TECHNOLOGY PLATFORMS

MAX DELBRÜCK CENTER

Discovery for tomorrow's medicine

IMPRINT

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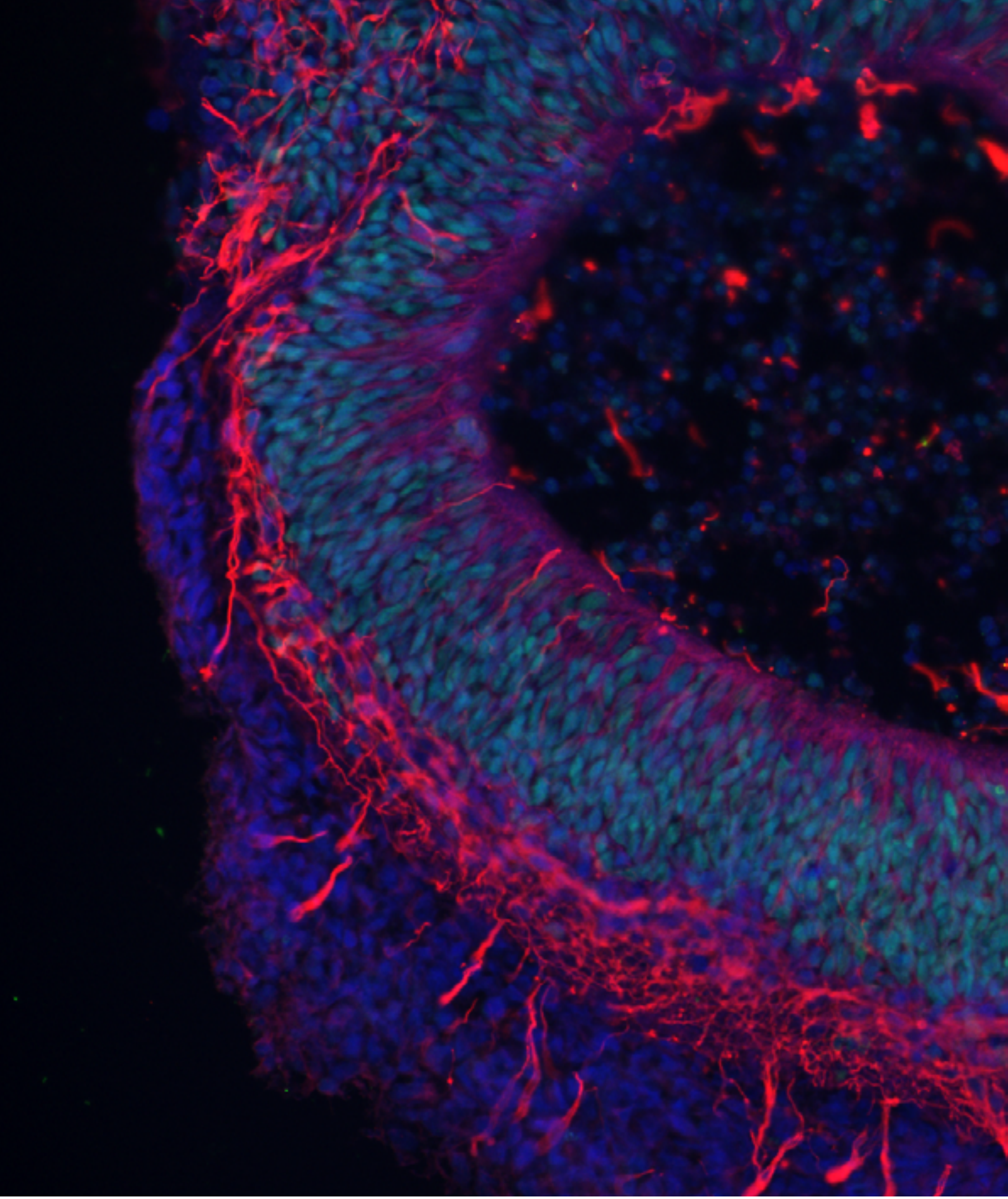
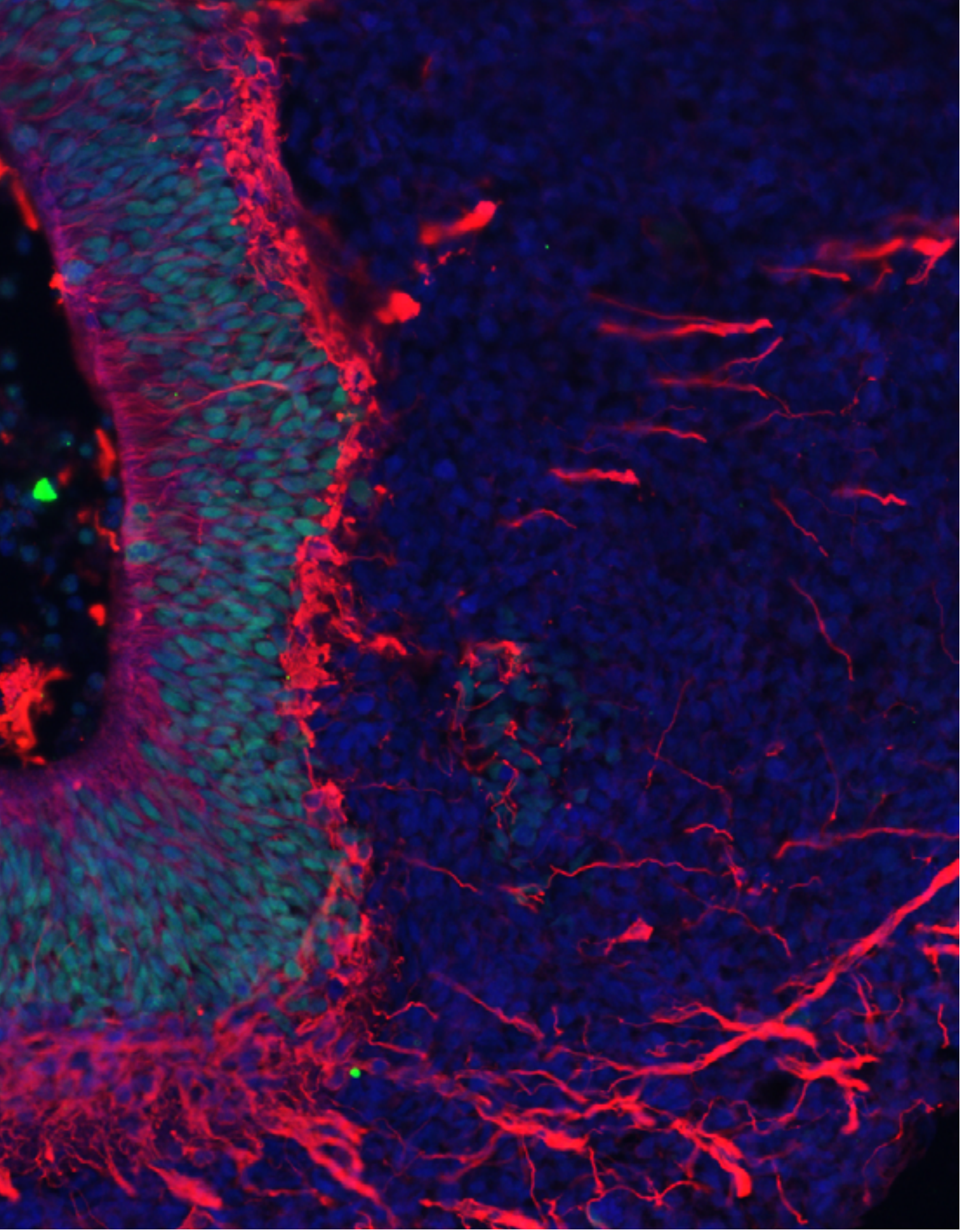
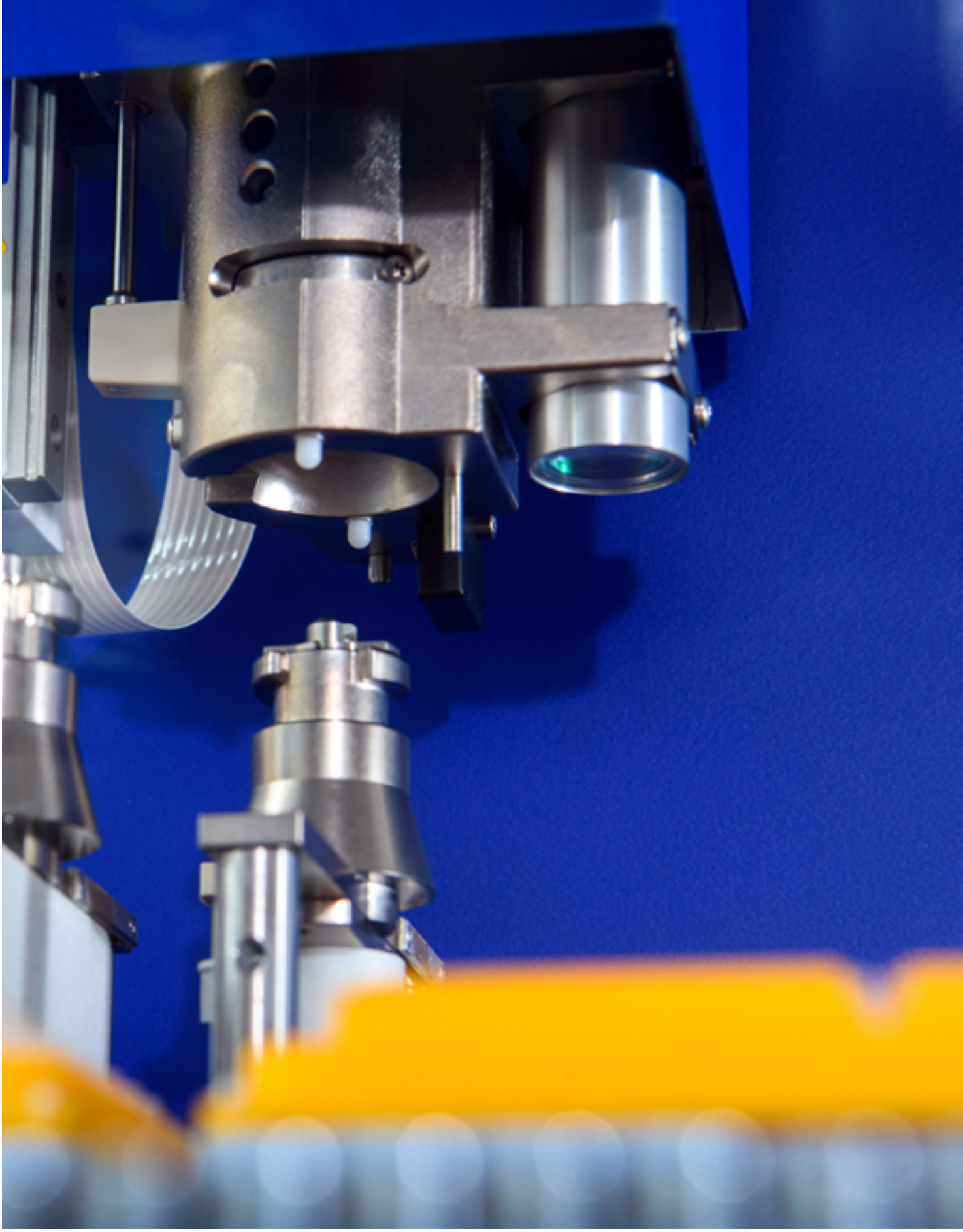






ENABLING DISCOVERIES IN BIOMEDICAL RESEARCH

Technology Platforms are integral to biomedical research at the Max Delbrück Center. When it comes to understanding the molecular basis of health and disease, scientists need access to high-performance instruments and methodological expertise. Technologies for imaging across scales, in-depth molecular analysis of processes in cells, tissues and disease models, computational approaches to analyze these data as well as access to biological material, models or samples from biomedical cohorts are all indispensable in answering today's life science research questions.

TECHNOLOGIES AND DISCOVERIES FOR FUTURE MEDICINE

The Technology Platforms at the Max Delbrück Center offer state-of-the-art imaging, multi-omics technologies and high-throughput analyses, bioinformatics and data analyses, stem cell and organoid technologies, transgenic animal models in combination with phenotyping technologies. They are located at the main Campus in Berlin-Buch as well as at the Berlin Institute for Medical Systems Biology of the Max Delbrück Center (MDC-BIMSB) in the center of Berlin. The Technology Platforms are run by scientists and technology experts. They are key to the implementation of innovative instrumentation and they drive technology development for specific research questions, supporting scientists at the Max Delbrück Center to be competitive and at the forefront of today's research. Scientific Infrastructures support the discoveries for future medicine.

