

Final Report

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1 Summary

In the frame of this Virtual Institute we brought together expertise in X-ray imaging, biology, chemistry, and chemical engineering to enable in-situ imaging of biological and chemical processes with the X-ray microscopes at PETRA III and FLASH, and at the European XFEL in the future. In example applications, we addressed important questions in biology and chemistry, adapting and developing further the X-ray microscopes and special sample environments, such as chemical reactors, microfluidic cells, and cryogenic sample environments. In the following, the most important results and future prospects are summarized:

In **Topic I, Bioimaging**, new microfluidic devices were used to study cells. In freeze-dried samples, we were able to visualize network structures inside cells in real and reciprocal space, including local structure, orientation, and characteristic length scales. In hydrated samples, we showed that chemical fixation alters intracellular nanostructures. X-ray imaging offers, for the first time, a method that can directly compare living, unfixed, unstained samples with samples that had undergone considerable sample preparation. New ptychographic, X-ray fluorescence (XRF), and full-field imaging techniques yield quantitative phase contrast and chemical images of cells at comparably low dose and with about 50 nm spatial resolution. The ultrastructure of melanosomes with different genetic background was analyzed with in-situ small-angle X-ray scattering, and the element distribution of marine adhesives was characterized with high spatial resolution.

In **Topic II, Chemical Processes**, catalysts were analyzed under ex-situ and in-situ conditions, from micrometer to nanometer scale and by 2D and 3D imaging techniques.

Realistic exhaust gas catalysts could be investigated and aging effects could be followed for individual samples. Various catalysts were investigated under working conditions, monitoring the temperature distribution by IR thermography in combination, the chemical state by X-ray absorption spectroscopy (XAS), and the catalytic performance by mass spectrometry. Information about reaction mechanisms, sintering and ageing behavior, and structural stability of catalysts were investigated on multiple length scales. Additionally, the formation of solid materials was studied by following the production of colloids by redox reactions or the formation of solids by precipitation within microfluidic devices. To follow these reactions and the growth inside the reactor, small-angle X-ray scattering (SAXS) as well as spatially resolved XAS measurements were applied.

In **Topic III, Methods & Instrumentation**, two major activities were pursued, the development of X-ray microscopy techniques, in particular with coherent radiation, and the development of special sample environments and sample delivery systems. In X-ray microscopy, the four X-ray microscopes that are part of the VI were developed further, both instrumentally and methodologically.

The developments within the VI are pursued further in several common projects. The X-ray microscopes at PETRA III are available through the DESY user program. Experiments requiring the special sample environments developed within the VI can be conducted in collaboration with VI members.

2 Work and results report

2.1 Starting point

Dynamics in both biological and chemical systems can only be fully understood if the relevant length and time scales are resolved. Biology, chemistry, and chemical technology have often developed separately in the past. However, at the microscopic level, these disciplines converge methodologically. This VI was implemented to foster the synergies in development and application of imaging techniques that are applicable to both these fields. To this end, the following aims were identified:

Topic I, Bioimaging: To study functions and structural rearrangements associated with membranes, we aimed at in-situ imaging of biological membranes as well as in lipid model membranes. To this end, advanced sample environments such as microfluidic devices and microscopy-compatible mountings had to be designed. A central challenge in life sciences is the imaging of cells and organelles at high temporal and spatial resolution. We had proposed to advance X-ray imaging as a novel, complementary set of high-resolution methods to study (living) cells, also with elemental (XRF) and structural (SAXS) contrast. In order to be able to eventually visualize dynamic processes in the system, appropriate sample environments were needed, which are compatible with X-rays but also with in-situ cell culture. The X-ray analytical imaging techniques were

applied to melanosomes and to marine biofouling organisms.

Topic II, Chemical Processes: Studies on chemical reactions, the formation of solids and derivation of structure-performance relationships are of utmost importance for chemical product design, energy storage/conversion and catalytic applications. In all cases, the study from the nanoscale to the real system is important. For example, considering that 95 % of all chemical products have seen a catalyst in at least one reaction step, any improvement has a strong impact. Particularly, in-situ imaging of these chemical processes has great potential. The aim was to study them in several parts of the project and to understand the formation of solids or of colloidal particles as part of catalyst synthesis and to understand catalysts on a nano- and microscale under operating conditions. To optimize catalytic processes, it is critical to understand the underlying phenomena both during synthesis and operation.

Topic III, Methods and Instrumentation: The aim of the VI was to further develop X-ray microscopy techniques, based on the expertise and instrumentation available within the team. In order to take full advantage of the in-situ capabilities of X-ray microscopy, a strong focus was put on the development of special sample environments, such as microfluidic chambers both for biological and chemical studies. In addition, cryo-preparation and liquid jet techniques were to be developed. The goal was to create a platform to make these instruments available to biology and chemistry.

