used to establish an analytical model that describes the analyte flux for a wide variety of nanoparticle shapes and sizes, array sizes, nanoparticle packing densities, flow rates, analyte diffusivities, and kinetic conditions [17]. The theory was verified in model oligonucleotide detection experiments using arrays of metal microwires, nanorods and nanodiscs [18]. These advances enabled the team to propose a novel unified model of nanoplasmonic biosensors that, by considering both mass transport

and optical response aspects, allowed the interrelation of performance characteristics of a biosensor with the design parameters of a plasmonic nanostructure [19]. In particular, the packing density of the array of nanoparticles has been shown to play an important role. It has also been demonstrated that a sensor based on an array of nanoparticles with an optimised packing density is able to exceed the performance of conventional SPR biosensors (based on continuous metal film) by an order of magnitude [19].

(3) Research of **functional biomolecular coatings** was focused mainly on the development of methods for the attachment of functional biomolecules (in the form of a functional coating) to planar substrates (with a nanostructured or smooth surface) and nanoparticles. In the area of functional biomolecular coatings for planar substrates, the team focused on the investigation of advanced functional biomolecular coatings based on polymer brushes [20] with the goal of providing functional coatings exhibiting low fouling in biological media, enabling applications of label-free affinity biosensors for the analysis of highly complex samples in medical diagnostics and food safety. In particular, the team studied carboxybetaine (pCB) homopolymer and copolymer brushes that combine the ability to resist fouling from complex biological matrices with the high capacity for the immobilisation of biorecognition elements (BRE). The biosensing performance of these pCB brushes was compared to that of conventional functional coatings based on self-assembled monolayers of alkanethiols. Characterisation of the prepared functional coatings with regard to their biorecognition and fouling properties was performed by using the SPR method, while their physicochemical properties were characterised by means of spectral ellipsometry, polarisation modulation infrared reflection/absorption spectroscopy, X-ray photoelectron spectroscopy and atomic force microscopy.

In collaboration with researchers at the Institute of Macromolecular Chemistry of the CAS in Prague (IMC), the team investigated functional copolymer brushes composed of the non-ionic N-(2-hydroxypropyl) methacrylamide (HPMAA) and carboxy-functional zwitterionic carboxybetaine methacrylamide (CBMAA). The evaluation of the sensing properties of this type of coating with a variety of food matrices suggested that fouling resistance of such copolymer brushes can be tuned by changing the ratio of HPMAA and CBMAA monomers [21]. The two research groups also jointly pursued research of molecular mechanisms involved in the functionalisation of polymer brushes, and showed that the BRE immobilisation capacity and resistance of the coating to the fouling from blood plasma is strongly influenced by the method of deactivation of pCB functional groups [22]. The collaborative research into functional coatings has resulted into two national patents covering advanced functional coatings; international protection for one of these inventions is also being pursued. In collaboration with IMC, the team also tackled the topic of material-specific functionalisation strategies for selective functionalisation of different materials in metal-dielectric plasmonic nanostructures. Various polymer brushes, as well as orthogonal chemistries based on thiols and silanes were investigated to enable the attachment of biorecognition elements to metal (gold) areas and the formation of a non-fouling coating on dielectric areas (SiO_2, TiO_2). Although several approaches have shown potential, the search for an optimal solution to this problem is still ongoing.

In addition, the team has pursued the development of functionalisation strategies for gold nanoparticles (AuNPs) that are widely used to enhance the response of label-free optical affinity biosensors. The team has investigated the effect of preparation

conditions (e.g. the concentration of antibody or activation reagents, the composition of buffer and reaction time), and demonstrated their significant impact on the performance of antibody-functionalised AuNPs and the applicability of functional AuNPs to biomolecular detection in complex matrices. The AuNPs prepared under optimised conditions were employed for the detection of carcinoembryonic antigen (CEA), and showed to enhance the SPR biosensor response to CEA by a factor of 1,000 [23]. The team has also demonstrated that the zeta-potential could be used to aid the development of functional AuNPs with optimal characteristics [24].

The team also contributed their expertise in functional coatings to a collaborative project with researchers at the Faculty of Mathematics and Physics, Charles University in Prague, comparing the performance of surfaces with different physicochemical characteristics with respect to their ability to preconcentrate molecules during the evaporation and formation of the so-called “coffee-ring” in the drop coating deposition Raman (DCDR) spectroscopy [25], [26].

(4) The team has targeted various applications in which **optical biosensors** can: a) provide capabilities opening new research opportunities in **medicine,** and b) provide superior analytical performance in areas such as **food safety**.

Applications of innovative biosensor technologies for unravelling biomolecular interactions related to the onset and progression of diseases, such as myelodysplastic syndromes (MDS) and Alzheimer’s disease (AD), have been pursued in close collaboration with researchers specialised in different branches of medicine. In collaboration with the research team at the Institute of Hematology and Blood Transfusion, Prague, the team has pursued applications of SPR biosensors for the detection of biomarkers related to MDS, and biomolecular interactions related to the onset and progression of the disease. MDS are a heterogeneous group of haematological malignancies with a high risk of transformation to acute myeloid leukaemia. On the molecular level, MDS are associated with posttranslational modifications of proteins and variations in the protein expression levels. The two teams developed and demonstrated two novel approaches to MDS diagnostics based on the analysis of the patient’s blood plasma. These new methods are based on the analysis of interactions between proteins and blood plasma [27] and the detection of misfolded proteins in blood plasma [28]. The teams demonstrated that the developed methods enable discrimination among different MDS subtypes and healthy donors. The teams have also demonstrated SPR biosensor-based detection of multiple microRNAs related to MDS at physiological (sub-picomolar) levels in erythrocyte lysate [29]. The team also applied plasmonic biosensors to the investigation of biomolecular interactions related to AD (in collaboration with the National Institute of Mental Health, Klecany, Czech Republic) [30]. The joint study showed, for the first time, that two proteins related to pathogenesis of Alzheimer’s disease, 17beta-hydroxysteroide dehydrogenase 10 (17β-HSD10) and cyclophilin D (cypD), interact and form a stable complex. It was also demonstrated that the presence of amyloid-β (Aβ) affects the binding between 17β-HSD10 and cypD, and that different fragments of Aβ influence the binding through different mechanisms [30]. In addition, the Optical Biosensors team has developed a plasmonic biosensor for the detection of pregnancy-associated plasma protein A2 (PAPP-A2) [31]. In the follow-up joint study with the researches at the Institute of Medical Biochemistry and Laboratory Diagnostics of First Faculty of Medicine, Charles University in Prague, the developed biosensor was applied to the analysis of blood serum from haemodialysis (HD) patients [32]. It was discovered that

HD patients exhibit elevated levels of PAPP-A2 (in comparison with healthy individuals), and that increased levels of PAPP-A2 in combination with decreased levels of PAPP-A2 analogue (PAPP-A) correlate with increased mortality of HD patients [32].