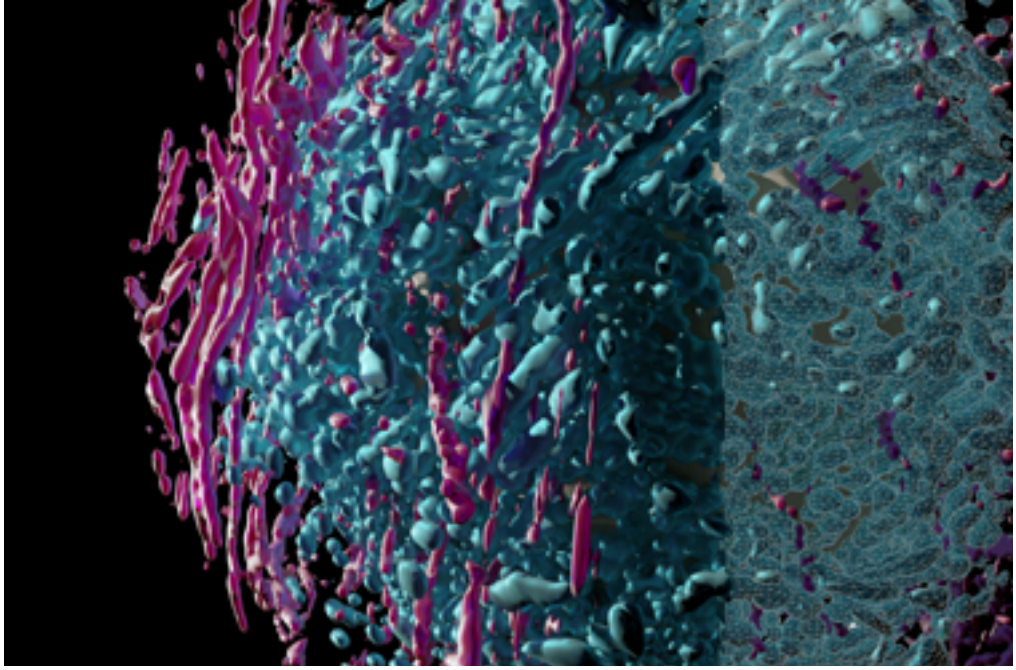
A stand-out characteristic of the Max Delbrück Center's Technology Platforms is the collaborative approach between the Max Delbrück Center and its academic partners. Together with the **Berlin Institute of Health at Charité (BIH)**, the **Charité – Universitätsmedizin Berlin** and the **Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)**, we jointly fund, manage and develop key technologies for the wider research community such as Proteomics, Genomics, Cryo-Electron Microscopy and Chemical Biology. These collaborations foster joint research projects in the Berlin community and provide access to a technology portfolio, which cannot be maintained by individual institutions.

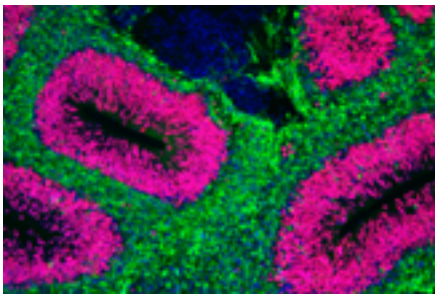
Collaboration within the Max Delbrück Center is also a key aspect, as we seek to integrate scientific approaches with state-of-the-art technologies, even across different Technology Platforms. Methodological workflows are adapted and optimized, and technological approaches are continuously developed in order to best address the research team's projects. The researchers leading the platforms help to design studies, analyses and contribute substantially to publications or publish their developments.

Since young scientists at the Max Delbrück Center need training on new methods and technologies, the Technology Platforms offer regular, general as well as highly specialized training in targeted seminars, workshops, digital materials and hands-on training. In collaboration with research groups, some platforms mentor PhD students and their technology applications. Several platforms also apply for third-party funding for technology-driven projects. Finally, all platforms and research groups are supported by specialized experts in research data management, in order to comply with the Max Delbrück Center's and European policies for managing the research data lifecycle and to enhance FAIR and Open Science policies.

COLLABORATION, TECHNOLOGY DEVELOPMENT AND TRAINING

It is precisely this blend of innovation and customization that makes the technological infrastructure of the Max Delbrück Center such a vital part of its research. The following pages provide an insight into the fascinating opportunities, the range of uses, and the scientific scope of the individual methods and technologies.





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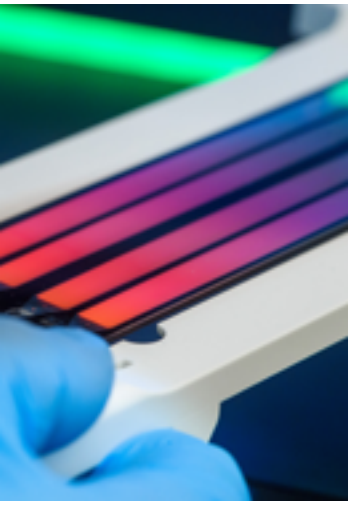
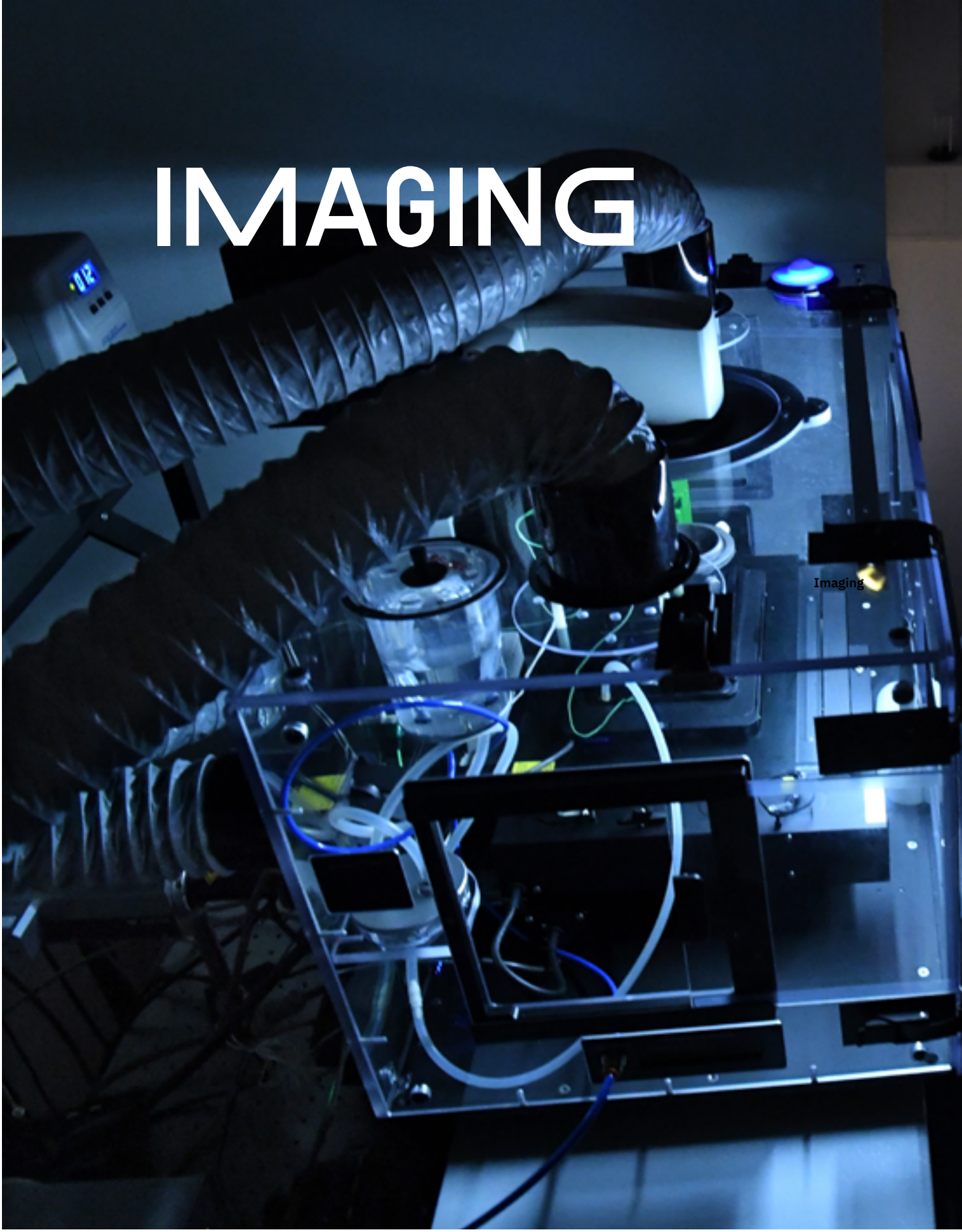
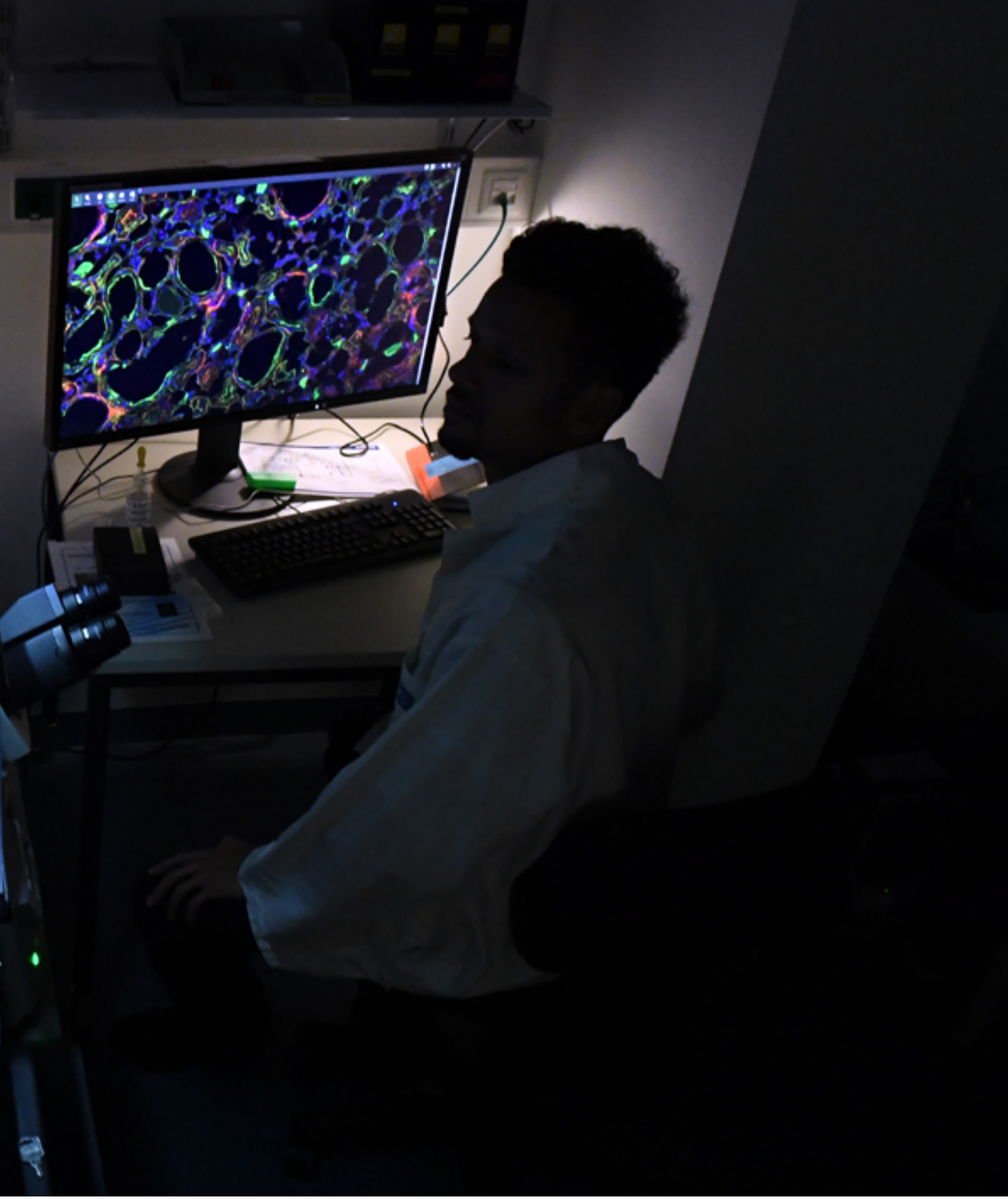


IMAGING





ADVANCED LIGHT MICROSCOPY

Assembly of a chamber for holding the specimen during light-sheet microscopy.

Fluorescence microscopy can reveal both structural and dynamic information about organs, tissues, cells, and even tiny molecular complexes. As such, the technique allows researchers to observe live molecular and cellular interactions in native biological environments.

Modern fluorescence-based microscopy technologies can image a wide variety of biological samples. These techniques use fluorescent molecules to generate images that provide not only structural information about the organization of cells, tissues and organs, but also about the interactions and dynamics of molecules and cells in live specimens. Thus, advanced light microscopy gives researchers the capability to quantitatively explore biological systems on a wide range of temporal and spatial scales.

Our platform at the Max Delbrück Center in Berlin-Buch provides techniques that can image samples from nm scale (super-resolution microscopy), through µm scale (confocal and wide-field microscopy), and up to mm/cm scale (multiphoton and light-sheet microscopy). In seminars and practical sessions, we help scientists to understand the theoretical principles of fluorescence microscopy and to use our microscopes independently. We offer advanced tools for quantitative image analysis and data processing as well as methodological support for research projects – including project planning, sample preparation, imaging workflows, and the establishment of new imaging methods.