2.2 Progress of the work carried out:

The major objectives of the project are reached. Most of the milestones have been reached with a few exceptions. The shutdown of PETRA III in 2014/2015 led to some shifts in the schedule but could be mostly compensated by making experiments at other sources. The shutdown allowed for instrumental improvements, leading to improved experimental conditions starting 2015. The project was extended by half a year until March 2017 to account for several parental leaves. In the following, we will briefly summarise the course of the project.

Topic I, Bioimaging: Membranes (Mem1a,1b,2a,2b,3a,3b,4) Both biological and model membranes have been studied with nano-focused coherent imaging and diffraction, circumventing the conventional ensemble averages. Myelinated axons in nerve fibers and in tissue slices have been scanned in precise areas of interest, such as the node of Ranvier (Mem1a, Memb3a). Different environmental conditions have been used, including freeze dried, frozen hydrated and solution states (electro-physiology sample chamber, microfluidic sample chamber). The scanning SAXS data was complemented by full-field phase contrast tomography. Non-equilibrium effects in model membranes were studied by time-resolved diffraction [pump-probe and stroboscopic experiments (Mem1a,Mem2b)]. A CDI experiment on membrane fusion stalk has been carried out (Mem4), but was not yet successful. Isolated black lipid membranes in an electrophysiology setup have been successfully imaged at P10 (Mem2a), and the analysis of data on fusion of monolayers

in a microfluidic chamber was completed. Nanoscale diffraction experiments on synaptic vesicles in microfluidic chambers were successful (Mem3a).

Cells (Cell1,2,3,4,5) Starting with in-vitro experiments and fixed cells, imaging experiments were carried out on model organisms, also on live cells. Experiments on (initially) living cells of relevant cell lines were carried out, and a platform was established for conducting cell experiments including data analysis (also offered to beamline users). All milestones for cellular imaging were successfully accomplished; we are now able to routinely image cells by X-rays, and other users have started to also employ our methods, partly in cooperation with our group.

Melanosomes (Mel3) Frozen hydrated melanosomes were successfully imaged at P10.

Algae (Alg3,4) Investigation of frozen-hydrated marine biofouling organisms and cells: frozen-hydrated diatoms and bacterial cells were successfully imaged with ptychographic and zone plate imaging at BESSY. Chemical imaging of biofouling organisms was demonstrated using X-ray fluorescence at ESRF beamline ID16-NI.

Topic II, Chemical Processes: Heterogeneous Catalysis & Sintering (Cat1,3,5 & Sin2,4) We have demonstrated X-ray microtomography of exhaust gas monolith catalysts by a quasi in-situ acquisition method. In-situ studies were performed related to the partial oxidation of methane, with observation of oxidation state and thermal gradients within a larger catalyst bed, and tentatively on an individual particle level. Several experiments are planned or in progress to address the structure and gradients of catalytic processes in 3D (cf. section 2.4). X-ray ptychography was used to investigate metal nanoparticle sintering, which will be extended to examine bulk catalyst samples such as nanoporous gold. Sequential time-resolved imaging of catalyst thermal ageing has also been investigated, both studies making use of special in-situ cells for high resolution-ptychography developed during this project.

Redox and Precipitation Reactions (React2,4) A microfluidic setup is available for in-situ studies of early stage kinetics for nanoparticle synthesis, colloid synthesis, and precipitation reactions. First in-situ XAS experiments on Au colloid synthesis have been completed, and further studies on precipitation reactions are underway (cf. section 2.4). The setup is flexible and may be applied for a range of colloid synthesis and precipitation reactions, for which early stage kinetics and reaction data is still not widely investigated.

Topic III, Methods & Instrumentation: Microfluidics (Mfluid1,2,3,5) Microfluidic devices were developed and tested to tailor them to experimental parameters (flow rates, pressure, time resolution) and to chose optimal window materials for given experiments/beamlines. These devices were then made available for experiment at beamlines P06, P10, and P11. The devices are available in a microfluidic platform and can be applied (also by beamline users) to a variety of chemical and biological experiments. All milestones for microfluidics were successfully reached.

Chemical reactors (CatRe3,5) In-situ reactor for 2D study of catalytic reactions.

Two types of chemical microreactors were realized for catalysis (gas flow) and in-situ liquid cell for chemical reactions. These microreactors are well suited for selective catalytic reduction of NO_x or for CO oxidation. A first reactor for in-situ tomography was designed and constructed based on experiments cells from external partners. Full 3D measurements are currently pursued in a followup project (cf. 2.4).

Methods & Instrumentation (Soft1, Ins1, Mdiff2,3,5, Horst5) The full ptychographic reconstruction chain for high-resolution X-ray microscopy was implemented for P06, P10, and P11. Reconstructions are routinely done during the experiment. In-situ sample environments are routinely used at these beamlines. Two large pixel detectors (Eiger X 4M) were installed (with slight delay due to the shutdown) at beamlines P06 and P10 for wide-angle X-ray scattering and diffraction. A liquid jet was implemented into the soft X-ray microscope HORST, and this microscope is now available to users.