The Optical Biosensors team has also pursued the development of plasmonic biosensors for the detection of foodborne pathogens [33], [34]. An SPR biosensor based on ultra-low fouling functionalised polymer coatings and a sandwich assay with functionalised gold nanoparticles was developed and evaluated for the detection of *Escherichia coli* and *Salmonella sp*. in crude food samples. It was demonstrated that the biosensor enables rapid and sensitive detection of *Escherichia coli* and *Salmonella sp*. with a limit of detection down to $<10^2$ CFU/mL and $<10^4$ CFU/mL, respectively [33].

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Research activity and characterisation of the main scientific results

Over the evaluation period, the research efforts of the Fiber Lasers and Nonlinear Optics team were mainly focused on the following key areas: (1) material science, (2) active fibres, (3) modelling and experimental research of fibre components and photonic devices, (4) fibre lasers, and (5) fibres and fibre devices for biophotonics. References to selected results are provided at the end of this section.

(1) The material research targeted the alternative synthesis of high-temperature-resistant rare-earth-doped ceramic nanoparticles with improved luminescence properties, and the exploitation of the achieved results in fibre optic technology. We elaborated a set of novel sol-gel methods allowing the preparation of nanocrystalline powders, and we studied the crystallisation kinetics of the investigated compounds to evaluate the mechanism of nucleation and crystal growth [1]. The crystallisation parameters were applied to prepare powders with a particle size below the scattering limits. The relationships between the structural and optical properties of the nanocrystals were evaluated, resulting in the preparation of highly transparent films and nanopowders with tailored optical properties [2].

A nanoparticle doping approach to the doping of optical fibres was established as a viable alternative to the widely used solution doping method, leading to active optical fibres with a more homogeneous distribution of rare-earths dopants in the fibre core and reduced background losses. Our extensive study of the effects of the chemical composition on the luminescence properties resulted in gaining comprehensive know-how of active optical fibres emitting in the “eye-safe” spectral range with improved luminescence efficiency that are necessary for the construction of advanced fibre lasers [3].

The scope of material research was expanded by the inclusion of new topics, such as magnetic [4] and radioluminescent [5] compounds for magneto-optical modulators and safety sensors for harsh environments. The intensive technological research of micropatterning [6] and laser-treatment [7] is shaping future applications of these materials in planar optical waveguides [8] and optical fibres for high-power energy transfers [9].

2) We invented and patented a new method for the efficient absorption of the optical pump in high-power double-clad fibres by their simultaneous coiling and twisting. We rigorously explained the principle of the invention by way of a theoretical model [10-12]. From fibres with enhanced pump absorption spectroscopy will profit pulsed fibre lasers and tandem thulium and holmium-doped fibre amplifiers, where the shortening of the fibre length plays an important role. Knowledge of managed pump absorption will also be used to uniform heat load distribution along the active fibre, without the need for water cooling, enabling applications with low power consumption within tightly limited space and weight. The optimised design will also minimise the risk of damage to the fibre during operation of the fibre laser.

Our research in optical fibre technology for energy-efficient fibre lasers has been appreciated worldwide. Our papers have been cited by researchers from world-famous laboratories, like Optoelectronics Research Centre in Southampton, UK, and others from the U.S., Europe and China. We were invited to participate in the prestigious project "Tactical Advanced Laser Optical System" (TALOS) financed by the European Defence Agency, and we will supply the fibres. The TALOS project will develop and

demonstrate some of the most critical Laser Directed Energy Weapon (LDEW) technologies, paving the way for the design and construction of an EU high-power laser effector that will be integrated into maritime, land and air platforms by the year 2027.

(3) Great effort has been devoted to the design, development and theoretical understanding of fibre components and photonic devices. We patented an innovative type of pump-signal combiner with a mode field adapter, which is useful for the pumping of the fibre lasers [13, 14]. Using the mode field adapter, we were able to decrease the insertion loss in signal branch below 0.2 dB for 6+1/1 type couplers. The patent is shared with the Czech company SQS Fiber Optics, a.s.

In the evaluation period, we developed a novel metal-dielectric grating with a high degree of diffraction efficiency over a broad spectral interval, applied for a patent that was issued in 2020, and demonstrated the application of the grating in a laser based on semiconductor optical amplifiers, which was tuneable over a wavelength range from 1,058 nm to 1,640 nm [15]. The grating requires no relief blazing to achieve high diffraction efficiency, and can be defined photolithographically. Large gratings are thus relatively easy to fabricate.

A new, fascinating and intriguing research field envisions a novel class of photonic devices integrated onto facets of optical fibres, allowing very compact and alignment-free architectures to be built. We studied subwavelength antireflection patterns, polarizers and wavelength selective filters nanofabricated at facets of optical fibres [16, 17] both theoretically and experimentally. The first experiments were performed in laser applications at moderate power levels of less than 20 W in order to demonstrate the potential of these devices.

Subwavelength integrated photonics is a highly innovative approach that significantly expands the applicability of CMOS-compatible silicon-on-insulator photonic technology, and helps to dramatically improve the technical parameters of many integrated optical devices. The subwavelength patterning of a silicon layer, attainable by contemporary nanolithographic techniques, makes it possible to tune the “effective” refractive index seen by the propagating optical wave to essentially any value between those of air and bulk silicon. The subwavelength grating (SWG) approach has been pioneered by the research group of Pavel Cheben from NRC in Canada in collaboration with the University of Malaga in Spain. We have recently developed a proprietary 3D numerical simulation tool, which appeared to be very suitable for modelling a particular class of SWG waveguide devices. This triggered the informal, but fruitful collaboration of our team with the teams at NRC and the University of Malaga in Spain. We jointly performed rather fundamental theoretical and experimental analysis of the effect of fabrication imperfections on the performance of SWG devices [18], and proposed a novel design of Bragg grating filters in SWG waveguides with minimum feature sizes large enough for fabrication with standard deep UV lithography [19]. SWG waveguide Bragg grating filters with bandwidths as narrow as 150 pm were designed, fabricated and successfully tested [20].

Research in the field of photonic devices was also complemented with theoretical research into novel non-conventional photonic and plasmonic structures with potential applications envisaged over longer terms. This includes, among others, research into the scattering properties of *PT*-symmetric dipoles, transverse Anderson localisation in plasmonic channel waveguides, and asymmetric propagation and nonreciprocal waveguiding of surface plasmons [21-25].