A key technology is confocal microscopy, which can visualize the distribution, mobility, and interactions of molecules within intracellular compartments, tissues, and model organisms. Some fluorescent molecules indicate changes in the intracellular environment (such as changes in pH or ion concentration), revealing the heterogeneity of physiological responses to specific conditions in a cellular population. In addition, spinning disk microscopy (a variant of confocal microscopy) allows very fast dynamic processes to be imaged in living specimens.

TRACKING SINGLE FLUORESCENT MOLECULES

Among the super-resolution techniques, total internal reflection fluorescence (TIRF) microscopy allows us to track single fluorescent molecules at the plasma membrane, while stimulated emission depletion (STED) microscopy provides detailed insight into the structure of cellular organelles.

The complex three-dimensional organization of cells within tissues and whole organs can be revealed using light-sheet microscopy. Also, multiphoton microscopy allows not only thick fixed tissue samples to be imaged, but it can also track cells and observe cellular processes in living model organisms.

ADVANCED IMAGING TECHNIQUES AND DATA PROCESSING TOOLS

In recent years, we have collaborated with other research groups to establish a number of new imaging techniques. Specifically, our group has implemented data processing and analysis tools for light-sheet microscopy that keep pace with the rapidly increasing volumes of image data. We are now able to perform 3D morphological analyses of, for instance, mouse spinal cords, hearts and brains, organoids, zebrafish hearts, or even very large samples such as rat hearts. Clearing and active labelling of different organs and organoids was established for user groups.

In multiphoton microscopy, our group has implemented customized methods for intravital imaging in mice that enable researchers to study the interactions between nerve cells and inflammatory cells while simultaneously obtaining functional information on neuronal processes. We have adapted a multi-color excitation and detection technique to image color-coded (“confetti”) mice expressing four different fluorescent proteins in their cells. Label-free imaging based on tissue autofluorescence has also been established.

New technologies are being implemented in our platform to better support research in areas such as translational medicine by imaging and analyzing highly multiplexed markers in mouse and human tissues to support, e.g. spatial omics and phenotyping. Together with the Technolgy Platform for Electron Microscopy, we will establish workflows for correlative light and electron microscopy and data analysis.

OUR TOOLS

- Confocal laser scanning microscopy
- Multiphoton microscopy
- Light-sheet microscopy
- Total internal reflection fluorescence (TIRF) microscopy
- Stimulated emission depletion (STED) super-resolution microscopy
- In-vivo and live imaging
- Fluorescence lifetime imaging
- Laser capture microdissection
- High performance work stations for image analysis and data processing including ML algorithms
- Tissue clearing and active labelling device

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SYSTEMS BIOLOGY IMAGING

Light microscopy is uniquely suited to probe how biological systems are composed and organized. Quantitating (sub-)cellular features and their relationships allows systems biologists to understand the molecular mechanisms that drive development, health and disease.

Light microscopy opens a window into the invisible world. Through technological advances, we now have the ability to visualize living systems across orders of magnitude. Light microscopy allows us to monitor the development of whole organisms, the structure and function of single cells, and the location and dynamics of individual molecules.

RECONSTRUCTING THE LIFE OF THE CELL

The Systems Biology Imaging Platform is part of the Berlin Institute for Medical Systems Biology at the Max Delbrück Center (MDC-BIMSB), located in the center of Berlin. We provide access to and expert advice on advanced microscopy methods, data processing and analysis tools. Our group is working to optimize imaging modalities, increase throughput, and fosters collaborations with scientists and industry partners to bring emerging imaging technologies to the Max Delbrück Center.

One of the most widely utilized instrument class within our platform is confocal microscopy to measure fluorescence from thin 2D slices within a sample. Assembling consecutive “optical sections” allows for reconstruction of 3D volumes that let us measure the abundance and distribution of fluorescently labeled molecules within a sample. For example, a protein that is of interest can be tagged with a fluorescent antibody and visualized to explore subcellular structures, distinct cell types within complex tissues, or disease-related biomarkers.

We also offer a number of technologies that are optimized for live cell imaging. These include wide-field microscopy, light-sheet microscopy, total internal reflection fluorescence microscopies as well as spinning disk confocal microscopy. Researchers often apply these tools to measure physiological processes in living samples, ranging from the biophysical properties of single receptors within the plasma membrane to cell division and lineage tracing in developing embryos.

In the past years, our platform has seen increasing demand to image larger samples with increasing resolution. Although we were able to meet some of this demand with conventional methods, we have adopted several technologies to better image thick samples. The first instrument, an active clearing device, facilitates the uniform removal of lipid membranes in fixed tissue. This dramatically reduces the scattering of light as it passes through the sample rendering it essentially transparent. The second key technology we have established in the platform is a meso-scale light sheet microscope (mesoSPIM) that allows for fast isotropic volumetric imaging of samples ranging in size from millimeters to centimeters.

IMAGING ACROSS SCALES AND MODALITIES

In addition, we have established the computational infrastructure and software tools to handle increasingly large data sets. We have developed specialized processing workflows for image reconstruction, analysis, and quantification. For example, we have established an analysis pipeline to stitch multi-tiled acquisitions of mouse brains, align them with the Allan Brain Atlas, and quantify cell number or expression levels across distinct brain regions.

We will continue to increase the ease of use of advanced microscopy-based assays through automation of instrumentation as well as image data processing and analysis. Our mission remains qualitative and quantitative imaging across scales and modalities.

OUR TOOLS

- Laser-scanning confocal microscopy
- Spinning disk confocal microscopy
- STED (Stimulated Emission Depletion) microscopy
- Lightsheet microscopy
- mesoSPIM (large-format lightsheet microscope)
- Total internal reflection fluorescence (TIRF) microscopy
- Laser Capture Microdissection / Live cell isolation microscope
- Live-cell wide-field microscope
- Tissue clearing / labelling devices
- 3D printing / microfluidics manufacturing

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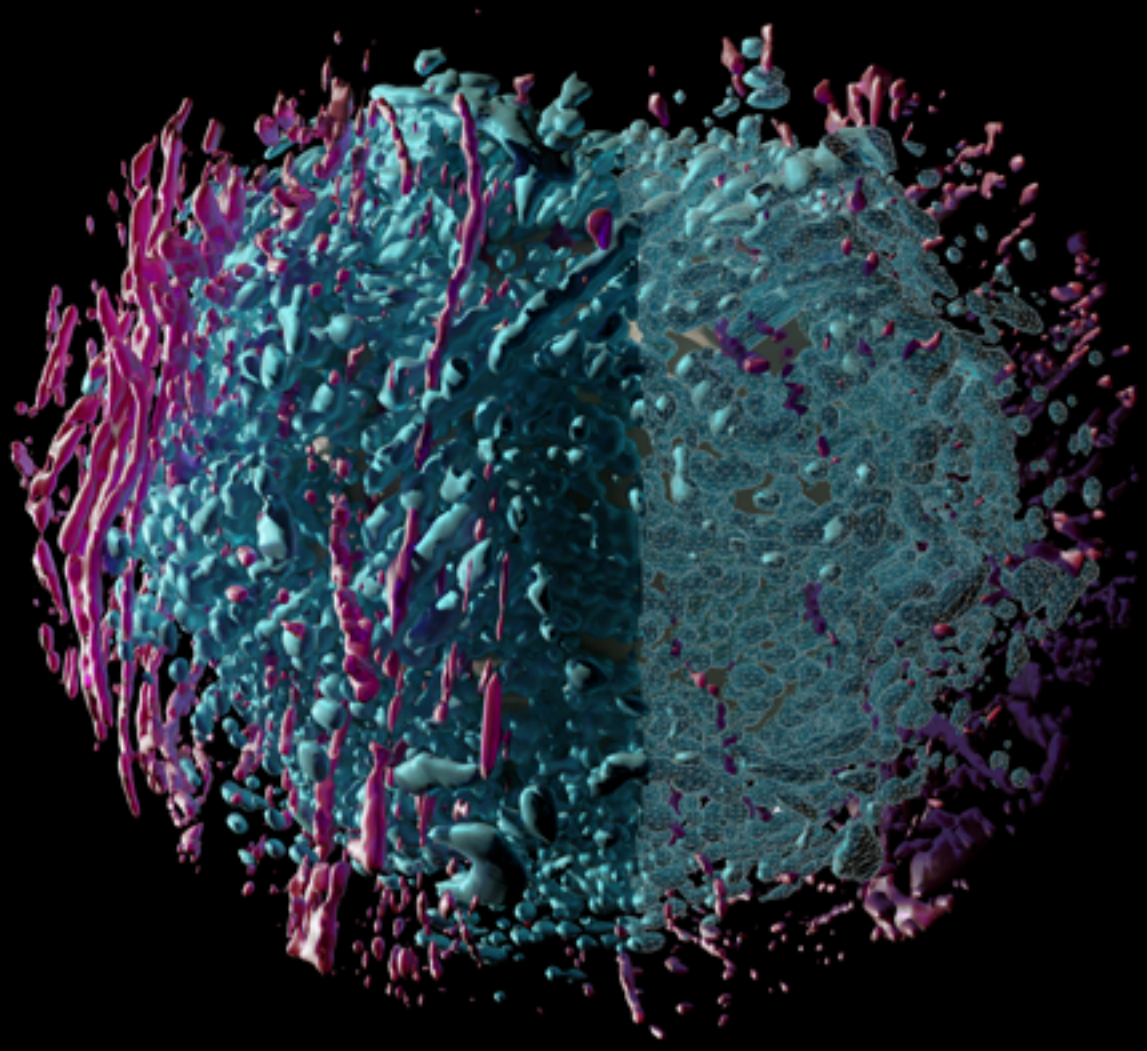
IMAGE DATA ANALYSIS

Pictures contain a wealth of scientific information. We build algorithms and other computational tools to efficiently extract and analyze data from images, which can help researchers see their data in entirely new ways and make unexpected discoveries.



To test the performance of our visualization algorithms we generated this synthetic image. The image data is 2-channel and 3D, we have also computed meshes for the segmentations of each channel.

From individual molecules and cells, to whole organisms and ecologies, image data is acquired across all scales. Images are distinctly useful because they capture spatial relationships, between, for example, two particles, or many cells in an organ. That information, especially when combined with other streams of data like gene expression, can tell researchers about what is happening, when and where in the body in much greater detail than before. We create the digital tools that help researchers access this deeper insight. As part of **Helmholtz Imaging**, all of the tools we offer benefit researchers across the entire Helmholtz Association.

A PICTURE IS WORTH A THOUSAND WORDS

Helmholtz Imaging is connecting scientists working with imaging data across all Helmholtz centers and beyond. Our team is visualizing data sets with a keen eye on the use case – when dealing with high-throughput imaging, processing speed is of priority, in other cases a time-consuming rendering can transport the beauty of a scientific discovery in detail. Sometimes specific attributes of detected objects in an image are highlighted, at other times the uncertainty of machine learning based results requires visualization.

Images are not only enabling scientific advances, they tell a story and can build bridges to society. Thereby generations, used to capture their environment on digital images, can be empowered to benefit from and contribute to scientific projects. Our platform offers support for image-based citizen science and frugal science projects by contributing our expertise in transporting messages through visualizations and in analyzing a variety of image data sources. Additionally, we establish contact to available shared infrastructure, provided by the Helmholtz Incubator Platforms.

FROM PIXELS TO INSIGHTS

The Image Data Analysis platform at the Max Delbrück Center explores computational strategies for processing, visualizing and analyzing diverse sources of image data. Image data may be 2D, 3D, or more generically N-dimensional, where additional dimensions may include time, channels, genes, or more. We use modern computing techniques, such as machine learning and virtual reality, to help researchers engage with their data in new ways.

Many teams are taking sequences of 3D images to capture dynamics of biological processes on the microscopic level. Often, this can generate volumes of data that quickly exceed the capacity of standard computer memory. We are developing solutions to enable researchers to view and analyze their extremely large data sets.

For example, we are incorporating machine learning to help detect cells in static images, track cells over time, infer properties, and make predictions. This allows us to transform large data sets into compact and efficient representations, such as cell trajectories, which can be easily analyzed.

The goal is to develop a universal framework that enables image analysis algorithms and tools across a broad range of spatial scales of image data. A key challenge will be to ensure the framework is adaptable and can keep pace with the rapid advancements in imaging and computing power.

