PURE diagnostics

Protein Research Unit Ruhr within Europe (PURE) – A European research consortium developing novel diagnostics for cancer or neurodegenerative diseases, as Professor Dr Klaus Gerwert explains

Proteins regulate various cellular processes as diverse as proliferation and cellular growth. Virtually all oncological and neurodegenerative diseases seem to be caused by altered proteins and their altered interactions within signal transduction pathways. A single mutated protein can cause severe diseases. However, these 'defect' proteins can serve as biomarkers, to recognise diseases in an early, yet clinically still symptomless stage. Technological innovations in protein analysis today provide insight into protein function and interaction with the highest spatio-temporal resolution ever seen. Such detailed understanding of protein interactions opens the door to an early detection of disease based on protein alterations. Once single proteins are identified as 'key players', we are able to intervene with the causing factors as well as with the pathological processes, with early therapy. Under the umbrella of PURE, six research areas provide the fundamental pillars. This unique, interdisciplinary, and integrative approach combines fundamental with applied and clinical research.

To create individual protein profiles for a personalised diagnosis is the vision of PURE's scientists. They are applying methods inspired by criminology: just as every human has a unique fingerprint, every disease has a characteristic protein profile. As a first step these protein profiles are identified in tissue, then they are identified in the next step in bodily fluids such as blood or urine.

A fundamental element of PURE is a comprehensive study centre with an integrated biobank. The study centre establishes standard operating procedures (SOPs) and co-ordinates recruiting samples from tissue as well as bodily fluids along with the corresponding patient data from the university hospitals of the Ruhr Metropolis in accordance with legal, ethical and epidemiological standards. The PURE study centre comprises the areas of cancer prevention (Professors T Brüning and T Behrens), clinical oncology (Professors W Schmiegel and A Tannapfel) and neurodegenerative diseases (Professors J Wiltfang and R Gold). These areas focus their scientific attention on different diseases and different research questions. The PURE study centre takes care of:

- **The systematic collection, processing, storage and distribution of bio samples**
  The study centre collects bio samples and corresponding patient data according to standard operating procedures (SOPs). Contrary to other approaches, both tissue and bodily fluids (e.g. blood or, in case of neurodegenerative diseases, cerebrospinal fluid) are collected. Currently, PURE scientists are focusing on specific types of cancers and neurodegenerative diseases. In oncology, the focus is directed towards tumours in the urinary bladder, lung and colon; in neurodegenerative diseases the focus is on Alzheimer's and Parkinson's disease as well as on multiple sclerosis. The comprehensive study centre assures the central co-ordination of sample collection as well as biobanking according to the rules for good epidemiological practice.[1]

Professor Dr Klaus Gerwert

- **Molecular and epidemiological characterisation of bio samples and the corresponding study collectives**
  The study centre takes care of the histopathological processing, characterisation and categorisation of the human samples in accordance with current gold standards at the Institute for Pathology at Ruhr University. Complementary to the molecular characterisation of the sample material, patients of the study collectives are epidemiologically characterised by epidemiologic questionnaires and documentation forms in the department for cancer prevention of PURE.
- **Validation of potential biomarker candidates in independent collectives of test persons**
  To monitor the diagnostic relevance of identified biomarkers, validation studies complement PURE's biomarker discovery. The typical validation process passes through different stages, ranging from cross-sectional or simple case control designs in independent patient groups (verification) to prospective observational studies with healthy study subjects at baseline (actual validation). The last stage of the validation process would be a randomised clinical trial, comparing a marker with a usual care arm, as study design with the highest level of evidence. For marker validation, the Department for Cancer Prevention in PURE has access to high risk study collectives of the German Social Accident Insurance (DGUV). Access to these internationally unique collectives is provided by the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA).

Cancer prevention

The Department for Cancer Prevention in PURE is focusing on oncological diseases of the bladder and lung. Scientists of the IPA, an Institute of the Ruhr University Bochum, headed by Professor Thomas Brüning are focusing on prevention of disease. From the proper surveying of test persons regarding data protection, over a quality assured handling of bio samples (tissue, urine, blood), all

the way to the administration of bio sample processing, the study centre lays a solid foundation for each research group in PURE.