Theory (Theo1,2,3,5) Partially coherent nanobeams were modeled. This provided the basis for improved ptychographic reconstruction, taking mechanical instabilities into account. Several algorithms were developed that cope with different types of instability. Multimodal tomographic reconstruction schemes, including X-ray fluorescence, small-angle and wide-angle X-ray scattering, and ptychography were developed and successfully implemented and applied in user operation. All theory milestones were reached, with combined tomographic reconstruction of different contrasts being routinely carried out.

2.3 Description of the results:

In the following, the main research results are summarized. The research is published in peer reviewed journals.

Topic I, Bioimaging A central goal of VI-403 was to probe local distributions of biomolecular structure in complex environments of cells and tissues, and to develop and adapt experimental tools for this purpose, based on recent advances in X-ray optics and microscopy. For many biological samples, global average in real space leads to a signal, which is difficult to interpret and to model. In practice, this entails a severe restriction of diffraction methods that require homogeneity, which is often not given in biological matter. For the case of biological cells and tissues, for example, function relies on both a specific and well-defined molecular nano-structure as well as on cellular compartmentalisation or well-defined cyto-architecture, respectively. Ideally, a wide range of length scales has to be probed simultaneously.

To achieve these goals, we have adapted and used advanced coherent X-ray diffraction and imaging techniques, and developed suitable sample environments. With these tools, the consortium has been able to investigate a wide range of biological samples. Starting from biomolecular model systems (membranes, protein networks [1, 2, 3, 4, 5, 6] and gels), to entire organelles (melanosomes, synaptic vesicles, cellular nuclei [7]), eukaryotic cells (epithelial cells lines [8, 9, 10, 11], cardiomyocytes, stem cells, macrophages), as

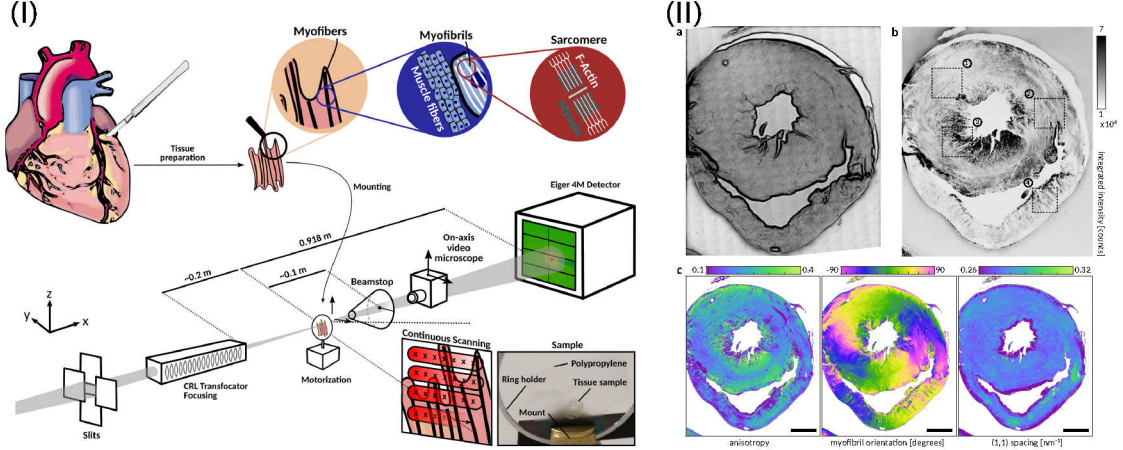


Figure 1: (I) Sketch of the scanning diffraction experiment on tissues. A tissue slice from an excised mouse heart, embedded in an agarose matrix, was mounted on a thin polypropylene foil and placed into the focus of the X-ray beam. Zoomed illustrations depict the highly regular arrangement of cardiac muscle fibres. Scattered radiation is detected using a 2D pixelated detector. The sample is swept through the focus of the beam and diffraction patterns are recorded at regular intervals during scanning. (II) Scanning SAXS dataset on tissue slices from mouse cardiac muscle. (a) Optical micrograph and (b) scanning diffraction image (darkfield contrast) of a mouse heart. Multiple scattering parameters are extracted in a fully automated manner, such as (b) integrated scattered intensity, (c) anisotropy of the scattering resulting from the $d^{(1,1)}$ reflection from the acto-myosin lattice, (d) the corresponding myofibril orientation, and (e) the extracted mean position of the reflection along q_r . More detailed views of the tissue within selected regions-of-interest (ROIs) have been acquired (data not shown). From Nicolas et al., unpublished manuscript

well as bacterial and yeast cells (*D. radiodurans*, *B. subtilis*, baker's yeast), we have now bridged length scales all the way towards 3D imaging of tissues, with sub-cellular (real-space) resolution, and molecular resolution in reciprocal space.