OUR TOOLS

- Algorithm design and implementation
- Image processing pipelines
- Visualization
- Machine learning analysis
- Image-based models
- Computer graphics

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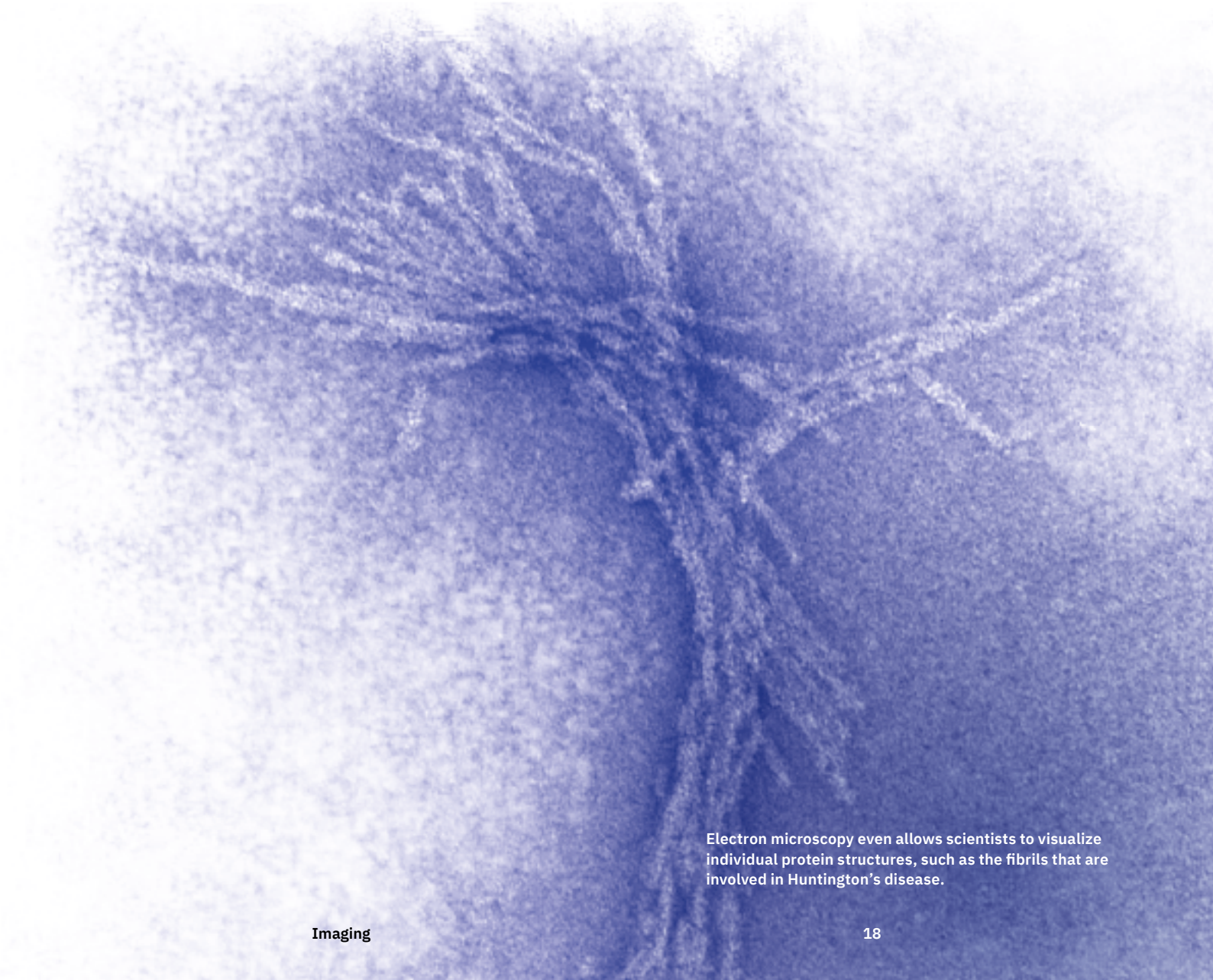
OUR PARTNER

HELMHOLTZ IMAGING

ELECTRON MICROSCOPY

With a resolution of less than one nanometer, electron microscopy allows researchers to peer deep into biological samples and visualize ultrastructural details without losing information about the cellular context.



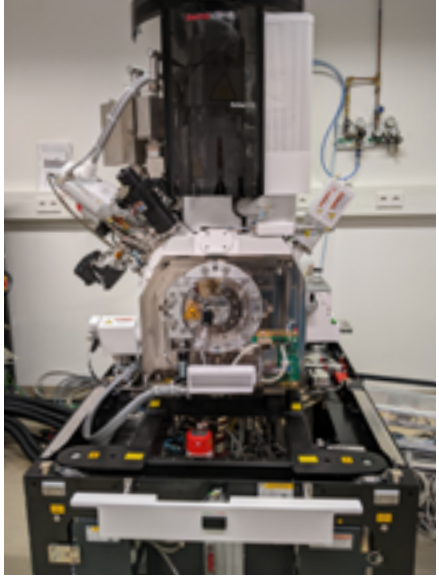
Electron microscopy even allows scientists to visualize individual protein structures, such as the fibrils that are involved in Huntington's disease.

Electron microscopy (EM) is an indispensable tool in many fields of cell biology and medicine for studying subcellular structures in normal and diseased states. However, since the electron beam is only stable in a high vacuum (a vacuum with very low pressure), it is impossible to image living cells or tissues. Samples have to be chemically fixed and embedded, or immobilized through very low (cryogenic) temperatures – while retaining biological structures in a close-to-native state.

DEPLOYING THE ELECTRON BEAM

Our group offers a set of EM methods to explore manifold specimen types spanning from human biopsies to model organisms, such as mice, zebrafish, and fruit flies. Imaging modalities range from Transmission EM (TEM) to Scanning EM of tissue blocs. The data obtained by EM offers detailed context information about the ultrastructure of cells and how organelles interact. Immuno-EM techniques or correlative light and electron microscopy (CLEM) methods combine functional information on protein identity with the underlying morphological features at nanometer resolution.

Using these tools, our group has been investigating heart and muscle cell disorders, stem cell architecture, and myelin defects in the brain. We have visualized neurodegenerative proteins as well as nanoparticles for drug development, and characterized various cell cultures as well as tissue models including organoids. EM is a powerful technique to investigate mitochondrial networks within the cell or organelle-organelle contacts. For many projects, available protocols had to be adapted or new sample preparation strategies developed. This includes high pressure freezing combined with freeze substitution which is particularly suited for preserving the subcellular architecture. Other improvements during sample preparation yield



Fib-SEM (Focused Ion Beam-Scanning Electron Microscope) capable of imaging cellular organelles in 3D at 3-4 nm isotropic resolution.

better preservation of antigenicity for immuno-EM studies. Our extensive knowledge on multiple sample types and numerous EM techniques makes it possible to adjust protocols for each research question.

A 3D PLUNGE INSIDE THE CELL

We offer several approaches to obtain high-resolution information in 3D, like tomography of sections with TEM or array tomography of serial sections by SEM. Especially suitable to resolve organelles or whole cells in 3D is FIB-SEM (Focussed Ion Beam Scanning Electron Microscopy). It can be used to achieve a resolution of up to 3nm isotropic voxel on volumes of 1-20µm^3. Our new device, installed in 2023, will be equipped with a powerful plasma-FIB, which is able to mill quicker and larger areas as well as improve working with CLEM-compatible resins. We will be thus able to visualize small cellular compartments, such as vesicles and mitochondria, as well as bigger cellular arrangements of complex tissue in 3D.

In the future, we will further strengthen the use of CLEM approaches to target rare or transient events in 3D to help answering complex research questions in health and disease.

OUR TOOLS

Electron microscopes:

- TEM @ RT:
  - 200kV JEM-2100 Plus (Jeol) equipped for tomography with 20MP CMOS camera “Xarosa”
  - 80kV Morgagni (FEI) with 11MP CCD camera “Morada”
  - 80kV EM 910 (Zeiss) with 11MP CCD camera “Quemesa”
  - TEM @ cryo temperatures:
  - 120kV Talos L120C (Thermo Fisher Scientific (TFS)) with 16MP Ceta CMOS camera
- SEM:
  - Helios 5CX (TFS). (Gallium-) FIB-SEM: detectors for SE and BSE, STEM and array tomography (Shared investment with FMP)
  - Helios 5 Hydra (TFS): Plasma-FIB-SEM

Sample preparation:

- Ultramicrotomes for room temperature and cryo sectioning
- High pressure and Freeze substitution systems
- Grid plunger for cryo immobilization
- Carbon coater & sputtering unit, Glow discharge unit
- BioWave for improved sample processing

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CRYO-ELECTRON MICROSCOPY

Cryo-electron microscopy (cryoEM) visualizes nanometer-sized biological molecules at a resolution as detailed as that of X-ray crystallography. Thus, it enables structural biology to venture inside the cell.

Cryo-electron microscopy (cryoEM) has become an essential tool in structural biology. It enables scientists to look at biological samples in their native state with near atomic resolution, allowing them to study the precise structure and function of complex cellular machineries such as the ribosome, the “protein producer” of the cell. More recently, a related method, cryo-electron tomography (cryoET), has been gaining attention as it allows these structures to be observed in their cellular environment. Here, we have a joint infrastructure and team, based on collaboration agreements between the **Charité – Universitätsmedizin Berlin**, the Max Delbrück Center as well as the **Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)**.

FLASH-FROZEN PREPARATIONS IN THEIR NATURAL STRUCTURES

In order to perform cryoEM, biological samples are cooled so quickly that ice crystals cannot develop. Instead, glass-like water forms, tightly enclosing the molecules in their natural state. The electron beam of the microscope can penetrate the amorphous layer. A camera installed in the lower part of the microscope records the image and a computer calculates an exact three-dimensional image of the molecule from the often hundreds of thousands of photos. For conventional electron microscopy, samples must first be dehydrated, chemically fixed and stained with heavy metals to enhance image contrast. Consequently, scientists have to interpret artefacts. CryoEM, on the other hand, makes it possible to analyze individual molecules in their native aqueous environment and observe how they interact with each other. This way, CryoEM bridges a gap to elucidate biological structures.

Glimpse into the vacuum chamber of the Aquilos2 dual beam microscope.

Our team has implemented a special tomography workflow: First, we make the sample carriers hydrophilic by glow-discharging in a plasma. Then, we mark the areas of the carriers on which the target cells should grow. To estimate the best time to flash-freeze the cells we use live cell imaging. In a next step, we identify the areas in the cells that should be imaged by confocal cryo-fluorescence light microscopy and mill these out using a focused ion beam (FIB). Finally, we can obtain three-dimensional images of these cell lamellae at high resolution using a Titan Krios cryo-transmission electron microscope. This microscope is also suitable for single particle analysis, a technique that allows structural analysis of biological samples at near atomic resolution.

TO MOVE FROM IN VITRO TO IN SITU

The facility with its exceptionally sensitive core instrumentation opened its doors for users in spring 2021. We provide data of highest quality to our users and streamline the new and challenging in-situ cryo-electron tomography workflow. Our aim is to advance structural biology in a methodical way, to move from in vitro to in situ, that is, to observe biological processes directly in the cell.

OUR TOOLS

- Thermo Fisher Titan Krios G3i 300 kV cryo-TEM equipped with energy filtered Gatan K3 and Thermo Fisher Falcon III direct electron detectors and Volta phase plate
- Thermo Fisher Aquilos 2 cryo-FIB-SEM equipped with a Kleindiek Micromanipulator
- Leica SP8 confocal cryo-light microscope for correlative microscopy
- Leica DMi8 light microscope equipped with incubation chamber and Alveole PRIMO micropatterning device
- High performance computing infrastructure including advanced Windows and Unix workstations, several PB storage, and a dedicated GPU / CPU cluster with 4.5 TB RAM, 240 CPU cores and twelve Tesla V100 GPUs.

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OUR PARTNERS

MAGNETIC RESONANCE

Our group carries out interdisciplinary imaging projects using small animal and whole-body human MR scanners with high and ultrahigh field strengths to explore organs and tissues in novel ways. Our aim is to support new approaches to imaging-based diagnosis, disease interception and therapy.

Clinical application of an in-house built radio-frequency coil array for investigating the heart at 7 Tesla.

Magnetic resonance imaging (MRI) is a mainstay of clinical diagnosis and biomedical research. The approach uses magnetic fields and radio frequency (RF) signals to depict tissues and organs. Research applications are rapidly expanding thanks to constantly increasing magnetic field strengths, customized RF antenna arrays, faster imaging and smarter image processing. Higher spatial resolution combined with the capacity to image new substances non-invasively is providing new insights into healthy and pathological processes under in vivo conditions.

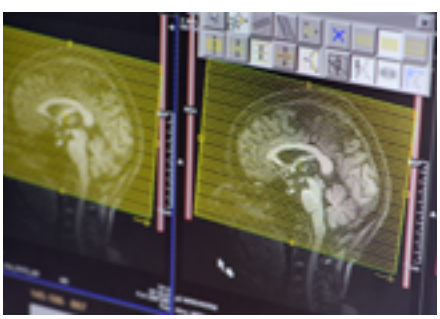
BRINGING MRI TO NANO-SCALE PROBES

The Berlin Ultrahigh Field Facility (B.U.F.F.) at the Max Delbrück Center provides advanced MRI for interdisciplinary research projects using small animal and whole-body human MR scanners. We carry out human MRI on 7-Tesla and 3-Tesla instruments, and animal MRI at 9.4 Tesla. Significant gains in field strengths are enabling us to image new substances and nano-scale probes. We are also expanding imaging to new organ and model systems by custom-designing novel types of MR detectors (RF antennae).

The facility has reception areas and changing rooms for volunteers, and all the technical prerequisites for clinical studies, including emergency equipment and extra patient monitoring units. This has allowed us to take on an important role in major research initiatives, including Helmholtz Imaging, the large-scale population imaging study of the German National Cohort, the Collaborative Research Center 1365, the Helmholtz International Research School iNAMES and a number of other national and international projects devoted to various health conditions.

