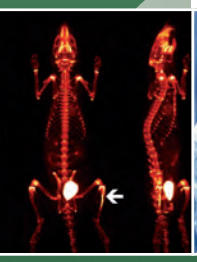
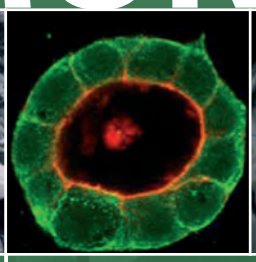
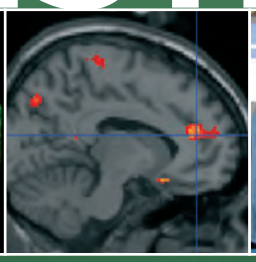
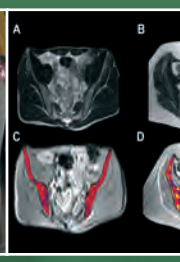
IZKF MÜNSTER



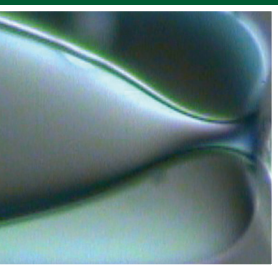
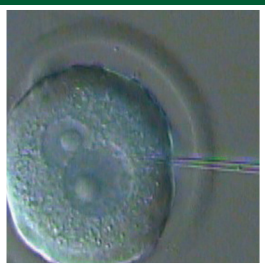


Technology Platform

Interdisciplinary Center
for Clinical Research
of the Medical Faculty Münster

State-of-the-Art Technologies

IZKF Technology Platform

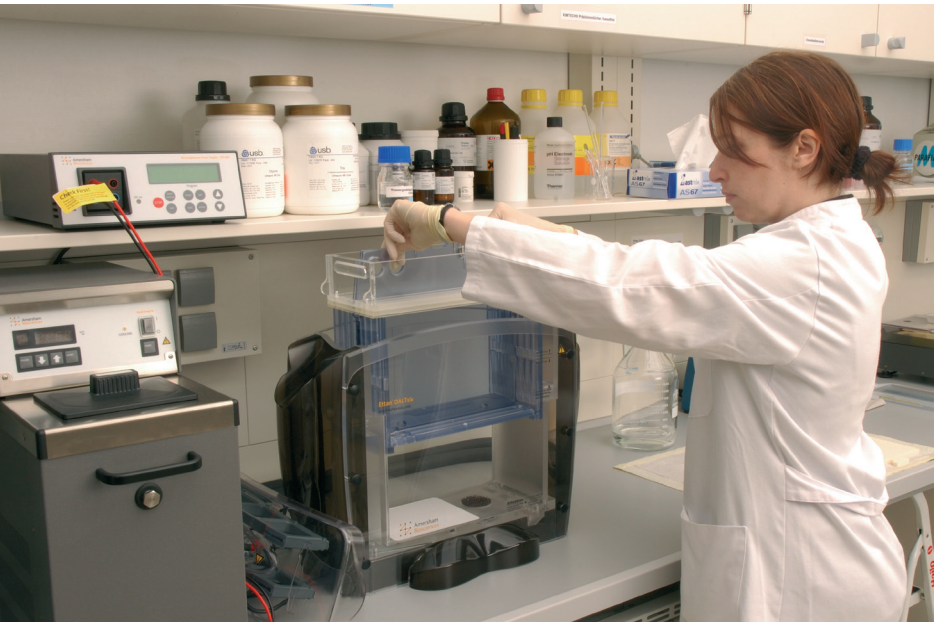



Our Goal

The rapid advancement in the field of biological and biomedical research has facilitated an in-depth understanding of cellular and molecular processes in health and disease. This has given rise to the need for researchers to have access to efficient high throughput services that are too cost- and labor-intensive for single university institutions. The Interdisciplinary Center for Clinical Research (IZKF) Münster operates several Core Units, which are a valuable resource for an effective research environment.

Principal Aims

› The Core Units specialize in providing State-of-the-Art skilled technical and instrument support for the Medical Faculty of the University of Münster, extramural faculty, external non-affiliated institutions as well as industrial researchers, at affordable rates.



› Specialists in the field assist researchers from the stage of project conception to its completion thus ensuring maximum efficiency.

› Researchers have open access to a large instrument pool consisting of expensive high-end equipment that has increased the versatile nature of the technology available to the institutions of the Medical Faculty.

› The Core Units operate on a cost recovery basis and charges reflect the actual expenses for consumables and service contracts.

Moreover, the Core Units promote translational research by interacting closely with academia and industry, ensuring that the technology available is continuously updated. Lectures, workshops and practical courses are organized on a regular basis to keep users informed about current developments.

This information folder provides an overview of the different facilities, their technical equipment and methodological support services available to researchers.