Live-cell imaging [9] has been a major emphasis, which has now been realized for several systems and contrast modes, including holographic and ptychographic imaging. New microfluidic devices have been developed and were used to study cells [8]. In freeze-dried samples, we were able to visualize network structures inside cells in real and reciprocal space, including local structure, orientation, and characteristic length scales [11]. In hydrated samples, we showed that chemical fixation alters intracellular nanostructures [12]. X-ray imaging offers, for the first time, a method that can directly compare living, unfixed, unstained samples with samples that had undergone considerable sample preparation [9]. Novel full-field holographic imaging techniques were shown to yield quantitative phase contrast images of cells at comparably low dose and with about 50 nm spatial resolution.

Since it is impossible to give a complete account, we restrict ourselves to an illustrative

example. The scientific question at hand is the physiological function of heart muscle contractility is supported by the structure of heart tissue, notably the 3D cyto-architecture. Classical diffraction studies on muscle (both for skeletal and cardiac muscle) have contributed to the understanding of the sarcomere as the basic structural unit of the myofibrils which make up muscle fibers. However, it is largely unknown how the molecular structure parameters vary within the tissue, in terms of local orientation, strain and variation in filament lattice spacing, and how they correlate to the textures visualized by histological microscopy. These questions can be answered based on scanning small-angle X-ray scattering (scanning SAXS) data of cardiac tissue, covering the entire cross section of a mouse heart slice, see Figure 1. To this end, different experimental advancements made by VI-403 have been combined: optimized focusing by compound refractive lenses (CRL) to micron spot size chosen to reduce radiation damage, low background sample mounts, continuous scanning, synchronized data acquisition by a fast single-photon counting pixel detector, and fully automated analysis scripts. The experimental workflow is sketched in Fig. 1(I). A surprising amount of structural data can be harvested from such scans, evaluating the local scattering intensity, interfilament spacing of the muscle tissue, the filament orientation, and the degree of anisotropy (J. D. Nicolas et al., unpublished manuscript).

A second example shall be given to illustrate the power of scanning experiments with nano-focused radiation, which has allowed us to precisely probe specific biological organelles, such as melanosomes. Melanosomes are membrane bound organelles hypothesized to be involved in the outbreak of the disease glaucoma which can lead to blindness. Nanoprobe X-ray fluorescence analysis revealed an enhanced Cu concentration in the periphery. Cu is a surrogate marker for eumelanin which surrounds a pheomelanin core. The data is the first experimental proof for the casing model suggested several times in the literature. Mice with a genetical disorder (D2) that supports the development of glaucoma show a higher granularity of the melanosomes in nanoprobe SAXS experiments. This granularity is hypothesized to be connected with a loss of integrity of the organelles and leakage of cytotoxic substances that cause clogging of the trabecular meshwork and the increased eye pressure [13, 14]. The combination of different X-ray imaging techniques (e. g., full-field methods and local, scanning techniques) has thus been successfully implemented for several highly relevant biological systems. For bioimaging are now able to routinely address systems and questions that we could only dream of ten years ago. Several national and international users groups have started to also employ the methods developed in VI-403.

In **Topic II, Chemical Processes**, in-situ sample environments for hard X-ray imaging of catalysts were developed. The study of catalysts under reaction conditions requires specially designed cells for gas flow and elevated temperatures. In this context, several reactors were developed. Firstly, a silicon gas phase microreactor allowing complementary spectroscopic analysis (e. g., XAS, XRD, Raman [15]) was used to study: (i) formation of thermal and chemical gradients in reactors, (e. g., during catalytic partial oxidation of methane); (ii) transient temperature regimes [136] typical of automotive catalysis. Such studies are difficult to perform in conventional cells, the microreactor therefore has good potential for future spatially resolved spectroscopy and microscopy

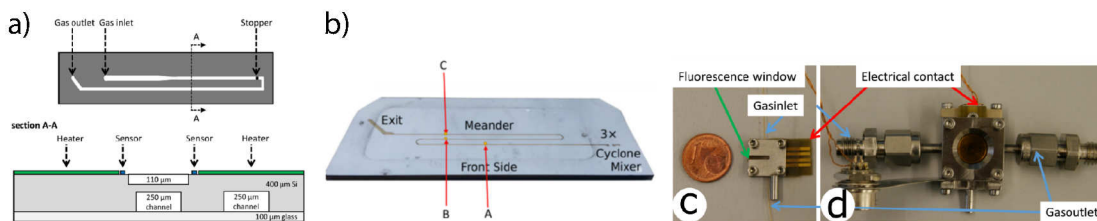


Figure 2: In-situ cells for X-ray microscopy/spectroscopy in catalysis. Si microreactor for (a) gas phase and (b) liquid phase studies. Cells for in-situ ptychography (c) new and (d) old version (DTU collaboration).