In parallel the Department for Molecular Tumour Biology of the IPA is focusing on the molecular characterisation of the bio samples, the identification of genetic biomarkers as well as their influence on signalling pathways related to tumourigenesis.

Clinical oncology

The Department for Clinical Oncology in PURE consists of the Institute for Pathology of the Ruhr University Bochum, headed by Professor Andrea Tannapfel, and the university hospitals Knappschaftskrankenhaus Bochum and Bergmannsheil, headed by Professor Wolff Schmiegel. At the Institute for Pathology tissue samples and cells are histopathologically assessed and characterised. Additionally, molecular profiles of tumour markers are developed and refined. The main focus is on colon cancer and pancreatic carcinoma. Bio samples are obtained from clinics of the Ruhr University's Comprehensive Cancer Centre (RUCCC), as well as other hospitals in the region. Supported by the Centre for Oncologic-clinical Studies of the RUCCC, affiliated scientists from Knappschaftskrankenhaus and Klinikum Bergmannsheil in Bochum evaluate and validate the newly developed technologies and biomarkers in PURE with respect to diagnosis, prognosis and response to therapies.[2]

Neurodegenerative diseases

The scientific focus of the Department for Neurodegenerative Diseases in PURE is on the identification and validation of molecular biomarkers for the enhanced early and differential diagnosis of neurodegenerative diseases. In this context the main focus is on predictive diagnostics of menacing neurodegenerative dementia (e.g. preclinical Alzheimer's dementia). Cerebrospinal fluid and blood samples are collected from patients suffering from neurodegenerative diseases. The samples are analysed with biophotonic and proteomic techniques in collaboration with Professor Jens Wiltfang, who heads the Department of Psychiatry and Psychotherapy at the University of Göttingen. In addition, the neurologist Professor Ralf Gold, head of the Clinic for Neurology of the St Josef Hospital in Bochum, has joined forces with PURE. He investigates the interaction of inflammatory and neurodegenerative mechanisms of multiple sclerosis and Huntington's disease.

Biophotonics

Located at the chair for Biophysics of the Ruhr University Bochum and headed by Professor Klaus Gerwert, the Department for Biophotonics in PURE analyses tissues and bodily fluids with state-of-the-art spectroscopic methods. In particular, label-free and non-invasive vibrational imaging (infrared and Raman) are applied. Thereby, histopathological alterations of tissue can be detected label-free, non-invasive and automated. In PURE, spectral histopathology (SHP) was established for both paraffin-embedded and fresh tissue for colon cancer.[3,4] SHP records spatially resolved vibrational spectra of a tissue sample using microscopic spectrometers. IR and Raman active molecular vibrations indicate the local biochemical composition of the sample. As such, each spectrum represents a characteristic,

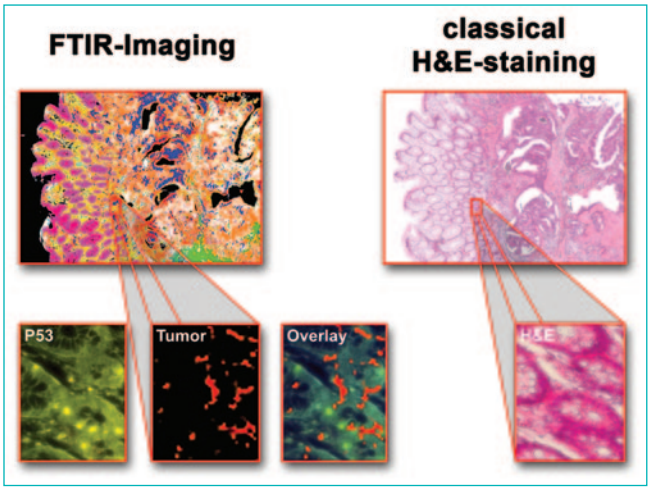

Fig. 1 Spectral histopathology: Fourier transform IR (FTIR) imaging analysis of colorectal carcinoma tissue sections (colon tumour). Image magnification increases from the top down. A classic haematoxylin-eosin (H&E) – stained section is shown on the right. FTIR imaging (left and centre) achieves a resolution of 5µm. Spectral histopathology reveals alterations in the crypts of the colon (red), which are confirmed by an immunohistochemical fluorescent stain (P53)