Contact and more information

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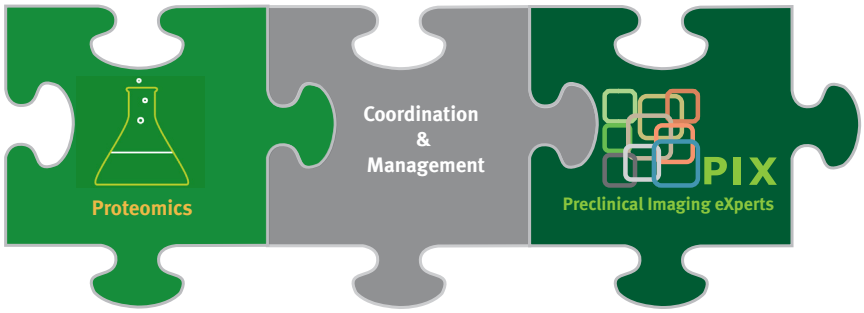
IZKF Technology Platform

The IZKF Münster Technology Platform provides users with state-of-the-art expertise and equipment and currently consists of two highly specialised Core Units (CU) - Proteomics and Preclinical Imaging eXperts (PIX). The Core Unit PIX now combines the expertise of the former IZKF Core Units OPTI, SAMRI and SmAP in the area of multimodal preclinical imaging. The IZKF Scientific Office coordinates and supports these Core Units and oversees their smooth operation.



Our information folder aims to provide users with an overview of the services offered by the Core Units. For special experimental paradigms not listed, users are requested to contact the Head of the respective Core Unit. It is mandatory for all users to strictly abide with the Terms of Use of the Core Units. The IZKF Scientific Office provides information on prices and issues invoices for services carried out. Users are advised to include costs for Core Unit services in their grant applications.

The logos on the upper right hand corner are specific to each Core Unit, enabling the easy identification of leaflets that have been updated. In addition, news regarding novel technologies will be communicated to users via a newsletter. As a user of our Technology Platform, we would appreciate your feedback on our Technology Platform and urge you to contact the IZKF Scientific Office if you have any concerns or constructive suggestions involving any of our facilities.

IZKF Technology Platform
Overview

Coordinators

Prof. S. König

The Core Unit **Proteomics** was set up with the aim of enabling the investigation of proteins and specializes in providing Stateof-the-Art skilled technical and instrument support for researchers. Open access to a large instrument pool consisting of expensive high-end equipment for proteomic research that is continuously validated according to the experimental needs of researchers is also available. The unit provides assistance with experimental design, project planning, data analysis and coordinates interdisciplinary research projects. Lectures, workshops and practical courses are organized to keep users informed about current developments. The facility is also used by a number of scientists and customers from national and European universities and industries. With high-ranking publications and patent applications the group has achieved an excellent standing in the community of international Proteomics Core Facilities.

Dr. S. Hermann

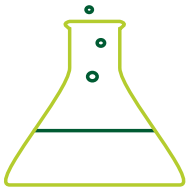
Preclinical Imaging eXperts (PIX) is a Core Unit for preclinical imaging that provides access to multimodal imaging technologies for cooperative research in a highly integrated structure. PIX is based on an already existing infrastructure and proven expertise for single preclinical imaging tools, namely magnetic resonance imaging, positron emission tomography, single-photon-emission-tomography, X-ray computed tomography, near-infrared fluorescence imaging, and bioluminescence imaging

PIX offers:

- Integrated expertise through a project consulting service to tailor the application of imaging tools to the scientific questions of research partners
- Integrated workflow for efficient multimodal imaging and data analysis
- Interdisciplinary training for scientists and technicians
- Quality control of project management, imaging workflow, data analysis, data logistics and training.

The imaging workshop *The Mouse Imaging Academy (MIA)* is a special workshop hosted annually by PIX. MIA aims at providing theoretical and practical training for young researchers and students in the field of small animal imaging.

Core Unit Proteomics



Proteins are the principal effectors in cellular function and the target of most pharmacological strategies. The goal of the proteomics facility is to provide cost-effective access to sophisticated technology for modern proteomics analysis in a central facility. Special emphasis is placed on assisting researchers in designing and evaluating their research projects and includes extra features such as advanced manual and bioinformatic data mining.

Biomolecular mass spectromery

Mass spectrometers are available (Q-TOF Premier, ESI/AP-MALDI (IR/UV) ion trap, MALDI-TOF), which can be flexibly used for different types of experiments such as the determination of molecular weights of biomolecules regardless of their size. Soft ionization techniques allow the analysis of intact proteins and labile modifications. Procedures have been developed to monitor enzymatic or chemical reactions or to fingerprint biological fluids. Not only proteins and peptides can be investigated; the analysis of certain types of drugs (e.g. Imatinib), fatty acids or other substances in complex mixtures is also possible.

Protein identification

The analysis of proteins pre-separated by electrophoretic or chromatographic means is one of the main service tasks. Reversed-phase nanochromatography coupled to high-end mass spectrometry (LC-MS; nanoUPLC/Q-TOF Premier) allows confident assignment of proteins after enzymatic digestion. Protein modifications such as phosphorylation, isoforms and unknown sequences are accessible in specially designed experiments.