measurements [16, 17]. A second liquid flow microreactor was designed for studying [137] precipitation or redox reactions in turbulent flow regime, enabling kinetic analysis of material or nanoparticle synthesis (e. g., Au colloid formation by QEXAFS at PSI), allowing correlation of metal oxidation state and mixing time with μm spatial and ms time resolution. Further studies will include bimetallic alloys synthesis and precipitation reactions (e. g., CuZnO). Imaging of catalytic materials with resolutions below 20 nm is usually performed by TEM under high vacuum, however, hard X-ray microscopy enables in-situ studies at ambient pressures or above. X-ray ptychography is particularly suited for investigating hierarchically-structured materials (e. g., core@shell particles) or porosity/mass transport effects relevant to catalysis (between 20 nm and $1\mu\text{m}$). In-situ X-ray ptychography cells developed with the Technical University of Denmark (DTU) offer the possibility to characterize congruent sample areas both with X-ray and electron microscopy. The cell was used to study thermal coarsening of nanoporous gold based catalysts [18, 19] in different gas atmospheres, and the stability of a core@shell catalyst for DME synthesis [159][20]. While ptychography and electron microscopy require relatively thin samples, X-ray tomography (CT) offers non-destructive 3D imaging of catalysts with numerous acquisition modes (e. g., absorption contrast, XRD, XRF). This technique was used to monitor particle size and porosity changes in exhaust gas monoliths on μm scale following thermal ageing [21][138], which will be expanded to single catalyst grains and packed bed systems down to nanometer resolution. An in-situ cell for X-ray CT is currently under development, although it was used for quasi-in-situ experiments so far (e. g., selective oxidation of propylene). Inspiration for the cell was drawn from beamlines ID15A (ESRF) and I18 (Diamond) [160, 161], where a core@shell catalyst was studied in situ (CO oxidation to dimethyl ether) by XRD-CT and XRF-CT, with $2\mu\text{m}$ spatial resolution [22]. Tomography can reveal information, which is unavailable to conventional 2D imaging or bulk analysis, which is important for characterization of heterogeneous catalysts with complex or hierarchical structures.

In **Topic III, Methods & Instrumentation**, two major activities were pursued: the development of X-ray microscopy techniques [23, 24], in particular with coherent radiation [25, 26], and the development of special sample environments and sample delivery systems.

In X-ray microscopy, the four X-ray microscopes that are part of the VI were developed

further, both instrumentally and methodologically. The Ptychographical Nanoanalytical Microscope (PtyNaMi) at the nanoprobe station of P06 [162][139] was upgraded to improve the sensitivity and resolution in scanning coherent X-ray diffraction microscopy (ptychography) [27, 28, 29, 30], reaching unprecedented sensitivity and spatial resolution for the imaging of catalytic nanoparticles [31]. At the GINIX endstation at P10, the modular optical design was extended and characterized by ptychography, allowing the compound optical system to overcome the limitations concerning resolution and efficiency of the single components. In this way, it was possible to generate a world-record 5 nm focus [32]. In addition, a variety of near-field imaging techniques were developed that enable dose efficient and fast imaging [163][33]. At P11 a scanning transmission microscope and a Zernike phase contrast full-field microscope was commissioned and first experiments with biological samples were carried out [86, 43]. Both instruments are specially designed for stability, allowing two- and three-dimensional imaging of biological samples. Spatial resolutions of 50 nm have been demonstrated and 30 nm isotropic resolution is targeted.

A major instrumental focus was laid on the development of various sample environments. Microfluidic cells for various applications were designed [1, 34, 8, 3, 5, 6]: microfluidic devices for X-ray experiments, which are compatible with biological systems such as proteins, DNA or cells, have been developed and tested at different beamlines. In addition, first prototypes of microfluidic cells for gas and liquid phase chemical reactions were developed, fabricated, and tested. A cryogenic sample preparation chain was implemented at DESY and at Ruhr-Univ. of Bochum, keeping biological samples at all times at low temperatures [35]. A liquid-jet sample delivery system for the soft X-ray microscope HORST was implemented and used for experiments at FLASH. An important goal of the VI was the roll-out of in-situ techniques based on the sample environments and delivery systems developed within the VI: this is described in the next section.

2.4 Outlook on future work, sustainability:

The VI has established strong collaborative ties between the partners that continue beyond the formal funding period of the VI. Besides the continued research in in-situ nano-imaging of biological and chemical processes the results are disseminated to a broader user community by a very popular yearly satellite workshop to DESY's Photon Science Users' Meeting (cf. section 5). The knowledge obtained on special in-situ/operando techniques and sample environments developed within the VI are made available to external users within collaborations with the project partners. Users can obtain information and contact the experts through the VI website (<http://vi-nanoimaging.desy.de/>).