integral proteomic and genomic fingerprint that is registered in a database. After expert pathologist training of dedicated classification algorithms, the status of unregistered samples are predicted with a high accuracy based on spectral similarity.[5] SHP is compatible with standard pathology procedures. Combining pathology, biophysics, and bioinformatics, a reliable algorithm was set up. Databases for the annotation of colorectal tissue classes were set up by allocating each acquired spectrum to a specific type of tissue. Every type was represented by a specific colour, for example red for carcinoma and white for muscle (Fig. 1). Combining all types of tissue, a spatially resolved, highly accurate and annotated image of the section was obtained with sensitivity and specificity over 95%. Fig. 1 depicts a specific discrimination of 14 tissue classes of colorectal tissue, including cancerous lesions and morphologically intact pre-cancer states by SHP.[3]

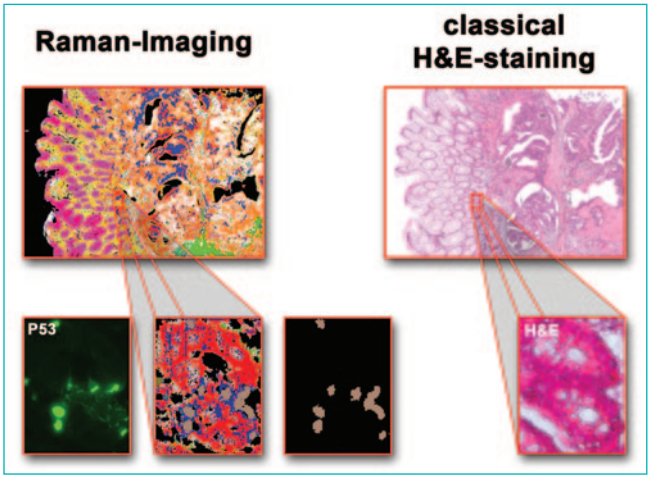

Fig. 2 Spectral histopathology: Raman imaging analysis of colorectal carcinoma tissue sections (colon tumour). Image magnification increases from the top down. A classic haematoxylin-eosin (H&E)-stained section is shown on the right. Raman imaging depicts the crypt alterations in more detail than FTIR imaging in Fig. 1. Altered nuclei (grey) in the altered crypts (red) were identified in comparison with the P53 stain (green)