Protein expression analysis

The The unit offers a choice of gel-based or MS-based differential analysis. The entire pipeline for the DiGE technology (Differential Gel Electrophoresis) is available. This is an advanced system solution based on 2D-PAGE (separation of proteins according to their isoelectric point (pI) and molecular weight), which enables the quantification of protein expression profiles of two samples (e.g. normal vs disease state) in a reproducible and statistically relevant manner. The methodology eliminates gel-to-gel variance and represents the state-of-the-art in gel-based expression analysis. DiGE gels are scanned with the Typhoon 9400 three-laser scanner, which is also versatile for use in other applications such as modification-directed staining. Regulated proteins are subsequently identified using LC-MS/MS.

A dedicated MS-based approach using Q-TOF Premier MSE technology allows label-free absolute and relative quantification of proteins. It promises comprehensive data with high reproducibility and reduced sample handling. Even less sample is needed (~2 µg compared to 500 µg for DiGE) and the data can be stored indefinitely for later mining. The identification of hundreds of protein components of one proteome is automatically performed. The results of the analysis include not only the fold changes in regulation but also provide clear insights about the magnitude of proteome differences and regulatory factors using principal component analysis (PCA)- see Bioinformatics section).

Core Unit Proteomics

Protein separation and gel electrophoresis

The unit assists in the preparation of tissue homogenates or cell lysates. 1D- and 2D-electrophoresis can be performed with customer samples. Moreover, the fractionation of proteins in the liquid phase based on their pI is possible, e.g. to exclude dominant proteins for subproteome analysis. Quality control of protein separations is carried out with a miniaturized capillary electrophoresis lab-on-a-chip system. This method serves to rapidly diagnose separation procedures or sample quality. Biological fluids such as urine, cell extracts or whey can be evaluated for their protein content.

Pre-fractionation of protein mixtures for sub-proteome analysis

Profiling of biofluids

LC-MS profiles are excellent means to study biological changes. In particular, urine profiles of low-to-medium molecular weight compounds have been shown to reflect health or hormonal status. Replicate samples are compared with statistical tools such as PCA and the results are evaluated further for biomarkers.

Protein arrays

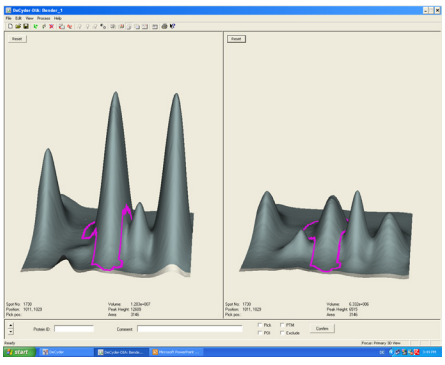
Protein arrays are increasingly used to miniaturize interaction experiments. They serve to detect proteins, study their expression levels, as well as their interactions and functions. Using protein arrays, efficient and sensitive high-throughput analysis of thousands of proteins can be achieved simultaneously. Protein arrays such as the FAST slides can be analysed with two microarray scanners. Protein arrays are commercially available for cytokine and biomarker analysis as well as autoimmune diagnostics.

Bioinformatics for proteomics and profiling

It has become increasingly necessary to evaluate and visualize proteomic or mass spectrometric data with statistical methods. A number of procedures have been established for that purpose:

Protein expression analysis using DIGE

The DeCyder software applies a gel comparison method that introduces zero statistical error, offering reliable data and analysis. Co-detection, background subtraction, normalization, and quantification of spots in images are improved based on a warping function. The software provides multivariate statistics tools and enables the combined analysis of different datasets, aiding the biological interpretation of results by matching with data retrieved from public and local databases.



Core Unit Proteomics

Protein expression analysis using LC-MS^E

The expression module of ProteinLynx-GlobalMiner 2.5 assists in the statistical evaluation of LC-MS runs of proteome digest replicate runs. Both relative and absolute quantification is possible without the need for sample labeling. Experimental data are further mined using tools such as Principal component analysis.

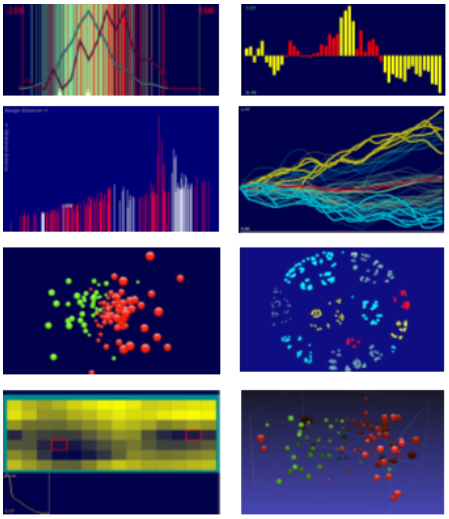
Principal component analysis (PCA)