MAPPING THE HEART AND KIDNEY IN NEW WAYS

B.U.F.F. is collaborating with other research groups to develop new methods for mapping anatomy, morphology, microstructure, function, physiology, and metabolism in animal and human subjects. By achieving new levels of spatiotemporal resolution, and imaging new substances such as fluorine, sodium and potassium, we are able to conduct groundbreaking studies on organs such as the heart, kidney and brain. We have opened an entirely new research field with our thermal phenotyping project, supported by an Advanced Grant of the European Research Council (ERC), to characterize the temperature profiles in biological systems and diseases. One aim of the project is to use MRI to manipulate the temperature of tissues and utilize this parameter as a potential diagnostic and therapeutic tool.



Examinations need to be carefully planned, as in the case of advanced brain imaging (above).

OUR TOOLS

- Human MRI at 7 Tesla
- Human MRI at 3 Tesla
- Animal MRI at 9.4 Tesla

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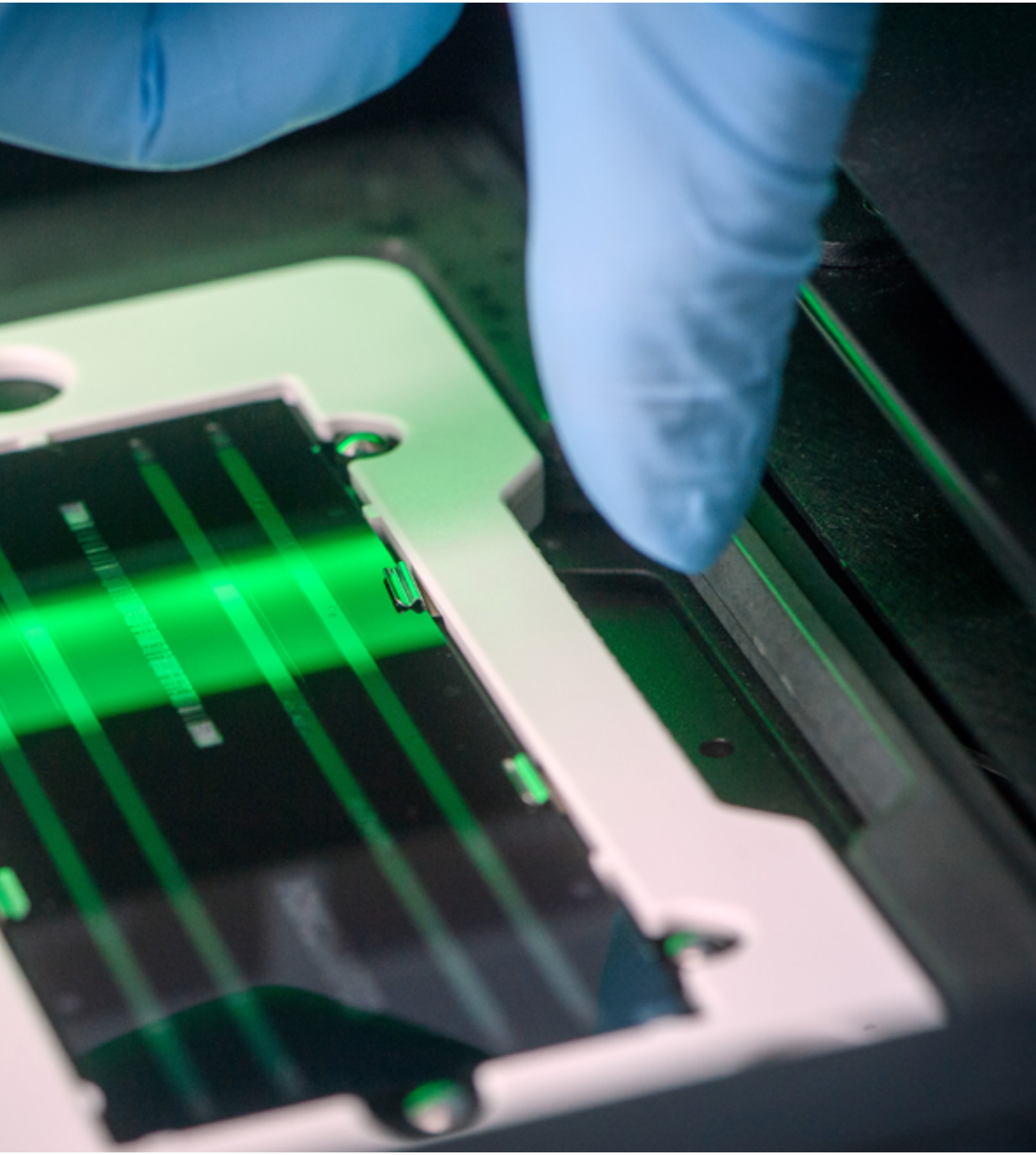
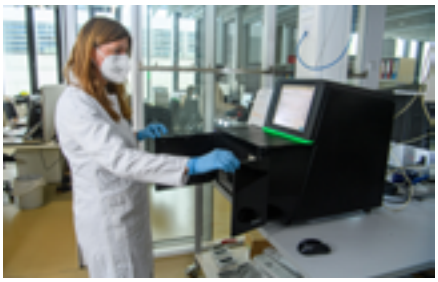
OMICS AND ANALYSIS



GENOMICS

Advanced DNA and RNA sequencing technologies can determine the genomic sequence and gene expression profiles of individual cells in a complex tissue, organ, or entire model organism. This enables researchers to study the genetic basis of health and disease at unprecedented resolution.



Oxford Nanopore Technologies PromethIon Chip (left), Illumina Next Generation Sequencing in operation (right)

High-throughput sequencing technologies have transformed biomedical research. Today, it is possible to analyze a human genome in a fraction of the time and cost that would have been incurred just 15 years ago. Tremendous improvements in sensitivity enable us to sequence the DNA or RNA that is present in the nucleus of a single cell. Genomics – the systematic and quantitative study of genes and how they are regulated and transcribed – has thus become an everyday tool in basic science and clinical research.

UNDERSTANDING THE GENOME AT UNPRECEDENTED LEVEL

The Genomics Platform is a joint facility of the Max Delbrück Center and the **Berlin Institute of Health at Charité – Universitätsmedizin Berlin (BIH)**. We provide support to researchers from the Max Delbrück Center and Charité and are committed to implementing key sequencing technologies and establishing state-of-the-art service pipelines.

Over the last decade, the spectrum of NGS methodology applications has increased considerably. Besides resequencing and transcriptome profiling, NGS readout can identify epigenetic modifications that regulate the activity of individual genes, determine chromatin structure and spatial organization, analyze nucleic acids binding properties of transcription factors, or monitor CRISPR-based gene editing, to name a few examples. Most of them currently rely on short-read sequencing (Illumina platform). A long-read approach is beneficial for phased and de novo genome sequencing, or to analyze mRNA isoforms and splicing (Oxford Nanopore and PacBio platforms). We operate all three platforms and offer commonly used as well as customized library preparation workflows to our users.

Single-cell technologies are a major focus of our work. Our integrated FACS unit enables us to select and isolate cells on site. A broad range of state-of-the-art single-cell genomics applications is available for downstream analyses, including the detection of gene expression profiles, open chromatin regions, DNA methylation patterns, cell surface epitopes, or T- and B-cell receptor sequences.

Combinations of these features can be detected from the same single cell, and for thousands of cells in parallel. These molecular signatures provide a comprehensive view on the individual cell types that form a complex tissue, on their functional states and developmental trajectories. New spatial transcriptomic approaches allow us to assay individual cells in their spatial context, to reconstruct tissue architecture and its changes in response to environmental cues or in disease states. Single-cell genomics technologies are evolving particularly rapidly. We constantly implement novel techniques in collaborative research projects.

TOWARDS PATIENT-SPECIFIC GENETIC PROFILING

The Genomics Platform is closely collaborating with clinical researchers to translate these technologies into systems medicine. By providing a multi-omics view on the pathophysiology of patient samples we help clinical scientists to delineate the underlying causes of diseases like cancer, cardiovascular events, or neurological disorders, to identify novel diagnostic markers, and to derive and evaluate new treatment options. Our long-term goal is to develop clinical pipelines that enable treatments tailored to the needs of individual patients.

OUR TOOLS

- Illumina short-read sequencing
- Pacific Biosciences and Oxford Nanopore long-read sequencing
- Single cell and spatial technologies (FACS, 10XGenomics Chromium, SmartSeq3, G&T-Seq, scEMseq-Seq, 10XGenomics Visium and Xenium, Nanostring GeoMx and CosMx)

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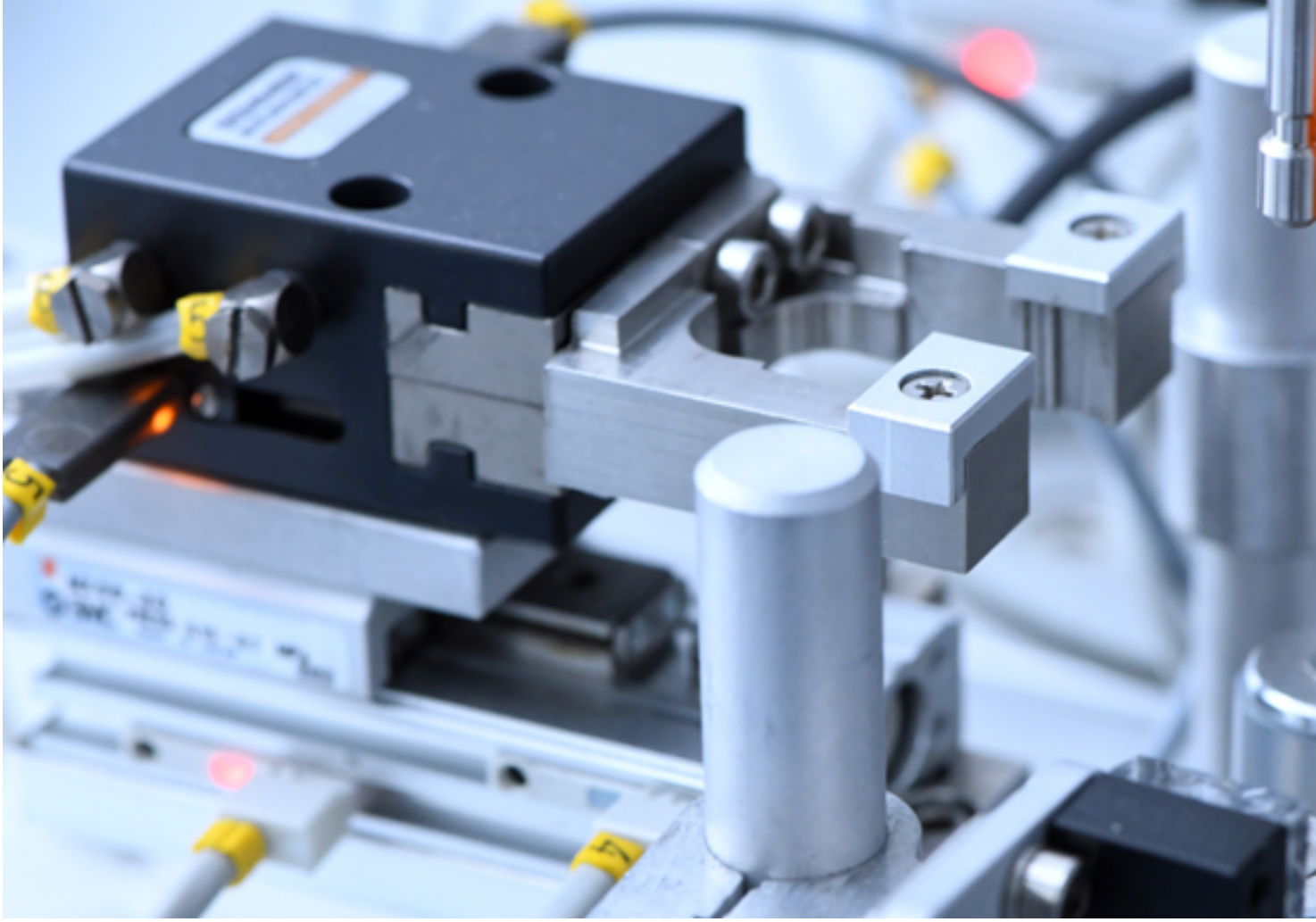

A custom-built robot enables automatic sample handling in large metabolomics studies.

METABOLOMICS

Metabolomics can tell complex stories about the interactions of small molecules – the metabolites – in cells and body fluids. This allows researchers to investigate diseases, their causes, and potential treatments based on a systematic global analysis of the biochemical changes that are associated with them.

During the life of an organism, a myriad of small molecules arise as intermediates and products of metabolism. Metabolomics – the systematic study of these metabolites – aims at elucidating the chemical fingerprints that specific cellular states and processes leave behind, thus enabling a complex and in-depth understanding of health and disease. Metabolomics significantly relies upon mass spectrometry, an analytical technique that measures large numbers of metabolites simultaneously to characterize the molecular

composition of a biological sample. Complex statistical and modelling methods are used to interpret the results and explore an organism's molecular response to change caused by disease, pharmaceuticals, diet or environmental factors.