(4) In the field of fibre lasers, the team focused its research efforts on thulium- and holmium-doped fibre lasers with an operating wavelength in the range of 2,000-2,100 nm, which is two to three times longer than wavelengths of common ytterbium-doped YAG and fibre lasers or Ti:sapphire lasers.

We contributed to the discovery of the self-sweeping effect, a special type of mode-instability in fibre laser devices. We were the first to observe the effect in ytterbium-, erbium- and holmium-doped fibre lasers, we explained the self-sweeping effect in ring lasers, and we discovered the sweeping effect in the reverse direction. In the evaluation period, we focused on the study of the direction of sweeping, self-sweeping outside the 1 μm wavelength range, and in the investigation of dynamic gratings responsible for the laser wavelength self-sweeping [26-28]. We assessed for the first time the reflectance of the dynamic Bragg gratings created in fibre lasers with longitudinal mode instabilities, as is the case with self-swept fibre lasers [28].

From the point of view of fundamental laser physics, research of self-sweeping helps to understand fibre laser instabilities and to find ways to avoid them. For example, self-sweeping or longitudinal-mode instabilities are undesired effects in fibre lasers that are intended for continuous-wave operation. In the case of self-pulsing instability, like self-Q-switching, it is even more important, as the peak power may damage components of the laser device itself or measurement devices. Our results contribute to safe laser operation and prevent damage to expensive devices or components.

From the point of view of applications, the self-swept fibre lasers may find similar use as other swept sources. They are attractive for their relatively high power, simple design and inherently narrow linewidths. It makes these swept sources interesting for applications in interrogation of optical fibre sensor arrays, component testing and in laser spectroscopy. Another field of practical exploitation is in all-fibre self-Q-switched fibre lasers where understanding of triggering mechanisms allows improvement of their stability.

We investigated single-frequency fibre lasers and methods of wavelength conversion of single-frequency radiation into the mid-infrared spectral range with important applications in molecular absorption spectroscopy. We studied the phase noise of our fibre laser in a comparative study involving a commercial semiconductor laser, and proved the excellent noise properties of the fibre laser [29].

We collaborated with the Laser & Fiber Electronics Group, of Wroclaw University of Technology in the field of a mode-locked laser based on graphene saturable absorbers, where we contributed our experimental holmium-doped optical fibre [30]. It was the first demonstration of a fully fiberised Ho-doped mode-locked laser based on real saturable absorption, and as such this publication quickly received significant attention within the fibre laser community. With Cyprus University of Technology, Limassol, we conducted joint research of monolithic CW and subpicosecond pulse fibre lasers based on normal and tilted fibre Bragg gratings, where we contributed our expertise in fibre optics and measurement techniques and built the fibre lasers [31-32].

(5) In the field of fibres and fibre devices for biophotonics, we investigated special bioresorbable optical fibres. Bioresorbable optical fibres can be employed for long-term in-vivo sensing, photodynamic therapy or optogenetics, because they decompose in the body over time and thus they do not require explantation after usage. Phosphate-glass based bioresorbable optical fibres offer good optical properties and a tailorable resorbability rate on the basis of the composition of the material. For the first time,

bioresorbable optical fibre samples were administered under the skin of male rats, and their in-vivo resorbability and potential toxicologic risks were evaluated after given time intervals over a period of several weeks. This research was performed within the international collaboration with researchers from the University Hospital Hradec Kralove, Faculty of Health Studies, University of Chemistry and Technology, Prague, Politecnico di Torino, and Instituto di Fotonica e Nanotechnologie, Torino [33]. Fibres used in the study were drawn in our team.

We also developed for commercial use a tapered fibre optical sensor for a physiological pH range of 5.75-7.25. The tapered fibre probes are suitable for the measurement of pH in very small volumes in the order of microlitres [34]

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Research activity and characterisation of the main scientific results

In the evaluation period 2014-2019, the team pursued research in the following key areas:

(1) Preparation and characterisation of nanomaterials and nanostructures and their applications.
(2) Charge transport in semiconductor heterojunctions.
(3) Optical characterisation of semiconductors, glasses, and ceramics.

The research approaches used in the key areas and representative results are presented below. References to the selected results are provided at the end of this section, where the names of the team members are highlighted in bold.

(1) Research into the preparation, characterisation, and application of nanostructures was focused on one-dimensional semiconductor nanostructures, the fabrication of nanostructures using focused ion beams (FIB), and the electrophoretic deposition of metal nanoparticles. The research into **one-dimensional semiconductor nanostructures** concentrated on nanowires and nanorods of ZnO and encompassed several topics, including the preparation of high-quality seed layers to control their nucleation, the fabrication of p-n homojunctions, the investigation of their optical properties, and their application in UV photodetectors and gas sensors. Vertically-oriented ZnO nanorods with uniform physical properties require high-quality seed layers with a narrow size distribution of the crystallites, strong c-axis orientation, and low surface roughness and porosity. We showed that such seed layers can be prepared by the sol-gel method, and identified critical parameters that control the properties of the seed layers [1]. On these seed layers, we fabricated p-n homojunctions formed by the n-type ZnO nanorods, and p-type seed layer doped by a unique approach using thermal diffusion of dopants from the underlying silicon substrate [2]. By measuring temperature-dependent photoluminescence spectra of solution grown ZnO nanorods, we demonstrated that after annealing in nitrogen and in a vacuum, the defect density in the nanorods is significantly suppressed, and that the defect nature is altered [3]. Understanding the role of defects is key to controlling the electrical and optical properties of ZnO nanostructures. It is particularly relevant in solution grown nanostructures, where the formation energy of defects is low. We further designed a novel structure for Schottky-junction UV photodetectors, where the Schottky junction was formed between a graphite layer deposited from colloidal suspension and the ZnO nanorod array. The photodetector is highly sensitive across a broad range of UV illumination intensities, and enables operation in a self-powered regime without consuming external energy [4]. To fabricate highly-sensitive hydrogen sensors, the interface between the ZnO nanorods and graphite was decorated with Pd nanoparticles. The sensor outperforms resistive sensors in sensitivity, and unlike conventional metal-oxide sensors, can be operated at room temperature [5].