PCA (SimcaP, RapidMiner) as a linear dimensionality reduction algorithm, which projects high dimensional data (replicate runs with hundreds to thousands of data points) to lower dimensional subspace spanned by the principal eigenvectors and assist investigators in finding major differences in their samples. It is an important step preceding biomarker assignment both in proteomics and the analysis of LC-MS profiles of urine or other biofluids

Exploratory analysis and data visualisation

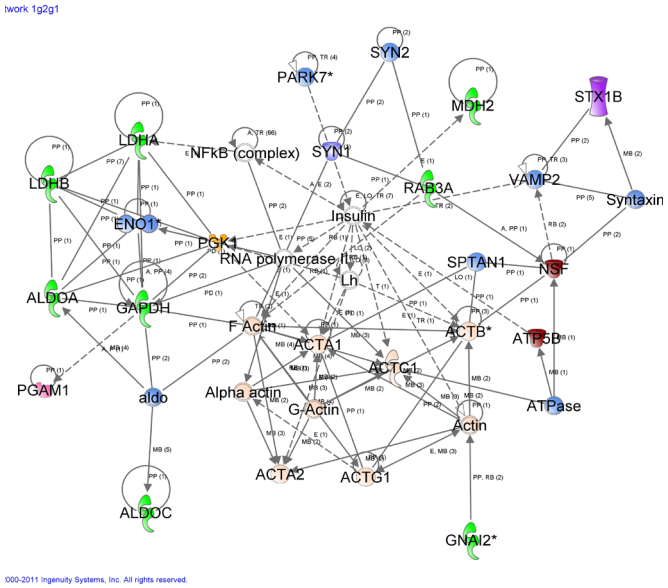
VisuMap software allows understanding data that otherwise resists easy interpretation.

It provides a novel insight into the patterns, relationships and correlations behind the data. In particular, relational perspective mapping and advanced clustering algorithms for high dimensional data like self-organizing map, affinity propagation, k-mean clustering are of great value for analysis of LC-MS data.



Pathway analysis

The information about regulated proteins is further processed identifying relationships, mechanisms, functions, and pathways of relevance using Ingenuity software. It delivers an assessment of signaling and metabolic pathways, molecular networks, and biological processes that are most significantly perturbed in the dataset of interest.



Core Unit Proteomics

Protein Histidine Phosphatase (PHP)