MASS PROFILING OF BIOCHEMICAL SYSTEMS AND PATHWAYS

Our platform is affiliated with the Max Delbrück Center and fully funded by the **Berlin Institute of Health at Charité – Universitätsmedizin Berlin (BIH)**. It provides a number of state-of-the-art mass spectrometers alongside bioinformatics and statistical support. Our tools are suited for both targeted and untargeted metabolomics approaches and we place a heavy emphasis on quality management. Untargeted approaches attempt a global analysis of a large numbers of metabolites in an unbiased way while targeted methods measure a smaller number of specific compounds, using optimized analytical methods to enable maximum detection sensitivity and quantification.

Our group specializes in targeted analyses based on gut-immune pathways, including short chain fatty acids and tryptophan metabolites. We also offer wider analyses of central carbon metabolites and Biocrates steroids, as well as bile acids. In addition, we support researchers who are interested in applying metabolomics approaches to lipid metabolism, a branch known as lipidomics. We work hard to develop new methods in response to the requirements of our collaborators.

We are currently developing new methods of analysis, including breath and volatiles analyses, and real-time analysis using rapid evaporative surface ionisation.

PICTURING CELLULAR AND SUBCELLULAR METABOLISM

Current challenges in the field of metabolomics include the need for better and more rapid identification of unknown or unidentified signals. In addition, there is increasing interest in metabolomics analyses of single cells, or even specialized subunits of the cell (organelles), such as mitochondria. These developments entail the need for new and refined approaches and protocols, including strong attention to quality management to ensure robust and reproducible results. We are developing metabolic imaging methods and are exploring new technologies for ever greater resolution of metabolic information for low volume, cellular and sub-cellular analyses. We are open for collaboration with other scientific groups that complement our research interests.

OUR TOOLS

- Gas chromatography mass spectrometry
- Liquid chromatography mass spectrometry
- Direct infusion mass spectrometry
- Liquid extraction surface analysis mass spectrometry (LESA-MS)
- Thermal Desorption for volatiles analysis
- Rapid evaporative ionization mass spectrometry (REIMS)
- Desorption ionization mass spectrometry (DESI)
- Ion mobility

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This precision device, which couples liquid chromatography and mass spectrometry, is used to identify and quantify proteins.

PROTEOMICS

Our platform provides high-throughput techniques to study the basic function and regulation of proteins in health and disease. This helps to identify new candidates for novel diagnostic and therapeutic approaches.

Proteomics enables the global and targeted analysis of all proteins and their functional states. Proteins are central to most biological functions within an organism and frequently alter in their abundance and in their activation status in disease. Thus, proteins are routinely used as disease biomarkers as well as drug targets. To elucidate how proteins work, proteomics approaches make use of mass spectrometers. These high-throughput instruments allow proteins extracted from cells, tissues, and blood samples to be identified and quantified. In addition, it is possible to explore the binding partners of proteins, their localization in cells and tissues, and their functional activity.

UNDERSTANDING HOW PROTEINS WORK

Our platform combines resources and services of the Max Delbrück Center and the **Berlin Institute of Health at Charité (BIH)**, develops mass spectrometry-driven proteomics methods and makes this technology available to partners at the Max Delbrück Center, **Charité – Universitätsmedizin Berlin**, and beyond. The most common application in our group is the generation of global quantitative inventories of all proteins in cell or tissue samples, with the highest possible coverage and throughput for various biological or clinical states. A special focus of our work is on improving multiplexing methods that enable us to analyze several samples at the same time, allowing us to either spend more time on an experiment and dig deeper into the proteome, or to analyze more samples at an even faster throughput.

We can also determine the activation status of the thousands of proteins we measure by detecting biochemical modifications, such as phosphorylation, ubiquitination, or acetylation groups in these proteins. Such modifiers act like molecular switches that turn functional states on and off.

Investigating proteins with sensitive and comprehensive methods (“deep coverage”) is crucial for connecting information about proteins with DNA-sequencing and RNA-sequencing information, since many genes that cause disease (and thus their protein products) occur at very low abundances. Reading out the consequences of genetic alterations on the protein level helps researchers to understand which mutations are possible drivers of disease and to identify potential drug targets.

For altered genes that have no known function in a certain context, it is also valuable to analyze the interaction partners of proteins. These may offer clues about specific roles in disease-causing processes. To validate initial discoveries, we employ targeted mass spectrometry methods that allow even higher throughput and could be used for biomarker analyses in future clinical studies.

CLOSING THE PERFORMANCE GAP

By augmenting throughput and coverage of proteomics approaches, we have contributed to closing the performance gap with regard to genomics techniques. Specifically, we have been able to demonstrate that proteomics analyses allow the reinforcement of findings from genomic sequencing studies (e.g. in cancer genomics), and enable researchers to gather additional information on precise disease mechanisms, such as how molecular signals and pathways are deregulated in a diseased state. A key task for the future will be to further increase the sensitivity of these techniques to make them applicable in routine clinical biopsy samples, possibly down to the single-cell level.

OUR TOOLS

- Proteomics of cell lines, tissues and plasma samples
- Label-free (DDA/DIA) and TMT-based global protein quantification
- Targeted protein quantification
- Post-translational modification analysis
- Ultrahigh-pressure liquid chromatography
- High-resolution orbitrap and timsTOF mass spectrometry
- Multi-omic and proteogenomic approaches
- Pathway and signaling bioinformatics
- Olink Target 96 assays

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PROTEOMICS AND METABOLOMICS

We use quantitative and time-resolved methods to understand the dynamics of biochemical pathways and networks at the molecular level. These studies will help us decipher the crosstalk between metabolism and gene regulation.

Interactions between genes, proteins, and metabolism are a key part of cellular processes. It is now possible to study the manifold molecular species and correlations in the cell at a systematic and quantitative level. Technologies such as mass spectroscopy allow for large-scale analysis of cellular systems. Whereas proteomics (a term coined on the analogy of "genomics") aims at the large-scale study of proteins and their functional networks, metabolomics is concerned with the intermediates and products of metabolism. Investigating the intertwined molecular systems permits a comprehensive and more complex understanding of metabolic regulation. We characterize the metabolic state of cells to elucidate its impact on basic processes such as cell differentiation and cancer formation.

CELLULAR CROSSTALK IN HEALTH AND DISEASE

The integrative proteomics and metabolomics research and technology platform is an integral part of the Berlin Institute for Medical Systems Biology at the Max Delbrück Center (MDC-BIMSB).

We are a team of biochemists, engineers, and bioinformaticians who have established a number of mass spectrometry-based proteomics and metabolomics methods that allow us

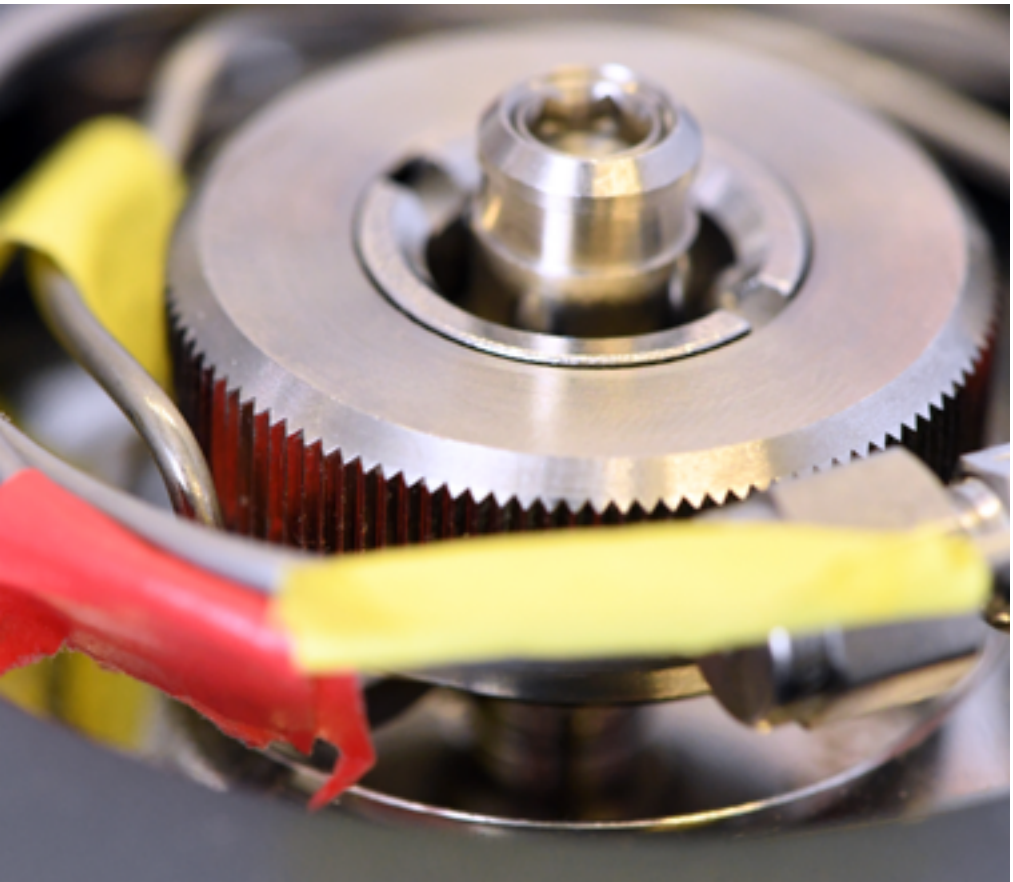
to analyze the dynamics of metabolism in vitro and in living organisms. For proteomics studies, we apply mass-spectrometric techniques that either use stable isotopes for labeling proteins or that are label-free in a global scale. We have developed and patented a specific workflow called pulsed stable isotope-resolved metabolomics (pSIRM). This method provides dynamic, time-resolved quantitative measurements of the central metabolism in in vitro and in vivo investigations.

Specifically, our group is interested in the crosstalk between metabolism and gene regulation during cellular differentiation and disease formation. We are focusing on central metabolism, a set of core biochemical pathways that is highly flexible and continuously adjusted to the physiological programming – and reprogramming – of the cell. Conversely, the metabolic state and metabolic modifications can influence gene and protein expression.

PINPOINTING THE ROLE OF METABOLIC STRESS

Moving forward, we will intensify our efforts to explore this crosstalk between metabolism and gene regulation using a systems biochemistry approach. The analysis of the biochemical network at multiple levels enables us to identify molecular targets and regulatory mechanisms, for example within the cancer metabolic network as well as metabolic regulation during the development and adaptation of immune cells. We are also part of the MSTARS consortium. We aim to further improve our methods and data integration workflows. Advances in proteomics and metabolomics techniques may allow us to derive interactions at the single-cell level in healthy and in cancerous tissues. This is a joint effort with the Systems Biology Imaging Platform, the Rajewsky lab and the single cell focus area at MDC-BIMSB.

Temperature-controlled injection port, which is an important instrumental component for performing gas chromatographic analyses.



OUR TOOLS

- Gas chromatography mass spectrometry based metabolic profiling
- Stable isotope resolved metabolomics analyses of central metabolic pathways
- Quantitative shot-gun proteomics analyses

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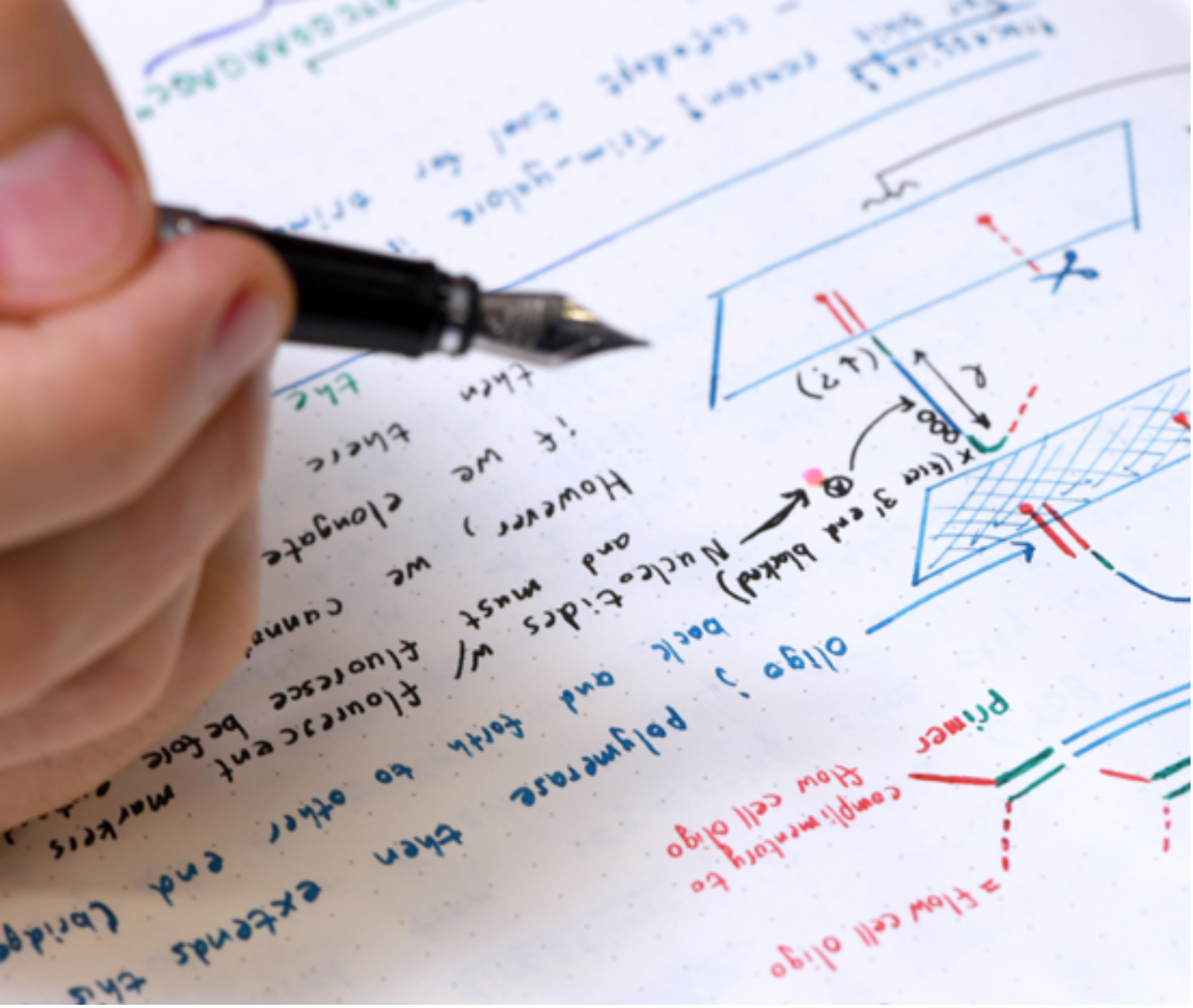