In the research of **nanostructures fabricated by FIB,** the team investigated the interaction of Ga+ FIB with solids. The team demonstrated that by locally modifying the surface of semiconductor substrates, the nucleation and growth of one-dimensional

nanostructures can be precisely controlled, which opens space for the position-controlled deposition of semiconductor nanostructures on lattice-mismatched substrates, even if the morphology of the substrate is nonuniform. This approach allowed us to prepare highly uniform hexagonal arrays of ZnO nanorods on GaN epitaxial templates, and to identify and control their growth mechanisms, which is a key step in controlling their morphology and physical properties. In collaboration with the Fibre Lasers and Nonlinear Optics research team, the FIB technique was employed for the fabrication of a sub-wavelength diffraction grating directly milled on a fiber facet [6]. In another collaboration with the ELI Beamlines research centre, using FIB, we fabricated nanostructured hollow targets to enhance the absorption of the laser pulse energy, while keeping the minimum target thickness. These targets enhance the acceleration of protons driven by high-power femtosecond laser pulses [7].

The team also pursued research of the **electrophoretic deposition of nanomaterials from nonpolar solvents** with the goal of depositing monolayers of metal nanoparticles. The preparation of nanoparticle monolayers is a subject of interest in many branches of research, with a variety of potential applications in photonics, electronics, medicine, catalysis, and sensing. In contrast with using more conventional polar solvents, the use of nonpolar solvents in the electrophoretic deposition: (a) limits the current between the electrodes, (b) allows for more homogeneous distribution of the composition and electrical conductivity of the suspension, and (c) suppresses electrochemical reactions at the electrodes. All of this translates into great control of electrophoretic deposition on a monolayer scale, and allows for the investigation of fundamental interactions between individual nanoparticles. We answered three key questions: how the nanoparticles acquire charge, by which mechanism individual nanoparticles are incorporated into a growing monolayer, and how to control their interactions and ordering. We also developed a mathematical model of nanoparticle charging in nonpolar solvents and an experimental approach to control the charge. We demonstrated for the first time the formation of Pt nanoparticle monolayers by electrophoretic deposition from nonpolar solvents [8]. The research was carried out in collaboration with the Centre for Functional Nanomaterials, Brookhaven National Laboratory, USA, where some of the experiments and characterisation by transmission electron microscopy were carried out.

(2) The investigation of **charge transport in semiconductor heterojunctions** was directed towards Schottky contacts on different semiconductor substrates and towards one-dimensional nanoscale heterojunctions. **Schottky contacts** are one of the key structures in semiconductor devices. We showed that conventional metal Schottky contacts to various semiconductors can be replaced with graphite deposited from colloidal solutions [9-11]. A thorough investigation of graphite Schottky contacts on ZnO by current-voltage, capacitance-voltage, and impedance spectroscopy measurements pointed to a significant difference in charge transport on different crystallographic orientations of ZnO. The difference in charge transport was assigned to variations in the defect nature in the near-surface region of ZnO [12]. Understanding charge transport on different crystallographic orientations is essential for the design of optoelectronic devices. In addition to investigating charge transport in bulk semiconductor heterojunctions and in ensembles of nanostructures [2], we developed

a method for the **in-situ electrical characterisation of individual semiconductor nanostructures** using a nanomanipulator in the scanning electron microscope. Using this unique method, we were able to identify charge transport mechanisms in ZnO/GaN nanorod heterojunction diodes grown on GaN substrates with a different local surface treatment. Moreover, we demonstrated that on the substrates locally modified by FIB, highly rectifying ZnO/GaN nanorod heterojunction diodes can be fabricated. In collaboration with the Institute of Physics, Polish Academy of Sciences, we also verified the in-situ electrical characterisation technique on AlGaN/GaN multiple quantum well structures, where we observed different electrical characteristics of the heterostructures with different polarities. The in-situ measurements show great promise in the characterisation of the arrays of nanostructures, where individual nanostructures possess different electrical properties and help identify the phenomena that are behind these differences.

(3) We further developed our established collaborations with domestic and foreign laboratories to characterise the **optical properties** of semiconductors, ceramics, and glasses doped with rare-earth (RE) ions. Low-temperature photoluminescence spectroscopy was employed to investigate radiative efficiencies of RE^{3+} ions in various glass matrices and/or the determination of the Stark level splitting of corresponding 4f manifolds by identifying the fine structure of relevant emission bands of RE^{3+} ions. We showed that the RE ions in different glass matrices are primarily excited via the electronic structure of the host glass, and provided direct experimental evidence of the energy transfer between the host glass and the REs dopants [13-15].

To enhance the application potential of Er^{3+} doped garnet structures in magneto-optical devices and in tuneable optical elements, it is essential to study the electronic state changes of paramagnetic Er^{3+} in the presence of an external magnetic field. Magnetically induced Zeeman splitting can influence the emission wavelength, as well as the lifetime, the latter through the remaining population in the excited states. In collaboration with our partners, we showed that the ab-initio calculation of energy levels of ground and excited multiplets of Er^{3+} and Yb^{3+} in aluminium garnet structures can be performed by combining a DFT assessment of crystal field parameters with local atomic Hamiltonian diagonalisation. The obtained energy levels and derived magnetic properties were compared with the experimental data from photoluminescence spectroscopy, magnetometry, and near-infrared spectroscopy [16].

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Research activity and characterisation of the main scientific results

The research activity followed the mission statement of the team: to develop enabling technology for, and collect scientific knowledge in, the analysis and influencing of biosystems using an electromagnetic (EM) field at the biomolecular level. To that end, we focused on ***passive* and *active* EM properties of biosystems** from the level of small biomolecule solutions up to cells and cell tissues.

We define **the *passive* EM properties** as those that describe the response of a system to an external EM field. In our research, we focus on the radiofrequency and microwave frequency bands, in which the degree of EM field interaction with biological materials vs. its penetration depth can be tuned across a wide range. Our interest is in the response of biosystems to the **electric component of the EM field**, because the response to the electric component is much stronger than to the magnetic component of the EM field. The fundamental quantity that determines the response of the matter to the electric field is the complex permittivity (referred to as permittivity in short in the remaining text), which is a frequency-dependent quantity often termed as a dielectric function. Therefore, **understanding the complex permittivity of biomaterials** is a crucial prerequisite for the development of the electromagnetic methods for novel, non-invasive and more effective methods for biomedical diagnostics and therapy. To that end, we developed **theoretical and computational tools** (i) to predict complex permittivity to rationalise the selection of appropriate frequency for microwave theranostics and biosensing, as well as (ii) to interpret the measured permittivity. Furthermore, we developed **experimental tools and devices** to measure the permittivity from microscopic volumes to save precious biosamples.