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1   GHHHHHHHHH HSSGHIEGRH MAVADLALIP DVDIDSDGVF KYVLIRVHSA
51  PRSGAPAAES KEIVRGYKWA EYHADIYDKV SGDMQKQGCD CECLGGGRIS
101 HQSQDKKIHV YGYSMAYGPA QHAISTEKIK AKYPDYEVTW ANDGY
```

PHP was discovered by the late Prof. Susanne Klumpp and studied extensively* in her group at the Institute of Pharmaceutical and Medical Chemistry at the WWU, Münster. As a result of earlier collaborations, the IFG continues to provide PHP for research purposes.

*Klumpp S, Krieglstein J (2009) Reversible Phosphorylation of Histidine Residues in Proteins from Vertebrates. Sci Sig 10: 2(61):pe13

The following products are available on request:

1. Dry recombinant PHP supplied in aliquots of 1 mg (or as required)
2. Glycerol culture of recombinant PHP supplied in aliquots of 1 ml (or as required)

Additional publications and further information can be found at http://php. uni-muenster.de

Representative Publications

Quiskamp N, Poeter M, Raabe CA et al. (2014) The tumor suppressor annexin A10 is a novel component of nuclear paraspeckles. Cell Mol Life Sci 71: 311-329.

Sehlbach M, König S, Mormann M et al. (2013) Arabinogalactan protein cluster from Jatropha curcas seed embryo contains fasciclin, xylogen and LysM proteins. Carbohydr Polym 98: 522-531.

Hahn A, Kaufmann JK, Wies E et al. (2012) The ephrin receptor tyrosine kinase A2 is a cellular receptor for Kaposi's sarcoma-associated herpesvirus Nature Med 18: 961-966.

Tekook M, Fabritz L, Kirchhof P, König S et al. (2012) Gene construction, expression and functional testing of an inotropic polypeptide from the venom of the black scorpion Hottentotta judaicus. Toxicon 60: 1415-1427.

Hermann A, König S, Lechtenberg M et al. (2012) Polysaccharides and glycoproteins from Boswellia serrata Roxb. and B. carteri Birdw. and identification of a proteolytic plant basic secretory protein. Glycobiology 11: 1424-1439.

Kummer MP, Hermes M, Delekarte A et al. (2011) Nitration of Tyr10 critically enhances amyloid beta aggregation and plaque formation. Neuron 71: 833-844.

Kriegeskorte A & König S, Sander G et al. (2011) Small colony variants of Staphylococcus aureus reveal distinct protein profiles. Proteomics 11: 2476-2490.

Wang W, Ackermann D, Mehlich A-M, König S (2010) False labelling due to quenching failure of N-hydroxysuccinimide-ester coupled dyes. Proteomics 7: 1525-2529.

Open access

Instrumentation such as array scanners, gel imagers and PAGE equipment are available for customer's use.

Getting started

Users can provide tissue, cells or protein mixtures for analysis and are advised to contact the proteomics staff who will be pleased to assist in experimental design and to discuss the parameters that samples have to fulfill to ensure smooth processing and excellent results.

Contact

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Core Unit Proteomics

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IZKF Münster (11/2014)

PET-CT / SPECT-CT

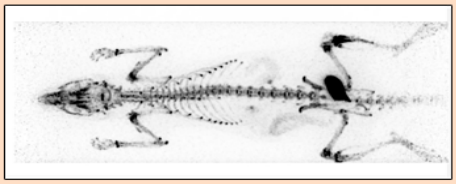
Molecular imaging covers a broad spectrum of applications ranging from imaging of single cells or even subcellular structures *in vitro* to clinical diagnostic imaging in patients *in vivo*. The aim of this core unit is the non-invasive phenotypisation of wild type, surgical and transgenic animal models using highly sensitive and high resolution dedicated small animal imaging technology. The CU offers both scientific expertise and technology access.

Small animal positron emission tomography (PET)

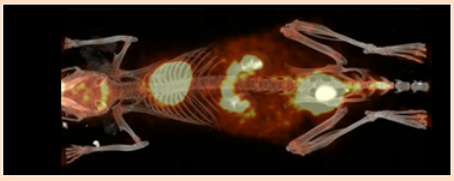
PET is a highly sensitive, quantitative imaging modality capable of assessing molecular dynamics *in vivo* with nano-/picomolar sensitivity. Within SmAP two dedicated high resolution small animal PET scanners with sub-milliliter resolution and a large field of view (28 cm * 16 cm) are available (quadHIDAC®, Oxford Positrons Ltd., Oxford, UK). In cooperation with the Department of Nuclear Medicine and the SFB 656 "Molecular Cardiovascular Imaging" this core unit is able to offer a wide range of different molecular imaging probes ranging from whole-body measurement of perfusion and metabolism down to cell imaging and imaging of targets involved in oncological, inflammatory, neurological, cardiovascular and other diseases.