BIOINFORMATICS AND OMICS DATA SCIENCE

The application of bioinformatics tools, statistics, and machine-learning methods is a prerequisite for today's data-driven biomedical research. Computational analyses of large data sets from high-throughput experiments may help to unravel complex diseases.

High-throughput technologies in molecular medicine, such as single-cell genome sequencing, produce vast amounts of data that may help to characterize complex diseases. However, powerful bioinformatics tools for data analysis and interpretation are necessary to understand these high-volume data sets. The application of statistics and machine learning methods to experimental results on a broad scale has thus become an essential part of biomedical research.

Bioinformatics team members are discussing data analysis strategies. Handwritten notes and sketches can help clarify various processes.

STUDYING BIOLOGICAL MECHANISMS WITH COMPUTATIONAL TOOLS

The Bioinformatics Platform and Omics Data Science is a research group within the Berlin Institute of Medical Systems Biology at the Max Delbrück Center (MDC-BIMSB). The platform creates and maintains bioinformatics tools and databases. It studies molecular mechanisms using externally available and/or in-house techniques. In addition, the platform provides collaboration opportunities and support for scientists at the Max Delbrück Center.

Specifically, we supply infrastructure and expertise for the bioinformatics and scientific IT needs of researchers, providing hardware and software for research-oriented tasks. We have various web-based interactive tools at our disposal, e.g. Genome Browsers (Shiny Server for interactive analysis, and an internal Galaxy server). We also develop and maintain reproducible software repositories for all users at the Max Delbrück Center.

We provide additional specialized IT services geared towards bioinformatics end users as well as a mobile teaching system for scientists. In addition, we offer courses and consultation sessions on bioinformatics, IT skills, and programming.

UNRAVELING COMPLEX DISEASE

As a general principle, we aim at gaining an understanding of genetic and epigenetic control mechanisms of cellular differentiation and of complex diseases, such as cancer, by using Artificial Intelligence to sift through high-throughput data sets. These data are derived, for instance, from microarrays, single-cell and other next-generation sequencing experiments. Ultimately, we aim to uncover genomic and epigenomic anomalies and the interactions between them that lead to disease. In doing so, we strive to maintain state-of-the-art expertise in computational biology and machine learning applications, as well as to develop bioinformatics tools and techniques that are relevant, reproducible, and well documented.

OUR TOOLS

- Local copy of UCSC Genome Browser and track hubs to display genomics data (genome.mdc-berlin.de)
- Galaxy server for guided user interface for bioinformatics analysis
- Shiny Server for interactive analysis
- Deep Learning tools such as Janggu & maui and R package such as methylKit; genomation; RCAS; netSmooth
- Reproducible bioinformatics software deployed at workstations and within the cluster via GNU Guix (guix.mdc-berlin.de)
- Virtual machines that can deploy publication-related web apps and databases such as DoRiNA and circBase

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TRANSLATIONAL BIOINFORMATICS

Our group provides bioinformatics expertise and workflows for turning large-scale biomolecular data sets into clinically relevant information. In close collaboration with researchers and clinicians, we aim to create knowledge and systems that lead to more patient-specific treatment regimens.

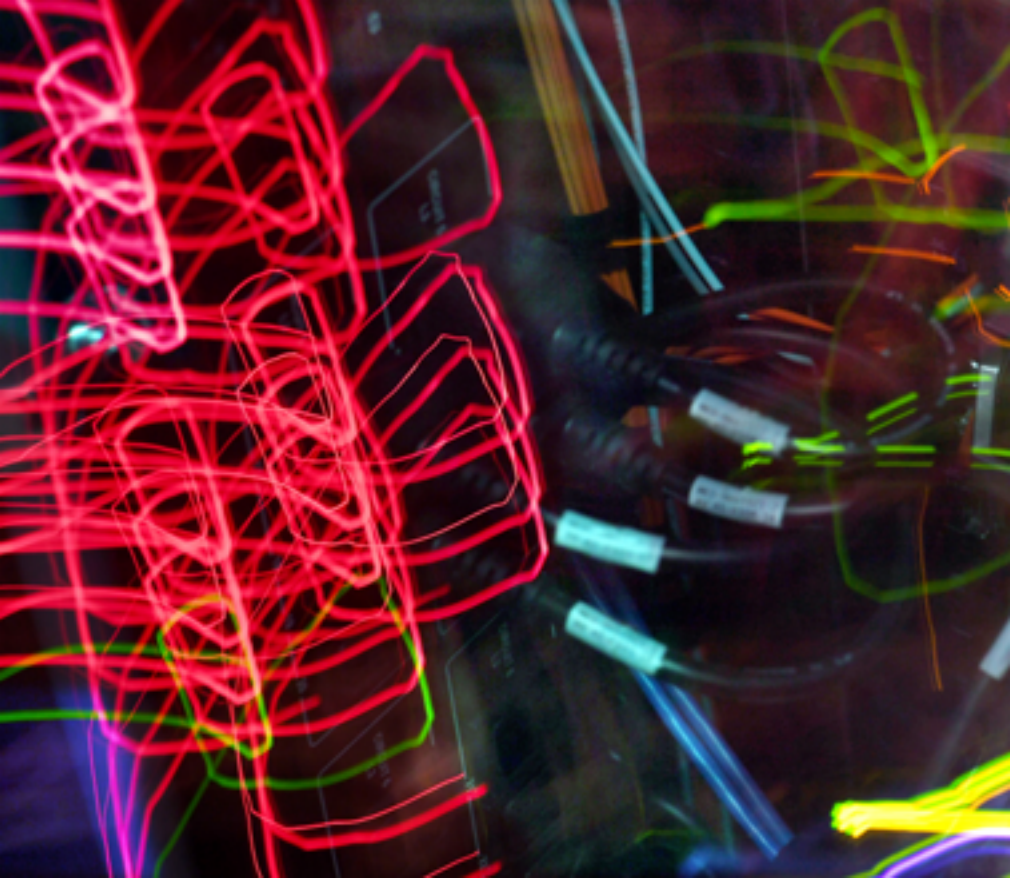
Discussing data analysis results and integration strategies forms an important part of the group's research and services.

Sophisticated bioinformatics analysis plays a key role in understanding the molecular mechanisms of disease and in translating complex biological data into clinically relevant information. While bioinformatics in general has long been essential for handling data sets from high-throughput (omics) experiments – such as genome sequencing or mass-spectrometric analysis of proteins and metabolites – the emerging science of translational bioinformatics focuses specifically on turning these data into actionable knowledge for clinical application. Translational bioinformatics thus aims to enhance human health and well-being as well as treatment outcomes through a range of computational methods.

FROM BENCH TO BEDSIDE

The Bioinformatics Core Unit provides bioinformatics and data analysis expertise for translational research projects. It is part of the **Berlin Institute of Health at Charité – Universitätsmedizin Berlin (BIH)** and affiliated with the Max Delbrück Center. To achieve this, we combine a growing portfolio of standardized data processing workflows with project-specific bioinformatics solutions, exploratory statistics, visualization, data-mining methods, machine-learning algorithms, and customized data integration. We perform data analysis projects in close collaboration with researchers and clinicians. This enables us to optimize data analysis strategies in an iterative and incremental manner. Our ecosystem data exploration and visualization tools empower independent data interpretation by biomedical users.

Established data analysis workflows cover a range of high-throughput sequencing technologies and application



areas. The rare disease workflows and tools allow the identification of relevant genetic variants. Our cancer genomics approaches provide rich annotations of variants that offer clinical intervention possibilities and aid researchers in defining immunological properties of cancer cells (neoepitope prediction, HLA typing). Workflows for RNA sequencing enable thorough statistical analyses and broad functional annotations for bulk as well as single-cell data.

We have designed and built the SODAR omics data management system according to the internationally recognized FAIR principles, whose aim is to make all research data Findable, Accessible, Interoperable, and Reusable. The system provides transparent long-term storage and enables cross-project data integration as well as efficient access to current analysis results. In addition, the Bioinformatics Core Unit organizes courses and workshops on high-performance computing, workflow management, and data analysis, while also consulting on method selection, experimental design, and analysis planning.

TOWARDS PERSONALIZED CANCER DIAGNOSTICS AND TREATMENT

Our group provides a local instance of a web-based cancer genomics platform (cBioPortal) that supports the analysis and visualization of large-scale cancer genomic data sets. We are continuously working to develop extensions and add-ons in order to facilitate better identification and interpretation of somatic variants and pathway dysregulation. The system is used not only by researchers to better understand cancer biology, but is also a key tool for the molecular tumor board at the Charité Comprehensive Cancer Center. We designed and developed VarFish, a software for comprehensive DNA variant analysis for research and diagnostics. The system is not only used in various research projects but also for rare disease case analysis in Medical Genetics at Charité – Universitätsmedizin Berlin and at other university hospitals.

OUR TOOLS

- Standardized processing: collection of established workflows for next-generation sequencing
- Scientific service: customized data analysis and cross-omics data integration
- SODAR: Omics data management according to the FAIR principles for projects, workgroups and large consortia
- VarFish: Comprehensive DNA Variant Analysis for Diagnostics and Research
- Full list of tools & services: https://cubi.bihealth.org

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DISEASE MODELS


Gilmore
6/12/20 (1)
SARSTEDT

PLURIPOTENT STEM CELLS

Pluripotent stem cells, which are capable of self-renewal and can differentiate into many cell types, are unique tools for understanding complex disease mechanisms and investigating novel therapies. As such, the technology allows the gap to be closed between basic research and clinical use.

Human pluripotent stem cells have the potential for various clinical uses as well as for establishing laboratory models of disease that overcome the limitations of animal models. Pluripotent stem cells can be derived from patient-specific somatic cells through genetic reprogramming (human induced pluripotent stem cells, hiPSCs). These hiPSCs are capable of self-renewal and have the potential to differentiate into virtually any cell type, opening up ways to better understand certain disorders and to investigate regenerative therapies and novel drug candidates. As such, hiPSCs are unique tools for closing the gap between the identification of disease-causing mechanisms and the development of suitable therapeutic approaches.