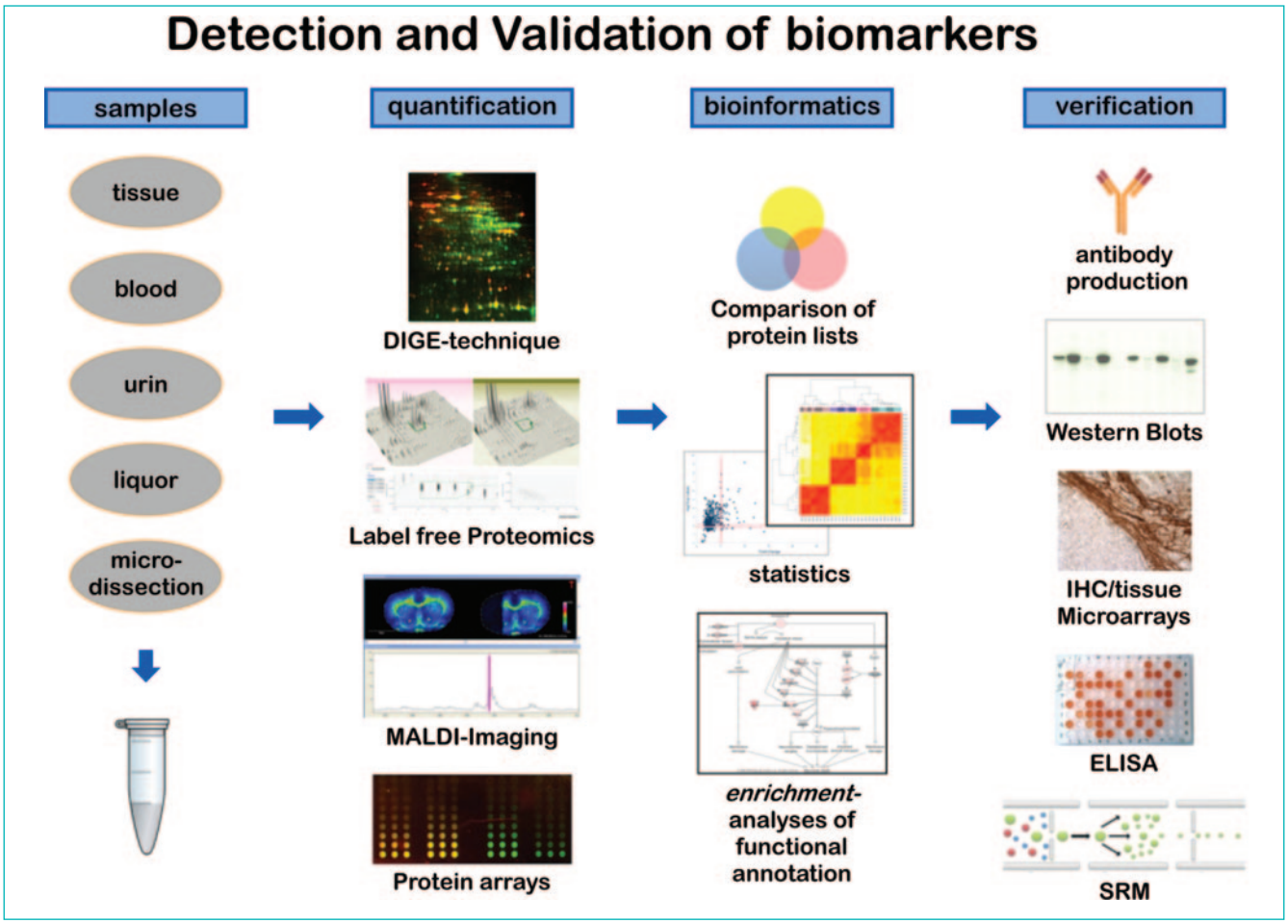

Fig. 3 Workflow for the identification and verification of disease-related proteins/protein biomarker candidates from tissue and bodyfluids, as optimised at the MPC

In comparison of SHP with classical immunohistopathology (IHP) methods, in which tumours were identified using fluorescent labels,[3,4] cancerous areas widely co-localised with p53-activated cells (as stained green in Fig. 1). IR SHP on colorectal tissues exceeded 95% sensitivity and specificity.

Microscopy with medium-IR radiation (4,000-1,000cm^{-1}, corresponding to 2.5-10µm) is resolution limited by the applied wavelengths. A deeper understanding of affected cells is gathered by Raman microscopy (e.g. 532nm or 785nm) (Fig. 2). With this enhanced spatial resolution, the automatic recognition of single cells and even intracellular structures including tumour characteristic nuclei was demonstrated.[4] This technique can also be used for monitoring distribution of a drug within a single cell.

SHP technology at the Biophotonic department of PURE is currently extended to become a fully automated high throughput technique.

Preparation of a thin tissue slice and its microscopic analysis requires a considerable amount of time. But in the operating theatre, speed is essential. Infrared fibre optics enable the flexible positioning of infrared and Raman probe heads for a localised, non-invasive SHP with real-time status evaluation. Such procedures are developed in PURE aiming at the introduction of spectroscopic techniques to endoscopy.