Small animal single photon emission tomography (SPECT)

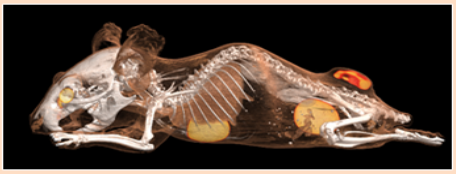
Recently a dedicated multi-pinhole SPECT/CT system for animals having a sub-millimeter resolution and good sensitivity was installed (NanoSPECT/CT, Bioscan, US). The SPECT methodology is basically a gamma camera system that provides tomographic images. Since this technology is the most common molecular imaging tool in patients a diversity of tracers/tracer kits are clinically available, which can be directly used in small animal SPECT. This will substantially broaden the spectrum of molecular targets and methodology.



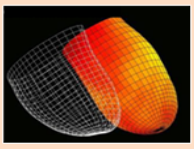
F-18-Fluoride-PET - bone metabolism
inflammation diagnostics (arthritis, rat)

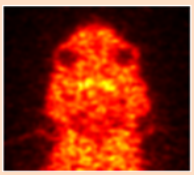


F-18-FDG-PET/CT - glucose metabolism
inflammation diagnostics (colitis, mouse)



F-18-FDG-PET/CT - glucose metabolism
tumour diagnostics (melanoma, mouse)



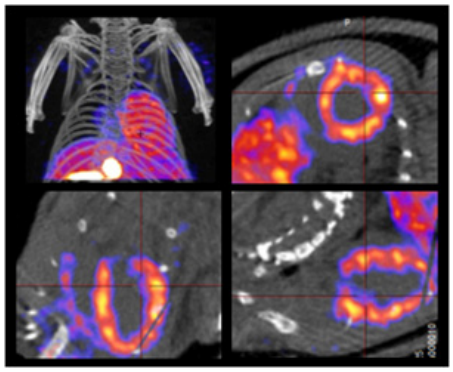


Right column pictures:

Top: F-18-FDG-PET, glucose metabolism, vitality diagnostic of the myocardium (normal)
Middle: F-18-DOPA-PET, dopamine receptor density, Parkinson diagnostics (normal)
Bottom: F-18-FDG-PET, glucose metabolism, inflammation diagnostics (explanted gut, colitis)

PIX
Preclinical Imaging eXperts

PET-CT / SPECT-CT



Tc-99m-Tetrofosmin myocardial perfusion imaging. Diagnostics for myocardial schemia/infarction (normal perfusion)

Small animal computed tomography (CT)

CT is an anatomic imaging modality with a very high spatial resolution. The core unit houses a dedicated small animal high-resolution CT device with a resolution down to 15 µm for in vivo and ex vivo applications (Siemens Inveon®). SmAP uses this method primarily to provide anatomical information in correlation to the distribution of specific molecular imaging probes in PET and SPECT. In general, small animal CT alone offers a spectrum of applications similar to CT in the clinical setting.

Ex-vivo autoradiography and biodistribution

Beside *in vivo* studies, questions concerning biodistribution and metabolism can be answered by high-resolution *ex-vivo* autoradiography (Biospace Micro-Imager®, 40 µm resolution) and tissue counting studies.

Statistics

In the last three years 4279 PET and CT measurements have been carried out on mouse (87%) and rat (13%) models of human disease.

Representative Publications

Vogl T, Eisenblätter M, Völler T et al. (2014) Alarmin S100A8/S100A9 as a biomarker for molecular imaging of local inflammatory activity. Nat Commun ug 6;5:4593. doi: 0.1038/ncomms5593

Steingräber AK, Schelhaas S, Faust A et al. (2013) Molecular imaging reveals time course of MMP activity in acute cutaneous vasculitis in vivo. Exp Dermatol 22: 730-735.

Claesener M, Breyholz H-J, Hermann S et al. (2012) Efficient synthesis of a fluorine-18 labeled biotin derivative. Nucl Med Biol 39: 1189-1194.

Bunk EC, Stelzer S, Hermann S et al. (2011) Cellular organization of adult neurogenesis in the Common Marmoset. Aging Cell 10: 28-38.

Law MP, Schäfers K, Kopka K et al. (2010) Molecular imaging of cardiac sympathetic innervation by 11C-mHED and PET: from man to mouse? J Nucl Med 51: 1269-1276.

Contact

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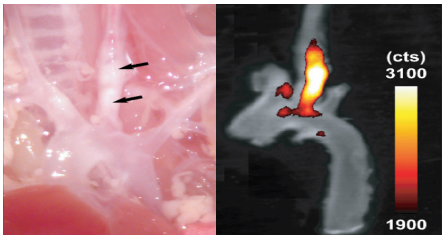
IZKF Münster (11/2014)

Optical Imaging

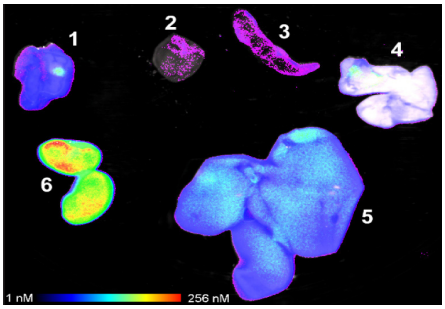