In (Havelka et al. 2018), we demonstrated the design, fabrication, and verification of a novel microwave chip for the dielectric sensing of biomolecules. The conceptual novelty was that, in contrast to most of the publications that have reported dielectric sensing, we showed how to rationally predict the spectral band of sensing interest based on the properties of a biomolecule. We then proposed a microwave sensing chip for the estimated frequency band, and evaluated its performance using both analytical modelling and numerical electromagnetic simulations. We fabricated the chip and experimentally demonstrated that we can extract the complex permittivity (0.5–40 GHz) of the water solution of alanine – one of the most common proteinogenic amino acids – without any calibration sample. From an engineering perspective, the novelty and advantage of our sensor was that it used a 20-fold lower volume than commercial techniques, while it required no microfluidics and could be fabricated with conventional printed circuit board techniques.

We strived to connect the molecular structure and dynamics of the biological samples to the macroscopically observable permittivity. To that end, we implemented classical molecular dynamics simulations, which employ full atom models of the biomolecules and water, to predict permittivity based on the correlation of dipole moments in our molecular systems. In (Havelka et al. 2018), we used this approach to demonstrate that the permittivity predicted from computer simulations agrees reasonably well with the experimental data. In contrast to experimentation, molecular dynamics simulations enabled us to dissect the contribution of any part of the molecular system to the permittivity. We found that it is not only the weighted contribution of the water and biomolecule fractions that determines the observed permittivity. It turned out that with

an increasing concentration of biomolecules, the interaction of the water and biomolecule phases (through cross-correlation functions of their dipole moments) starts to have a more significant contribution to the overall permittivity than previously expected. This finding is important to rationally develop any dielectric sensing method. The procedure that we described in this paper is rather universal and can be applied to the development of a dielectric sensing method of any polar biomolecule solution.

Molecular simulations employ various water models to fit various experimental observables, and it was not clear how various water models affect the computed permittivity. In (Cifra et al. 2019), we focused on the role of a molecular model of water in computational simulations, because it is the major compound that determines the microwave dielectric properties of biological tissue and wet samples. In that paper, for the first time, we analysed how the most common molecular water models (SPC/E, TIP3P, and TIP4P) affect the complex permittivity of biomolecular solutions predicted by molecular dynamics simulations. We found that the type of molecular water model used in the simulation affects not only the water contribution, but also the biomolecule contribution to the permittivity spectra. Our results contributed to an in-silico prediction and understanding of the dielectric properties of biomaterials, with a potential impact on the development of microwave diagnostic and therapeutic methods.

The high-frequency (optical) limit, which can also be expressed via the refractive index, plays an important role in the dielectric function of any material. The refractive index of a protein tubulin is an important parameter underlying the fundamental electromagnetic and biophysical properties of microtubules – protein fibres essential for several cell functions including cell division. Yet, the only experimental data available in the current literature includes values of the tubulin refractive index, which are much higher than what the established theories predict based on the weighted contribution of the polarizability of individual amino acids that constitute the protein. To resolve this controversy, in (Krivosudský et al. 2017), we reported the modelling and rigorous experimental analysis of the refractive index of a purified tubulin dimer. Our experimental data revealed that the refractive index of the tubulin is much closer to the values predicted by the established theories than those obtained by the earlier experimental data. The results are important for the mechanistic understanding of a range of electrostatic interactions as a contributor to the energetic balance in microtubule lattice stability, with a potential impact on fundamental biophysics, as well as computational drug design.

In general, the dielectric function of any material is composed of relaxation and resonance contributions. Although there is quite substantial knowledge on relaxation phenomena in biomaterials in the microwave band, little is known about potential resonant effects. One of the possibilities that can bring about a resonant effect is the coupling of microwaves to vibrations of nanoscopic structures via the dipole moment associated with vibrations. In aqueous environments, such as biological systems, viscous damping seems to be the limiting factor of the quality of resonances of electromechanical nanoresonators. In (Krivosudský and Cifra 2016), we elucidated the physical and chemical parameters that govern the viscous damping of nanoresonator vibrations and their coupling to electromagnetic radiation. We developed an analytical model for microwave absorption of a longitudinally oscillating and electrically polar rod-like nanoresonator embedded in a viscoelastic fluid. We showed that the slip length, which can be modified through surface modifications, controls the quality factor and coupling of nanoresonator vibration modes to microwave radiation. We demonstrated

that the larger the slip length, the sharper the frequency response of the nanoresonator vibration and electromagnetic absorption. Our findings contributed to the understanding of the behaviour of matter at the nanoscale with a potential impact on the fundamental biophysics and design guidelines of fluid-embedded nanoresonator devices and biosensors.

We showed in (Havelka et al. 2017) that microtubules (ubiquitous cellular self-assembled tubes with nanoscopic diameter) have their dominant vibration modes in the GHz frequency range. Our analysis connected the macroscopic deformations owing to microtubule vibration modes to local deformations on the molecular level. These results, beyond a theoretical understanding of the microwave spectra of the microtubule vibration mode, contributed to the elucidation of the mechanical properties of microtubules, with a potential impact on their biological function and for applications in artificial devices. However, the effective quality factor of microtubule vibrations remains elusive. In (Kučera et al. 2017), we critically analysed models of microtubule vibration dynamics, mainly focusing on limitations of particular modelling approaches and their relevance to observed and hypothesised biological phenomena. The results were important in assessing new research directions and experimental approaches that could resolve controversies about microtubule vibrations. Consequently, in collaboration (Barzanjeh et al. 2017), we formulated a detailed theoretical description of a new experimental optomechanical method for monitoring mechanical vibrations of a single microtubule. Our method opens up new possibilities for gaining information about the physical properties of microtubules, which will enhance our capability to design physical cancer treatment protocols as alternatives to chemotherapeutic drugs, which we have described in detail (Salari et al. 2018).

As a new research direction in our team in the 2015-2019 period, we also identified another perspective of how biosystems respond to an EM field, which goes beyond the analysis of complex permittivity and its relaxation and vibration components. This perspective focuses on the functional **response of the biosystems at the nanoscale** and molecular level to a very short pulsed (**nanosecond** scale), **intense** (> MV/m) **electric field** (in short: nsPEF). We carried out theoretical and experimental research within this topic. The theoretical work was focused on non-equilibrium (external electric field) molecular dynamics simulations of protein systems.

We selected protein systems that have a potential use in nanotechnology for nanoscale force generation and transport. The idea was, instead of creating devices such as nanomotors *de novo,* to employ biological nanomotors perfected by billions of years of natural evolution and control them to carry out required tasks. We focused on a kinesin protein, which is a biological molecular nanomotor that converts chemical energy into mechanical work. To fulfil various nanotechnological tasks in engineered environments, the function of biological molecular motors can be altered by artificial chemical modifications. The drawback to this approach is the necessity to design and create a new motor construct for every new task. To overcome this limitation, in (Průša and Cifra 2019), we tested whether an intense nanosecond-scale pulsed electric field could modify the function of nanomotors. Using molecular simulations, we demonstrated for the first time that an intense electric field can affect the structural properties of a kinesin nanomotor on a nanosecond time scale. We found that the contact surface area between the nanomotor and its track is affected. This effect might influence the processive motion of the nanomotor. Our results provide a mechanistic

physical foundation for a potential new method for parallel wireless control of the nanomotor systems for bionanotechnological applications.