Vibrational spectroscopy, particularly Fourier transform IR (FTIR), is also used to analyse body fluids, although some obstacles have to be overcome. Unlike tissue analysis, spectroscopic body fluid analysis is limited to only one representative spectrum per sample of the patient's health status. Further, a possible disease-associated signal can be expected to be subtle as compared to a comparably huge biological variation of the fluid itself. For an utmost reproducible spectrum acquisition, an automated spectroscopic system and data processing algorithms were developed. Characteristic marker patterns for an accurate, sensitive, and specific discrimination of urinary bladder cancer patients within a risk group were identified.[6] As such, the less invasive FTIR blood test could spare a lot of bladder cancer patients from repeated cystoscopy.

In summary, the biphotonic department of PURE applies a wide range of sophisticated non-invasive techniques to the routinely applied diagnostic instruments aiming at new versatile, easy-to-use, objective, and reproducible diagnostic tools for the personalised medicine.

Proteomics

The Medical Proteome-Centre (MPC) of the Ruhr University Bochum, founded by Professor Helmut E Meyer and headed by Professor Katrin Marcus from 1 March 2014, is responsible for proteomic studies in PURE. It houses an excellent infrastructure and all state-of-the-art proteomics technologies. The MPC focuses on protein

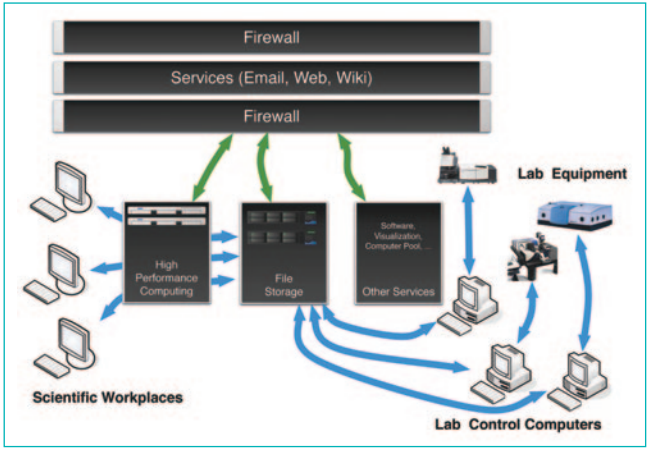

Fig. 4 IT infrastructure at the Department for Bioinformatics

analysis of biological systems. Methods used include gel- and chromatography-based separation techniques with subsequent mass spectrometry. In particular fresh tissue e.g. label-free characterised from the biophotonics department serves as primary material for subsequent proteomic analyses. (Fig. 3) The obtained protein profiles provide a detailed insight which proteins are misregulated in diseased persons. Identified proteins are evaluated using bioinformatics analyses. The obtained biomarker candidates are then validated in tissue and body fluids of a large number of patient samples via immunological and mass spectrometric techniques. The workflow described above was successfully applied resulting in several publications such as *PLoS One 2013.*[7]

Bioinformatics

The Department for Bioinformatics in PURE analyses the data obtained from the other PURE departments. Based on cross-platform statistical, mathematical and bioinformatical analyses, new and improved methods and algorithms, in particular for the analysis of the multispectral image data, are developed and implemented in the group of Professor Axel Mosig.

The constellation of clinicians and experimentalists within PURE creates data analysis challenges that can be solved, only up to a certain extent, by existing computational approaches. To fully exploit the availability of data from different microscopy and proteomics platforms, it is an inevitable task to develop new algorithms and computational tools that are dedicated to the specific and to date unique constellation of collecting data in PURE. This has led to the development of cross-platform analysis methods, such as registration algorithms that can overlay and thus co-localise and correlate observations from different types of microscopic images of the same sample. This has been successfully established for combining Raman image spectra with fluorescence microscopic images, and is currently being extended towards other types of microscopic data established in PURE.

The long term roadmap for bioinformatics within PURE is to establish specific cross-platform analysis algorithms for all constellations of microscopy and proteomics that are combined in studies investigated in PURE.

In order to facilitate joint storage and analysis of data from different platforms, a substantial amount of data storage and computing power is required. To this end, an IT infrastructure has been established involving more than 120TB capacity of data storage and high performance computing facilities with more than 1,500 CPU cores.

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