Small animal optical imaging allows quantitative data on key diseases and therapeutic response profiles to be generated *in vivo*. Fluorescence imaging in the near-infrared range (700-900 nm), also called „optical window“, is characterised by low absorbance through oxy- and-deoxy-hemoglobin (i.e. good tissue penetration) as well as low levels of autofluorescence, yielding high contrast to noise ratios. Thus, even picomolar amounts of fluorochromes can sensitively be detected without ionising radiation (permitting continuous or repeated exposures) so that molecular structures can be resolved *in vivo* using this technique. With the available fluorescence imaging systems - in combination with near-infrared emitting fluorophors tailored to specific biological applications - biological targets and pathways can be monitored and quantified even in deeper tissue sections. Beside the access to two state-of-the-art optical *in vivo* imaging methods, the core unit offers scientific expertise for experimental design and data analysis in optical imaging.

Fluorescence reflectance imager

A fluorescence reflectance imaging (FRI) system is available on-site for fast and convenient acquisition of 2D fluorescence images. The Kodak In-vivo Imaging Station FX Pro combines advanced multispectral fluorescence, luminescence, digital x-ray and radioisotopic imaging in a single system. Thus, multichannel and multimodal imaging capabilities are available. The system is ideal for rapid evaluation of superficially located processes such as subcutaneous tumors. In addition to *in vivo* imaging, the system is suitable for *in situ*, and *ex-vivo* fluorescence applications, e.g. biodistribution studies. A wide range of filter sets are available that are suited for different fluorochromes or fluorescent proteins.



In vivo and ex vivo FRI images: alpha(v)beta(3) expression of artherioscleorotic plaques.

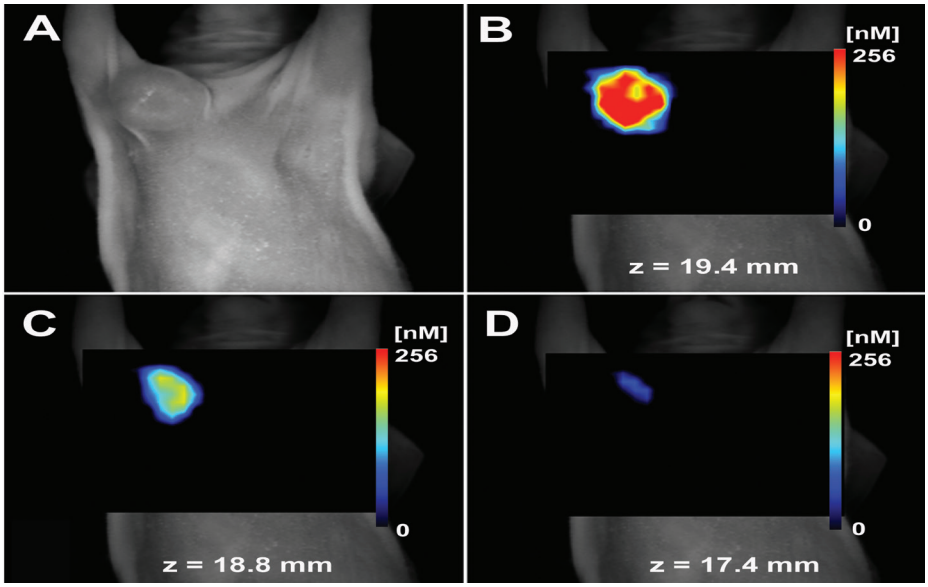


Biodistribution study

Fluorescence mediated tomograph

In comparison to 2D techniques, fluorescence mediated tomography (FMT) offers superior quantification accuracy and can yield three-dimensional determination of contrast agent uptake. Two state-of-the-art FMT systems for small animal imaging are installed in the core unit to yield 3D quantitative tomographic images of small animals. Data can be acquired at two different wavelengths: Excitation: 670 nm/ Emission: 700 nm and Excitation: 745 nm/Emission: 780 nm. Co-registration of FMT data and e.g. MRT data is possible.

Optical Imaging



White light image and FMT reconstructions of in vivo fluorescence signals indicating tumor-driven angiogenesis.

Contrast agents

In addition to commercially available fluorescent contrast agents, the core unit offers access to different fluorescence imaging probes that were developed in cooperation with the Department of Clinical Radiology, the Department of Nuclear Medicine and the Collaborative Research Centre SFB 656 (MoBil) „Molecular Cardiovascular Imaging“ at the University of Münster. In detail, markers of tissue perfusion, targeted probes for imaging angiogenesis and MMP-expression are available.

Representative Publications

Hahnenkamp A, Alsibai W, Bremer C, Höltke C (2014) Optimizing the bioavailability of small molecular optical imaging probes by conjugation to an albumin affinity tag. J Control Release. 186: 32-40. doi: 10.1016/j.jconrel.2014.04.053

Waschkau B, Faust A, Schafers M, Bremer C (2013) Performance of a new fluorescence-labeled MMP inhibitor to image tumor MMP activity in vivo in comparison to an MMP-activatable probe. Contrast Media Mol Imaging 8: 1-11.

Neesse A, Hahnenkamp A, Griesmann H et al. (2012) Claudin-4-targeted optical imaging detects pancreatic cancer and its precursor lesions. Gut 620: 1034-1043.

Getting started

The investigator is requested to contact the Facility staff to discuss specific needs and to design the project.

Information about costs and issuing invoices are given by the IZKF Scientific office.

Contact

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IZKF Münster (11/2014)

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is an extremely versatile pre-clinical diagnostic technique. Besides morphological imaging, MRI allows for functional and metabolic imaging in non-invasive longitudinal studies, aiming at both phenotyping or molecular imaging in mice, rats, or guinea pigs.