Our further computational work (Marracino et al. 2019), carried out in collaboration, focused on the analysis of the nsPEF effect on a single protein tubulin, the building block of microtubules. First, we performed a bioinformatic analysis that resulted in a remarkable observation: the proteins from the tubulin family have exceptional electric properties, namely a few-fold higher net charge and dipole moment than the average across all other protein types (N = 63 000). This finding suggests that tubulin is a susceptible target to an electric field. Using molecular simulations, we then demonstrated multiple effects of an electric field on the tubulin dipole moment, shape, structure and drug binding sites. The results have an impact on new electromagnetic bionanotechnology and biomedical methods. Guided by this computational work, in our experimental work, we made the greatest breakthrough in the research of our team. We discovered that intense nanosecond electric pulses can modulate the self-assembly of protein tubulin into nanoscopic cytoskeleton structures (Chafai et al. 2019). We consider the impact of this discovery to be significant, because self-assembly and self-organisation of simpler blocks into more complex units are the design principles of living cells and organisms. Inspiration from these biological principles is also an important topic in nanotechnology, where it allows the creation of nanoscopic devices and nanorobots. To control the nanoscopic self-assembly, chemical changes in the building blocks are typically needed, which are often irreversible. In our findings (Chafai et al. 2019), we overcame this limitation, as we found that the effect on the tubulin structure self-assembly capability is irreversible or reversible, depending on electric pulse parameters. We provided evidence of the PEF effect on the tubulin using a variety of methods, including 2D-electrophoresis, zeta potential, dynamic light scattering, and autofluorescence. Furthermore, the impact of the electric-pulse-modified tubulin on its self-assembly (probed by turbidimetry) was not only in terms of kinetics, but also in terms of the type of the self-assembled structure (probed by atomic force microscopy). These results have an impact on new bionanomaterial development and can lead to new electromagnetic procedures in biomedical therapeutic methods, such as cancer treatment, because microtubules are also essential in correct/incorrect cell division.

A detailed knowledge of the passive electromagnetic properties of biosystems described above, particularly polarisation and vibrational processes at the molecular level, provides a rigorous foundation for research into **active electromagnetic properties** of biosystems. We summarised the challenges and opportunities of this research field in a book (Fels et al. 2015), which was co-edited and also contained several chapters by members of the team. This research area, which encompasses the generation of (above-a-thermal level of) the EM field by endogenous processes in biosystems, has great potential for discoveries, but is also known for its notorious experimental challenges and controversies. The major challenges in the field are related to mechanisms of EM field generation and in the instrumentation and methods for its detection and analysis. We contributed to a roadmap for how semiconductor devices could address these challenges in (Tian et al. 2018). The potential applications are obvious and paramount: the detection and analysis of endogenous EM signals would enable completely novel label-free and perturbance-free monitoring methods of underlying biological processes for biosensing and biomedical diagnostics. In the evaluation period, we contributed to this research field through several outputs. In

(Kučera et al. 2015), we summarised the current hypotheses, theories and experimental evidence concerning the electromagnetic activity of living cells. We systematically classified the bio-electromagnetic phenomena in terms of frequency, and we assessed their general acceptance in the scientific community. We showed that the electromagnetic activity of cells is well established in the low frequency range below 1 kHz and at optical wavelengths, whereas there is only limited evidence for bio-electromagnetic processes in the radio-frequency and millimetre-wave ranges. In (Kučera and Cifra 2016), we discussed perspectives in the research of nanoscale electromagnetic interactions between biosystems in the radiofrequency and microwave spectral range. Based on our analysis, the main perspectives are in (i) the micro- and nanoscale characterisation of both passive and active radiofrequency properties of biomacromolecules and cells, (ii) the experimental determination of viscous damping of biomacromolecule structural vibrations, and (iii) detailed analysis of the energetic circumstances of electromagnetic interactions between oscillating polar biomacromolecules.

The active electromagnetic properties on which the team primarily worked are a phenomenon of **biological autoluminescence** (BAL), i.e. luminescence that is excited by endogenous energy sources in biological samples. In contrast to classical bioluminescence, which often refers to organisms (such as fireflies or some jellyfish) with special enzymatic apparatus to generate light intense enough to be seen by the naked eye, BAL is ubiquitous for all organisms and only detectable by a sensitive light detector. BAL stems from electronic excited states generated chemically during oxidative metabolism and stress. Thus, BAL can potentially serve as a method for non-invasive biomedical diagnostics of oxidative processes and oxidative stress, which underlies many cardiovascular, neurological, and oncological diseases. Although the fundamental generating mechanisms of BAL are fairly well elucidated, together with their approximate ranges of intensities and spectra, the statistical properties of BAL are still a highly challenging topic. In (Cifra et al. 2015), we reviewed claims about nontrivial statistical properties of BAL, such as coherence and squeezed states of light. After the introduction to the necessary theory, we categorised the experimental works of all authors to those with solid, conventional interpretation and those with unconventional and even speculative interpretation. The conclusion of our review was twofold: while the phenomenon of BAL from biological systems can be considered experimentally well-established, no reliable evidence for the coherence or non-classicality of BAL has actually been achieved up to now. Furthermore, we proposed prospective avenues for the research of statistical properties of BAL. Along this line, we worked on various methods for BAL signal processing and analysis. In (Poplová et al. 2017), we addressed a common scenario, in which the intensity of the photonic signals is low, and a nonstationary trend of the signals needs to be removed for any further analysis, which creates an obstacle: because of the dependence between the mean and variance typical for a Poisson-like process, information about the trend remains in the variance even after the trend has been subtracted, possibly yielding artefactual results in further analyses. Commonly available detrending or normalising methods cannot cope with this issue. To alleviate this issue, we developed a suitable pre-processing method for signals that originate from a Poisson-like process. In our (Poplová et al. 2017) paper, a Poisson pre-processing method for nonstationary time series with Poisson distribution is developed and tested on computer-generated model data and experimental data of luminescence from human neutrophils and mung seeds. The presented method transforms a nonstationary Poisson signal into a stationary