By appointing Christian Schroer as leading scientist for PETRA III at DESY and full professor for X-ray Nanoscience and X-ray Optics at the University of Hamburg, DESY has strongly extended its X-ray microscopy program in Hamburg and consolidates the scientific support of the user instruments at PETRA III. The group that moved in parts from TU Dresden to Hamburg continues to develop methods and instrumentation

for in-situ imaging with hard X-rays, building upon the results of this VI. The scanning microscope PtyNaMi at the nanoprobe station of beamline P06 is continuously developed further with the focus on X-ray scanning microscopy with highest spatial resolution. This is done in strong collaboration with partners of the VI, i. e., KIT (J.-D. Grunwaldt) and Ruhr-Universität Bochum (A. Rosenhahn). In this context, the group serves as local partner for both university groups and is involved in common followup projects.

The group at of J.-D. Grunwaldt continues the development of in-situ and operando imaging techniques for catalytic and other chemical reactions through a new BMBF project (2016-2019), which was initiated in collaboration between KIT and DESY (beamline P06, the X-ray Nanoscience and X-ray Optics group led by Christian Schroer and the X-ray Physics and Nanoscience group led by Andreas Stierle). The central aims include further development and application of cells for high-resolution X-ray imaging of catalysts under reaction conditions (in-situ / operando); linking analysis of real catalysts with model systems by preparation of size-selected metal clusters; and further optimising beamline P06 for future nanoimaging studies in catalysis. The project goes along with a long-term proposal (LTP) for beamtime at P06.

For the group of Axel Rosenhahn at Ruhr-Universität Bochum (RUB) the VI initiated a dedicated use of several beamlines with very good long-term perspective. Before the VI, the activities at DESY were focused on FLASH. Within the VI experiments at FLASH, P04, P06, P10, and P12 were performed. The involvement at PETRA III continues within a joint BMBF project in collaboration with P06, in which the cryo-environments developed in the VI will be implemented at the P06 microprobe experiment. The goal is to implement a highly efficient detector and a cryogenic platform to make full use of the high brilliance of P06. On-the fly XRF will be combined with ptychographic imaging schemes to provide high-resolution XRF and quantitative structural data in 2D and 3D.

The work on protein assembly will be carried further by characterizing the mechanical properties of the emerging filamentous structures within the ERC Consolidated grant MECHANICS (Sarah Köster, funding from 2017 to 2022). The goal of this project is to link cell mechanics via filament mechanics to the architecture of the filaments, which was one of the research foci of this VI.

The Salditt and Köster groups have recently successfully applied for BMBF funding (Project “Strukturanalyse in biologischen Zellen: Rasterkleinwinkelstreuung und Diffraktion mit nano-fokussierter Undulatorstrahlung”, 2016-2019) to realize the integration of super-resolution (STED) microscopy in the GINIX setup at beamline P10 at PETRA III. First experiments on the newly installed setup in May 2017 were already very successful. The project leaders have furthermore acquired a long-term proposal (LTP) for the next two years to perform experiments using the new instrumentation.

In 2014 **Suna Precision GmbH** (<http://www.suna-precision.com>) was founded by one of the members of the VI (A. Meents) as a spin-off company of DESY. Suna Precision commercializes high-precision mechanical systems for nano-positioning, automation, and

synchrotron radiation instrumentation.

2.5 Potential for application

A large part of the VI was the development of X-ray microscopy techniques for imaging biological and chemical systems and processes under in-situ / operando conditions. These techniques were implemented at the four X-ray microscopes

- PtyNaMi at the nanoprobe station of beamline P06 at PETRA III,
- GINIX at the coherence beamline P10,
- the tender X-ray microscope at beamline P11,
- the soft X-ray microscope HORST that can be installed at various X-ray sources, such as beamline P04 at PETRA III, FLASH, or at BESSY II.

The first two microscopes have become an integral part of the user program at these beamlines with about one third of the total beamtime allocated to these instruments. They are also open to industrial use through the Innovation and Technology Transfer group at DESY. They have thus found broad application. The two other microscopes are not directly part of the user program and can be accessed by collaborations with beamline staff at P11 and with the group of A. Rosenhahn, respectively.

In the framework of this VI we have developed together X-ray compatible microfluidic devices that are now available to the scientific community and applicable to a large variety of problems in biology, biochemistry, and chemistry. Due to the specific fabrication methods we employed (including photolithography as a geometry-defining step), the channel designs can be adapted to virtually any scientific question. We have put large efforts in rendering the fabrication methods reliable and adaptable and also straight forward. Therefore, other users can quickly learn and use the steps involved.