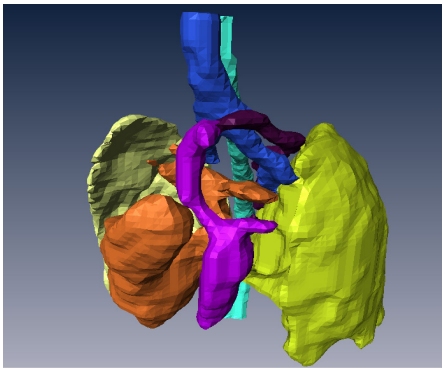
MRI - A tool in pre-clinical research

With 3D spatial resolutions approaching 10 µm in fixed specimens and 50 µm *in vivo* MR microscopy provides valuable information for phenotyping novel transgenic animals either *in utero*, during development, or following disease onset. Additional functional parameters such as cardiac volumes in models of heart disease or tumor tissue characterisation in cancer models are readily available from standard measurement protocols.

Cell tracking MRI allows for visualisation of labeled tumor cells or grafted stem cells over a time course of several weeks. Morphological and functional data is complemented by metabolic information, which is available from MR spectroscopy measurements, providing metabolic data with sub-millimeter resolution.

Furthermore, fMRI is a valuable tool in neurophysiological research, detecting either the hemodynamic response of neural activity via the BOLD (blood oxygen level dependent) effect, or activity of Ca^{2+}-channels via MEMRI (Manganese-enhanced MRI). Since fMRI is a non-invasive techniques these data can be collected over a time course and compared to behavior experiments in the same animal.

9.4 T Biospec with cryoprobe

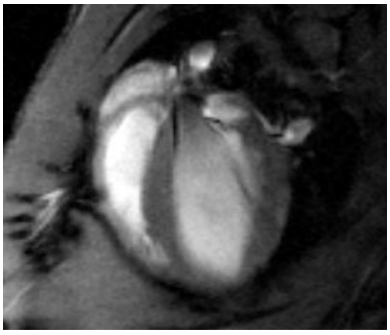


3D reconstruction of the murine thorax

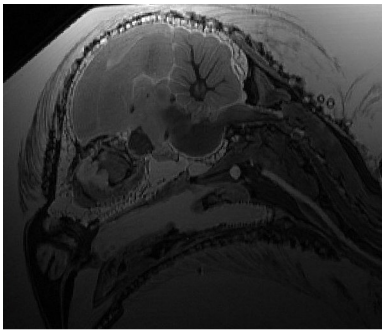
Infrastructure

The core unit SAMRI is equipped with a state-of-the-art small animal MRI system (Bruker Biospec 94/20), operating at a magnetic field strength of 9.4 tesla. Dedicated probes for mice and rats and high-performance microscopy gradient systems provide optimum preconditions-for numerous applications. Installation of a CryoProbe (Helium-cooled detector) affords highest sensitivity for ultimate temporal of spatial resolution. For cardiac MRI and fMRI studies ECG and transcutaneous blood gas monitoring devices are available.

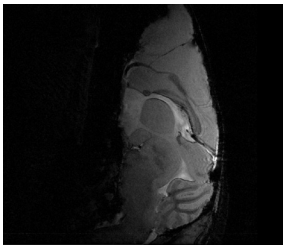
Magnetic Resonance Imaging



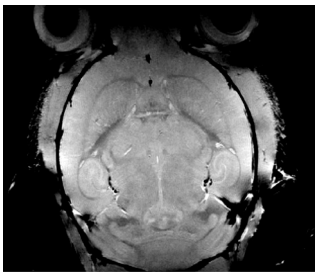
Morphological imaging of the mouse heart in vivo



Morphological imaging of the zebra finch brain in vitro



Sagittal image of the mouse brain in vivo



Coronal image of the mouse brain in vivo

Service

SAMRI offers a number of routine protocols for high resolution morphological imaging, as well as protocols for functional parameters in cardiac, developmental or oncological models. Protocols for fMRI studies or cell tracking applications are devised on demand. MR spectroscopy protocols can be established in cooperation with the interested user. To allow for a wide range of applications, including infection models, the MR-system is installed in a S2-laboratory.

Representative Publications

Ring J, Hoerr V, Tuchscherr L,Kuhlmann MT, Löffler B, Faber C (2014) MRI Visualization of Staphyloccocus aureus-Induced Infective Endocarditis in Mice. PloS One 9(9):e107179. doi: 10.1371/journal.pone.0107179.

Hoerr V, Tuchscherr L, Hüve J, Nippe N, Loser K, Glyvuk N, Tsytsyura Y, Holtkamp M, Sunderkötter C, Karst U, Klingauf J, Peters G, Löffler B, Faber C (2013) Bacteria tracking by in vivo magnetic resonance imaging. BMC Biol 11: 63-75.

Hoerr V, Purea A, Faber C (2010) NMR Separation of intra- and extracellular compounds based on intermolecular coherences. Biophys J 99: 2336-2343.

Getting started

The investigator is requested to meet with the facility staff to discuss specific needs and to design the project.

Information about costs and issuing invoices are given by IZKF Scientific Office.

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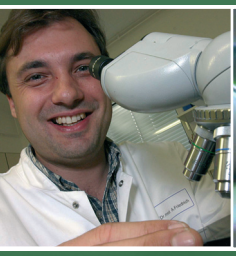
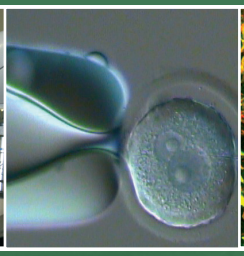
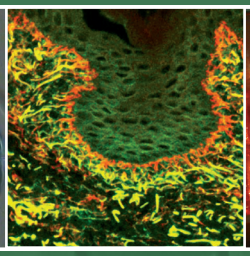
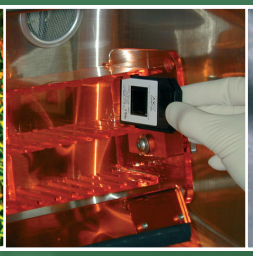
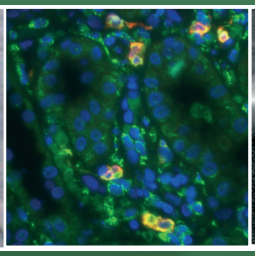
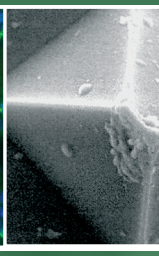
MÜNSTER IZKF

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