signal with a Poisson distribution, while preserving the type of photocount distribution and phase-space structure of the signal. The importance of the suggested pre-processing method is shown in Fano factor and Hurst exponent analysis of both computer-generated model signals and experimental photonic signals. We demonstrated that our pre-processing method is superior to standard detrending-based methods whenever further signal analysis is sensitive to the variance of the signal. In (Rafieiolhosseini et al. 2016), we introduced negative binomial distribution, which gave a better and more rational fit for BAL photocount statistics than Poisson distribution or other distributions borrowed from quantum optics, which employed more fitting parameters (Cifra et al. 2015). In (Saeidfirozeh et al. 2018), we addressed the question of whether BAL from germinating seeds display different information content than just random computer-generated Poisson signals. We implemented an approximate entropy method for this purpose. Although the values of the entropy for the detector and the sample signal were different, when we applied appropriate corrections and computer-generated signals developed in (Poplová et al. 2017), we did not find any indications of the BAL having different properties from a random computer-generated Poisson signal. In (Dlask et al. 2019), we applied rigorous statistical methods and advanced reference signals to test the hypothesis as to whether the time series of BAL from germinating mung beans displays any intrinsic correlations. Utilising the fractional Brownian bridge that employed short samples of time series in the method kernel, we suggested that the detected autoluminescence signal from mung beans is not totally random, but it seems to involve a process with negative memory. Overall, our results (Cifra et al. 2015; Rafieiolhosseini et al. 2016; Poplová et al. 2017; Saeidfirozeh et al. 2018; Dlask et al. 2019) contribute to the development of a novel methodology of signal analysis not only of BAL, but also any photonic biosignals. The potential impact is that not only the light intensity and spectra (Nerudová et al. 2015), but also the statistical properties of photonic signals, could serve as parameters in novel biophotonics-based diagnostical methods in medicine and biology.

Apart from BAL signal processing and analysis, we also performed collaborative research to enhance the mechanistic understanding of processes underlying BAL. In (Rafieiolhosseini et al. 2016; Saeidfirozeh et al. 2018), we demonstrated that the growth of the two types of plant seeds is correlated with an increase in BAL intensity. We also showed that BAL intensity from seeds increases after the topical application of hydrogen peroxide in a dose-dependent manner (Saeidfirozeh et al. 2018). These results support the notion that BAL originates from plants during metabolism, particularly an oxidative one, which occurs at a high rate during growth, and strengthens the position of BAL as a potential method for the non-invasive label-free assessment of oxidative processes for agricultural and biotechnological applications.

While it is accepted that BAL is generated during oxidative metabolic reactions, the specific pathways involved in BAL remain poorly understood. To deepen the understanding of the underlying biochemical pathways of BAL, in (Burgos et al. 2016, 2017), we used HL-60 cells, a human promyelocytic cell line that is often used to study respiratory burst (part of the immune response), as a model system to measure BAL kinetics together with metabolic changes. In these works, the BAL intensity was correlated with broad profile metabolomics for the first time. We identified correlations of the BAL intensity with several key biochemical factors that modulate the function of an NADPH oxidase complex system, which is responsible for generating reactive

oxygen species leading to BAL. Overall, our results show that BAL can be used as a non-invasive and label-free readout for measuring oxidative stress metabolism and related processes in the biomedical context.

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(*Authors from the team in **bold.** Qualitative (and quantitative, if available and confirmed by all authors) contribution is specified at the respective reference.*)

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Research activity and characterisation of the main scientific results

The Nano Optics research team was formed in 2016 to pursue a new line of research at the institute. The team leader joined the institute following his postdoctoral position at the Max Planck Institute for the Science of Light (Erlangen, Germany), where he contributed in part to research that he had started before the transition. One of the major challenges in these research efforts was in demonstrating the detection of single unlabelled proteins as a valuable bioanalytical tool. Therefore, a single-cell secretion was explored as the minimal source of information-rich biomolecular material, and the research aimed to contribute important knowledge in the chain of immune response by analysing the direct reaction of human T cells and B cell lines to an external trigger (1). This study was conceived by the team leader and carried out at the Max Planck Institute for the Science of Light and the Clinic of Internal Medicine in Erlangen.
The scope of the research activities of the new team grew progressively over the years 2016-2019, together with the establishment of new laboratory resources and the acquisition of technologies, personnel and skills for the projected research programme. During the period of evaluation, the Nano Optics team pursued research on ultrasensitive microscopy, high-speed imaging of single-protein interactions, nanoscale quantitative phase imaging, and direct label-free monitoring of single protein dynamics. The methodical research was closely interlinked with applications in biology and biophysics.

The central technique acquired in the first years of the team's research was interferometric scattering microscopy with a single-protein sensitivity pioneered by the team leader (M. Piliarik, V. Sandoghdar, *Nature Communications* **5** (2014) 4495). In the course of the set-up phase of the new laboratory, we have advanced the technology of ultrasensitive microscopy, not only allowing the smallest objects imaged in an optical microscope to be detected, but also allowing them to be observed and tracked for prolonged periods of time (2). In this proof-of-concept work, we have demonstrated direct optical detection of gold nanoparticles as small as 2 nm in diameter using interferometric scattering microscopy. Additionally, the new detection concept allowed for continuous imaging of the nanoparticle position. In the imaging and localisation experiments, we have explored parameters identifying the potential of an extremely small scattering label having a size comparable to or smaller than the molecule of interest. Sub-protein-sized labels are expected to allow an experiment in which the physical properties of the system under study are not altered. We believe that the implementation of extremely small scattering labels may indeed open up new possibilities in the high-fidelity tracking of dynamical systems in biophysics and biochemistry.

To leap further into the understanding of the single-cell machinery, we made use of the new level of performance in ultrasensitive microscopy developed at UFE, and enabled it with high-speed data acquisition reaching up to a microsecond in temporal resolution. We used the technique in a study of the diffusive motion of single non-motor proteins on a surface of single microtubules to decipher the underlying mechanisms of the