As a results of this VI, nano-diffraction on cells is now well established as a method, complementary to fluorescence microscopy and electron microscopy, which are already widely used by biologists and biophysicists. From a niche application for a small number of specialists, this method has now developed in an almost routine technique at dedicated beamlines, as can be seen from the increasing number of proposals submitted in the field. Apart from the actual data acquisition, we have put effort into developing analysis tools, which are available for other users as well.

X-ray microscopy has excellent potential within the field of catalysis, and is very much in the early stages of development. In particular, the advantages offered by in-situ imaging methods with X-rays are unparalleled for delivering insights into catalysts at work. From hierarchically-structured catalysts, to nanoparticle or colloid synthesis, studies under transient temperature conditions such as exhaust gas catalysis, to processes with elevated pressure such as Fischer-Tropsch or DME synthesis, the infrastructure developed during the project has the potential to address a wide range of complex structural

and mechanistic problems in catalysis. Testing, validation and several groundbreaking studies have already been completed using the in-situ cells developed, including the lithographically-etched silicon microreactor, in-situ cells for high-resolution ptychography, microfluidic synthesis of colloids and cells for 3D or tomographic imaging. The stage is now set to apply in-situ X-ray microscopy for more complex catalyst systems, in order to deliver structural, performance and mechanistic information. These studies will be achieved through follow-up projects including a new BMBF project, an accepted Long-Term Proposal at DESY P06, and other planned DFG funding projects.

Screening of antimicrobial drugs by SAXS has the potential to be applied in drug screening. Currently many companies stop their effort in developing new drugs. As step in between the conventional MIC tests and the time and money consuming tests against multi-resistant strains it could contribute to an enhanced drug discovery success rate. Key technologies for such efforts are high-brilliance beamlines such as P12 at PETRA III and a fully automatized autosampler environment for large q -range SAXS experiments.

The new cryogenic in-situ sample environments developed and currently implemented at P06 will be a key tool for the analysis of biological specimen in the future.

3 Qualification of junior researchers:

Professorial appointments

- In 2012, PD Dr. Axel Rosenhahn was appointed as W2 professor for analytical chemistry at the Ruhr-University Bochum.
- In 2015, Hudson W. P. Carvalho (KIT) was appointed Asst. Prof. in Applied Spectroscopy for the Study of Nanomaterials in Agriculture and Environment at the University of São Paulo.

The following measures were taken to support young scientists:

- Dr. Thomas Sheppard was promoted to group leader in X-ray microscopy at ITCP/KIT through research activities on this project (07.2017).
- Dr. Amélie Rochet held a Humboldt research fellowship at ITCP/KIT for studies in X-ray microscopy (09.2013-08.2014) and was promoted to group leader during this period.
- PhD students were encouraged to present their work at international conferences to discuss their results with experts in the field.

PhD

20 PhD students were supported fully or in parts by the VI, 16 of which graduated during the period of the VI. Another 21 PhD students funded by other projects contributed to

the research of which 13 graduated within the period of the VI.

Additional qualifications for PhD students:

- Sina Baier visited DTU-CEN for a PhD exchange for a few weeks for in-situ cell design and testing in 2013. She also stayed at DTU-CEN for two months for complementary electron microscopy and preparation of samples used for ptychographic experiments (2015).
- Sina Baier participated in the “São Paulo School of Advanced Sciences on Recent Developments in Synchrotron Radiation (SyncLight) 2015” in Campinas, Brazil.
- Sina Baier, Amélie Rochet, and Georg Hofmann visited the TU Dresden group for training courses in ptychography and tomography.
- Georg Hofmann stayed several weeks at DTU-CEN for preparing and characterizing the nanoparticle samples for ptychography (2012).
- Ghazal Tofighi has attended several courses (Research skill development course (2015), presentation and communication skills course (2016), as well as summer and winter schools) of the “Helmholtz Research School Energy-Related Catalysis”.
- Susanne Klare, née Hönig, attended the HERCULES course for X-ray science in Grenoble, France, 2012.
- Felix Wittwer attended the RACIRI Summer School in Rügen, Germany, in 2015 and the HERCULES course for X-ray science in Grenoble, France, 2017.
- All PhD students performed research stays at several synchrotron radiation sources: SLS at PSI, PETRA III at DESY, ESRF, BESSY, and Diamond.

Bachelor, Master, Diploma

Within the VI the following university degrees were awarded:

- 2 diplomas in chemistry at KIT
- 7 diplomas in physics at TU Dresden
- 12 master degrees in physics at University of Göttingen, TU Dresden, and University of Hamburg
- 15 bachelor degrees in physics at University of Göttingen, TU Dresden and University of Krakow, Poland

4 Publications

Scientific Publications

- [1] M. E. Brennich, J.-F. Nolting, C. Dammann, B. Nöding, S. Bauch, H. Herrmann, T. Pfohl, and S. Köster, *Lab on a Chip* **11**, 708 (2011).
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- [4] C. Y. J. Hémonnot, M. Mauermann, H. Herrmann, and S. Köster, *Biomacromolecules* **16**, 3313 (2015).
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5 Public relations:

5.1 Press releases

We have launched the following press releases concerning highlight publications:

- Press release DESY about publication [26]
http://www.desy.de/news/news_search/index_eng.html?openDirectAnchor=1191&two_columns=0
- Press release DESY about publication [61]
http://www.desy.de/news/news_search/index_eng.html?openDirectAnchor=1103&two_columns=0
- press release about publication [9], Univ. Göttingen, DESY. This release received broad attention (e. g., highlight in Nature Materials).
- press release (DESY, Uni Göttingen) about publication [32]
http://www.desy.de/infos__services/presse/pressemeldungen/@@news-view?id=6381&lang=ger
 highlighted also in many newspapers and physics information services, e. g.:
http://www.pro-physik.de/details/news/5307151/Drahtscheibe_fokussiert_Roentgenstrahl.html
- press release (DESY) about publication [27]
http://www.desy.de/infos__services/presse/pressemeldungen/@@news-view?id=3421&lang=ger
- Press release at KIT about start of the project
<http://www.kit.edu/kat/8049.php>

5.2 Lectures at research schools and public presentations

- C. Schroer, “Wie Dinge im Kleinen aussehen: Mikroskopie und Beugung mit Licht und Elektronen’,” lecture within the series “Physik am Samstag” that was held for high-school students at TU Dresden, Dec. 8, 2012
- C. Schroer, “Microscopy with Coherent X Rays,” Cheiron School 2014, SPring-8, Harima, Japan, Sept. 29, 2014
- S. Baier (AG Grunwaldt) gave a lecture for 16 high-school students on X-ray microscopy at the Chemie-Olympiade at KIT in Karlsruhe, Sept. 2014
- C. Schroer, “X-ray Microscopy with Coherent Radiation: Ptychography,” Debye Spring School, University of Utrecht, Apr. 10, 2014
- C. Schroer, “X-ray Microscopy,” Course on Modern Concepts and spectroscopic methods in materials science and catalysis, KIT, 18. Feb., 2014
- J.-D. Grunwaldt, “Fahrt in die Welt der Katalyse,” FChO-Workshop, Münster, Jan 3-6, 2014
- S. Köster, Course on Modeling Cellular Systems in Space and Time, Poquerolles, France
- C. G. Schroer, “X-ray Microscopy & Tomography: Sharp Views into the Nano Cosmos”, RACIRI School, Rügen, Germany, Aug. 25, 2015
- C. G. Schroer, “Spatially-Resolved Materials Characterization with Synchrotron Radiation”, EMU School, Vienna, Austria, Sept. 20, 2016
- S. Köster, “X-ray Microscopy”, Bad Honnef Summer School on “Advanced Microscopy – Physical Concepts and Impacts in Life Sciences”, June 2017

5.3 Conferences and Workshops:

Each year since 2012 a satellite workshop to the Photon-Science Users Meeting at DESY in Hamburg was organized by the VI-403 entitled “X-ray nano-imaging of biological and chemical systems at PETRA III”. It aimed at the Photon Science user community interested in nano imaging, giving an overview over the possibilities of nano imaging at the X-ray microscopes at PETRA III and the in-situ capabilities made available by the VI. Each year, the users of the instruments present their results. Over the years, the workshop was well attended with over 80 participants per year since 2014. This workshop is continued beyond the VI and organized by the DESY group ‘X-ray Nanoscience and X-ray Optics’ (AG Schroer).

In 2016, the international X-ray absorption conference XAFS16 was organized in Karlsruhe with Jan-Dierk Grunwaldt as chair person [165]. A special session was dedicated to spatially resolved XAS and X-ray microscopy and organized by Gerald Falkenberg,

Anna Zimina, and Christian G. Schroer with Koen Janssens as keynote speaker. Session chairs were Christian G. Schroer, Gerald Falkenberg, Axel Rosenhahn and Ulrike Boesenberg (www.xafs16.org). In addition, X-ray microscopy was also one of the topics of the “Sino-German Workshop on Catalysis and Membranes” (August 28 – 29, 2015, Karlsruhe).

Sarah Köster organized the 623. WE-Heraeus-Seminar: Cellular Dynamics, bad Honnef 4.9.–7.9.2016 as well as of the international conference Physics of the Cell, Bad Staffelstein, 20.8.–4.9.2015, as well as a symposium on synchrotron radiation in biophysics at the DPG annual meeting 2017 in Dresden.

Tim Salditt organized a satellite workshop at the DESY user meeting 2017 of X-ray holography and tomography, and a symposium on “X-ray microscopy: past, present, and future” in honor of the 80th birthday of G. Schmahl 2016.

5.4 Website

<http://vi-nanoimaging.desy.de/>

The website contains information on the VI and contact data of the project partners to get access to in-situ techniques.

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