interaction. While it is possible to understand much of the atomic structure and chemical interactions of biomolecular species from ensemble measurements, understanding the mechanics of molecular motion relies on single-molecular approaches, where the sensitivity and level of detail are severely limited. Within the family of microtubule-associated proteins, non-motor proteins, such as the microtubule crosslinker Ase1, are characterised by a diffusive motion along the microtubule driven by the thermal energy of the environment. Although it is hypothesised that the interaction allowing the free diffusion of Ase1 along the microtubule surface has electrostatic bases, a single step of Ase1 diffusion has never been resolved experimentally, and the exact molecular mechanism of the motion remains speculative. We achieved direct visualisation of the motion of Ase1 during its three-dimensional diffusion along microtubules with a spatial and temporal resolution of nanometres and microseconds, respectively. We unwrapped cylindrically confined trajectories and derived longitudinal and transverse diffusivities regulated dominantly by the interaction of Ase1 with single tubulin dimers. We discerned nanoscopic confinements in the diffusive trajectories having a mean dwell time on the order of 1 ms, and we associated them with individual equilibrium positions of Ase1 interacting with single tubulin dimers within the microtubule lattice. Finally, we reconstructed the periodic interaction potential of the diffusive motion resolving confinement peaks corresponding to a single Ase1-single tubulin interaction. In parallel to the development of these experimental studies, we collaborated with the Lansky-Braun group (Institute of Biotechnology of the CAS) to provide a control experiment supporting our findings where our collaboration was developing specific mutations of the diffusive microtubule cross-linkers influencing the protein-microtubule interaction. Despite this highly multidisciplinary work not having been finished before the end of the evaluation period, we collected all essential components of the experiments and novel means of data acquisition and processing which were previously never achieved at this level of detail. We are convinced that the results will lead to a major scientific publication in the near future.

In another research effort undertaken in 2019, we focused on developing a new strategy for quantitative phase imaging for three-dimensional wave-front reconstruction with a very high degree of sensitivity. The principle of interferometric scattering microscopy offers a unique insight into the variation of the scattering phase, which can be recalibrated into the height coordinate of the scattering origin. However, the signal fluctuation only offers a relative quantity and a very limited range of a few tens of nanometres. To reach towards fully-quantitative phase measurement comprising true holographic 3D-rich information, we sought inspiration in the quantitative phase imaging methodology. In order to bring the quantitative phase imaging to a level of performance compatible with imaging ultraweak scattering, we started with a novel approach to high-resolution and speckle-free spatial light modulation. Spatial light modulators have become an essential tool for advanced microscopy, enabling breakthroughs in 3D, phase, and super-resolution imaging. However, continuous spatial-light modulation without diffraction artifacts or polarisation dependence, and with the ability to capture sub-ms microscopic motion is challenging. We demonstrated a new concept of spatially-resolved optical phase modulation based on the photothermally driven thermo-optic effect, enabling the rapid and highly spatially

confined phase adjustment of a free-space optical beam. The new photothermal spatial light modulator (PT-SLM) maximises the phase gradient at the edges of the modulated areas, suppressing the effect of the thermal lens commonly associated with thermo-optics modulators. It is worth mentioning that iSCAT imaging is highly sensitive to any wavefront perturbation. To date, any wavefront shaping involving liquid crystal-based (LC) SLMs failed in integration with iSCAT due to insufficient phase stability and diffraction speckles. The laboratory prototype of the phase modulator featured phase stability in the mrad range with marginal distortion of the optical image of less than 0.5%, it worked in transmission, and it suffered from no grating or polarisation effect. In our proof-of-concept experiment, we achieved a phase switching time of as fast as 70 µs, which is faster than conventional LC-SLM devices by two orders of magnitude, and one order of magnitude faster than previously reported thermo-optic spatial-light modulation techniques. As the new technology of photothermal phase modulation holds broad potential in multiple fields of research, a patent application was filed (4), followed by a proposal for licensing, which is currently under negotiation.

We implemented the newly developed PT-SLM in new nanoscopic quantitative phase imaging to extract the full phase information of the light scattered on nanometre-sized objects. We demonstrated the sensitivity of the nanoscopic quantitative phase imaging to accurately characterise the scattering phase of single gold nanoparticles, and managed to obtain a 3D map of a microtubule network. We demonstrated the sensitivity of the nanoscopic QPI to accurately characterise the scattering phase of single gold nanoparticles with a precision of 10 mrad, corresponding to a vertical resolution of the particle localisation of 0.4 nm. This new level of performance pushed the limits of quantitative phase imaging deep into the sub-diffractional regime. Indeed, there are numerous other applications foreseen to exploit the potential of this PT-SLM technique, ranging from microscopy, biomedical imaging, and digital holography, to astronomy, that will be enabled and explored in follow-up studies. We supplemented the new developments in nanoscopic quantitative phase imaging with the 3D tracking of single proteins on a 3D surface of single microtubules, revealing the previously unseen character of the interaction. In these experiments, we offered an understanding of the diffusive behaviour of microtubule crosslinkers playing a crucial role during cell division. These additional experiments delayed publication beyond the evaluated period. However, the combined work acquired a significant degree of cross-disciplinary overlap, as well as biological impact, and is aimed for high-visibility publication in a leading multidisciplinary journal.

In parallel to the high-speed tracking and quantitative phase imaging research efforts, we focused on an ambitious and novel detection concept in optical microscopy, resolving the fluctuation of conformational changes of single proteins. To date, there exists no label-free method for the real-time detection of conformational changes within single proteins or small protein structures. Furthermore, it is not possible to observe the dynamics of single proteins at their characteristic time scales. We introduced a new method capable of detecting and tracking the conformational dynamics of proteins at a single-molecular level, at their native time scales, and without the use of any labels. Specifically, we were able to capture the choreography, rate, amplitude and spatiotemporal displacement of transient structural changes at the tip of disassembling

microtubules, and describe mechanisms enabling the switch from microtubule disassembly to microtubule assembly, termed rescue. We achieved an ultra-sensitive label-free optical microscopy method capable of capturing rapid changes in the nanoscale geometry of disassembling microtubule tips. This was performed by exploring the scattering anisotropy of tubulin oligomers at the tips of disassembling microtubules at sub-millisecond timescales. Our data provided quantitative information about the immediate cumulative length of microtubule protofilaments undergoing conformational changes. Our method allowed us to reconstruct a super-resolved image of the disassembling microtubule in a way similar to stochastic optical reconstruction microscopy (STORM), but without the need for any labelling. This imaging revealed the frayed shape of the disassembling microtubule tip that was previously suggested by EM imaging. We were able to discern the delay between protofilament conformational changes and tubulin detachment from the microtubule, and to observe long segments of protofilaments, closely matching the mean length of the dissociating tubulin oligomers, coiling momentarily from the microtubule shaft. Our work goes far beyond the proof-of-concept experiment, delivering substantially new knowledge in cell biology underpinning microtubule dynamic instability, and therefore required numerous tedious control experiments and collaborations to support the findings. This work was conceived in collaboration with the Lansky-Braun group at BIOCEV, and the Stefan Diez group at TU Dresden (Germany), with the lead role of the Nano Optics team covering the vast majority of experiments and all data processing and interpretation. This fruitful interaction yielded fundamentally new technology and strikingly new and topical results, but also resulted in a further delay in disseminating the results into the public domain.

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