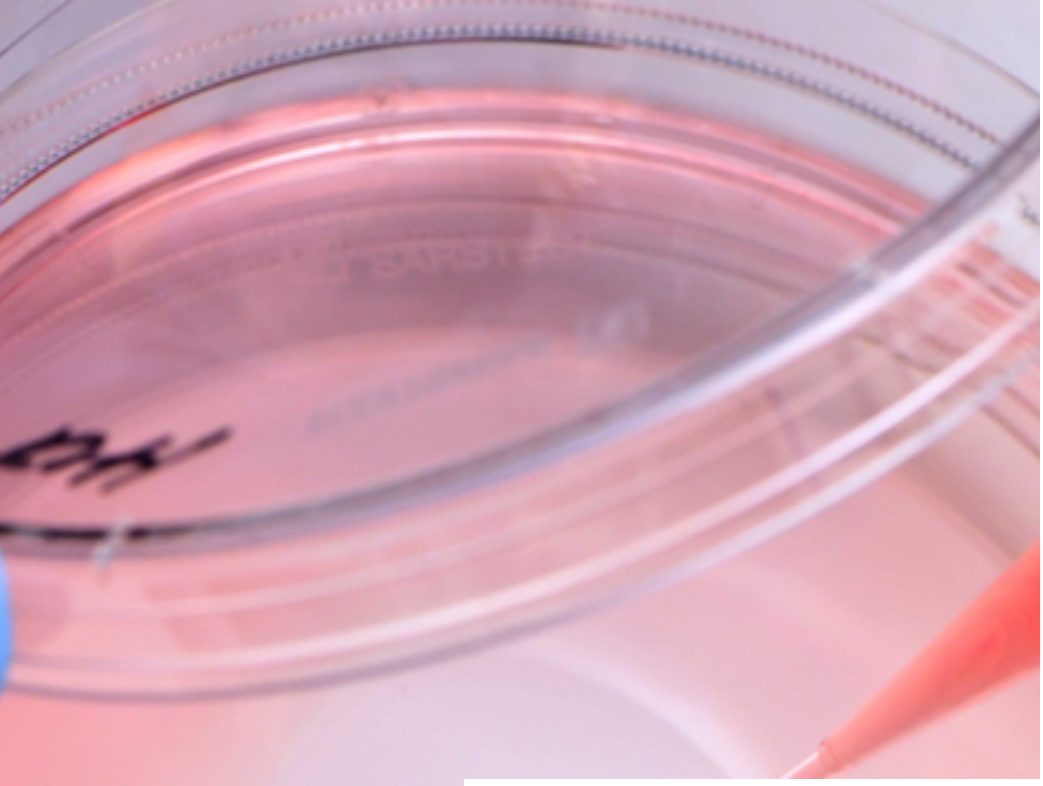
BASIC AND TRANSLATIONAL STEM CELL RESEARCH

The Stem Cell Research and Technology Platform is funded by the Max Delbrück Center and by the **Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK)**.

We support basic and translational research by facilitating all aspects of hiPSC technology, including the derivation, differentiation, and distribution of human stem cell lines. Additionally, the platform provides scientists with state-of-the-art protocols and techniques for proper handling and manipulation of hiPSCs.

We interact closely with international stem cell facilities to address the constant innovations in the field of reprogramming, differentiation, and genome editing. In recent years, our group has established a feeder-free cultivation system of hiPSCs that does not require foreign feeder cells and instead uses chemically defined culture media. In collaboration with other teams at the Max Delbrück Center, we developed neuronal, cardiac, endothelial, and muscle cell differentiation protocols. In addition, the platform has performed multiple genome engineering experiments on hiPSCs, and we were able to reprogram various disease-specific and non-disease-specific cells from patient and animal samples.

The Stem Cell Research and Technology Platform also organizes regular training courses on stem-cell culture techniques, as these methodologies



Induced pluripotent stem cells normally grow on cell culture dishes, which are coated with special nutrient matrices.

are distinct from those used in other mammalian cell cultures and require a profound depth of knowledge and hands-on expertise. The training courses are intended for researchers and technicians who plan projects using hiPSCs.

SCALING UP STEM CELL PRODUCTION FOR CLINICAL USE

Internationally, we collaborate with the CorEuStem and Stem cell Coordinates network to make research with pluripotent stem cells more sustainable and reproducible. One big problem is the maturation of the differentiated stem cells, which we are trying to address using 3D and suspension culture systems. Moreover, we are working to find ways, and are testing suitable machines, to allow for the automated, high-throughput functional analysis of differentiated stem cells. We are also interested in developing good manufacturing practices for producing stem cell lines for future clinical applications and in establishing a certified lab for quality-controlled hiPSCs.

OUR TOOLS

- Isolation of primary cells from patient samples
- Reprogramming of cells into human induced pluripotent stem cells (hiPSCs)
- Gene editing and cell labeling
- Provision of hiPSC reference lines and banking
- Provision of hiPSC-derived differentiated cells
- Derivation of organoids
- Bank of animal iPSCs

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OUR PARTNER

DZHK

30 days old brain organoid immunostained with neuronal marker TUJ1 (green), progenitor marker SOX2 (red), and nuclei DAPI (blue).

ORGANOIDS

Organoids are stem cell-derived 3D culture systems which mimic the cellular complexity and functionality of human organs. They are bridging the gap between in vitro and in vivo research. Our platform provides services for organoids derivation and their further characterization.

The organs of the human body consist of distinct cell types that are organized in a specific manner to form a multi-layered network. Today, it is possible to reconstruct organ-like tissues (organoids) from stem cells in the lab using molecular signals to drive cellular differentiation and the culture systems to promote their three-dimensional self-organization. Organoid technologies are rapidly developing and allow to phenotypically copy the cellular composition and, to a certain degree, also the functionality of various human organs such as brain, thyroid, thymus, intestine, liver, pancreas, stomach, lungs, kidneys, and even early embryos. As near-physiological 3D culture systems, organoids open up new possibilities for investigating how healthy and diseased organs develop and they offer great potential for translational research.

ENGINEERING BRAIN ORGANOID CULTURE SYSTEMS

Established in 2019, the Organoid Platform aims to support organoid-related research. We provide expertise on deriving organoids from pluripotent stem cells or progenitor cells and develop methods for their subsequent characterization. Our platform is part of the Berlin Institute for Medical Systems Biology at the Max Delbrück Center (MDC-BIMSB). Currently, we are focusing on different types of brain tissue models (including unpatterned and patterned brain organoids, assembloids, bioengineered neuronal organoids or BENOs, silk-based 3D neuronal cultures, tumoroids), but the range of simplified mini-organs will expand in the future.

A major goal of our platform is to further engineering of brain organoid culture systems and to improve in-vitro neural tissue maturation. We are currently implementing technologies to vascularize brain organoids (assembly of brain organoid and blood vessel organoid) and to incorporate microglia (a type of non-neuronal cells in the central nervous system) into our systems in order to realize long-term maturation of three-dimensional neural tissues.

In addition, we establish published methods and develop new approaches for organoid research. One particular focus is on single-cell technologies.

A COLLABORATIVE EFFORT TO DISSECT MECHANISMS OF DISEASE

In close collaboration with the Pluripotent Stem Cells Platform and the Transgenics Platform we will use genome editing techniques to model and study genetic brain diseases. Examples include Leigh syndrome (a hereditary psychomotor regression in infancy) and Alzheimer‘s disease: We will analyze the underlying molecular mechanisms and screen for drug candidates.

Ultimately, we would like to create a blueprint for gene therapy for genetic brain diseases. To this end, we intend to combine personalized, patient-derived brain organoid models with the latest genetic tools for gene repair.

Brain organoid on its way to slicing for Air liquid interface cerebral organoid culture.

OUR MODELS

- Unpatterned/patterned organoids
- ALI/enCOR organoids
- Assembloids (dorso-ventral)
- Bioengineered Neuronal Organoids (BENOs)
- 3D silk scaffold brain tissue models
- Blood vessel organoids
- Tumoroids

OUR TOOLS

- Organoid derivation
- Organoid preparation for spatial transcriptomic
- Organoid transgenesis (electroporation, lipofection, viral transduction)
- Organoid characterization:
  - nanostring analysis
  - immunohistochemistry/ISH
  - single cell/single nuclei RNAseq
- Disease modeling using patient-derived iPSC lines
- Gene editing/clonal expansion

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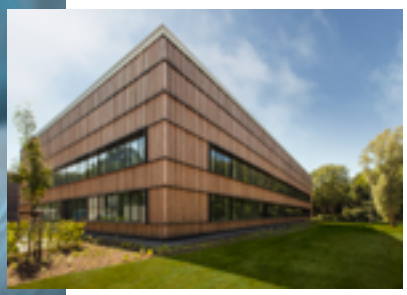
ANIMAL PHENOTYPING

Our platform provides a large set of validated approaches for investigating physiological and morphological characteristics in mice and rats and sensitively screening for phenotypic variations. This gives researchers a valuable resource for studying animal disease models.

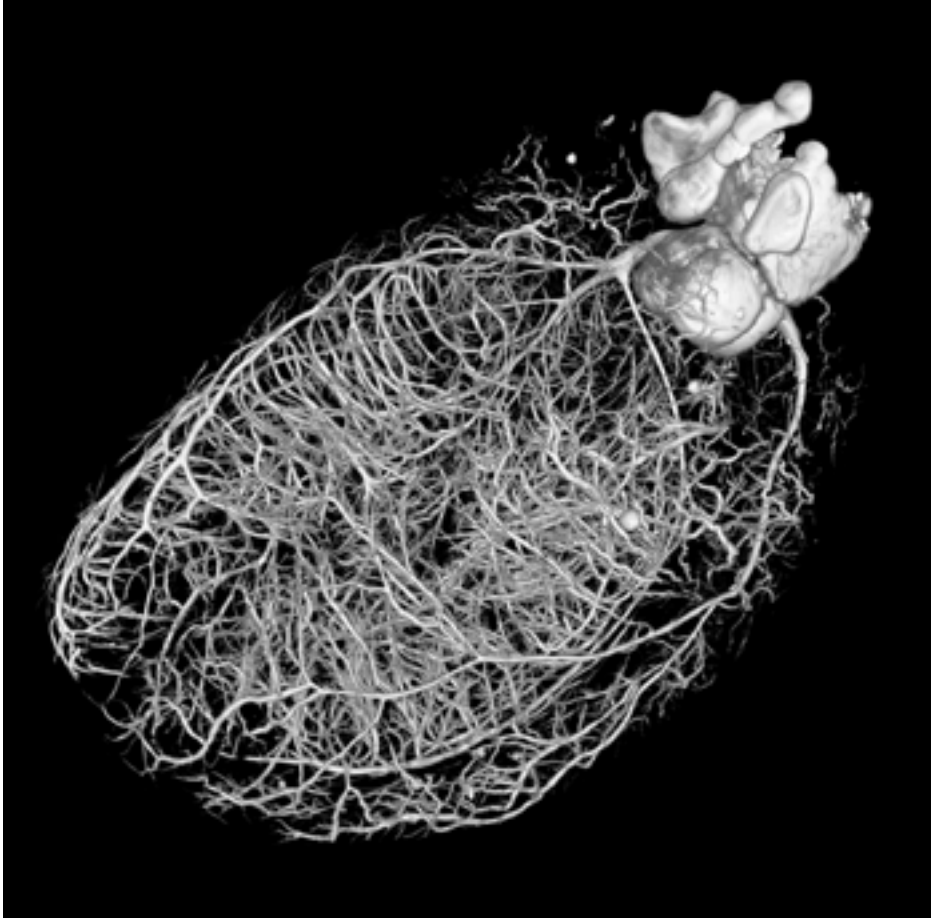


Bar-coded chips enable automated blood testing (left). The Preclinical Research Center was established in 2020.

Despite great progress in research techniques, such as in vitro methods and advanced computer modeling, that avoid the use of animals, there is still a need in preclinical research to investigate the morphology, physiology, metabolism or behavior – the phenotype – of animals. Such a holistic approach to health and disease yields uniquely complete data that would otherwise not be available. Animal phenotyping provides a better understanding of the complex interactions between the cardiovascular, respiratory, and central nervous systems, which is critical for the development of various drugs and therapies for humans.

STUDYING MODEL ANIMALS IN NON-INVASIVE WAYS

The Animal Phenotyping Platform at the Max Delbrück Center houses a comprehensive collection of tools for the physiological and morphological assessment of experimental mice and rats. We focus on techniques that minimize animal use and enable researchers to obtain comparable levels



High-resolution micro-CT imaging of a rat heart.

of information from fewer animals or to obtain more information from the same number of animals.

By using a wide variety of preclinical imaging methods – including high-frequency ultrasound, micro-computed tomography, magnetic resonance imaging, photoacoustic imaging, quantitative bioluminescence and fluorescence imaging, and time-domain nuclear imaging – scientists can precisely characterize disease progression and therapeutic effects.

Additionally, we apply numerous non-invasive in vivo procedures to monitor physiological, metabolic, and bioelectrical variables in conscious animals (e.g., blood pressure and heart rate measurements, electrocardiography, and respiratory analysis). We provide state-of-the-art services, innovative techniques, and helpful advice to both experienced and novice investigators.

A WHOLE-ORGANISM APPROACH TO FUNCTIONAL GENOMICS

In summer 2020, the Max Delbrück Center opened a new research building for in vivo pathophysiology experiments: the **Preclinical Research Center (PRC).** It promotes preclinical translational research and functional genomics. Our platform pools and further improves well-established phenotyping approaches, ensuring the highest technological and quality standards. The PRC gives investigators the capacity to accurately assess developmental, behavioral, cardiovascular, and metabolic characteristics in rodent disease models over extended periods of time and to sensitively screen for phenotypic variations. The new research building has multiple benefits for researchers at the Max Delbrück Center and for collaborating external scientists.

OUR TOOLS

- High-frequency ultrasound
- Photoacoustic imaging
- Micro-computed tomography
- Magnetic resonance imaging
- Bioluminescence and fluorescence imaging
- Clinical chemistry
- Hematology
- Metabolic phenotyping
- Body composition analysis
- Electrocardiography (ECG)
- Blood pressure measurements
- Respiratory analysis

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TRANSGENICS

Our group provides services for engineering the mouse genome, creating “knock-out” and “knock-in” mice. A powerful molecular tool – the enzymatic CRISPR-Cas9 system – enables highly precise gene editing in a single-step procedure.

Team members perform microinjections on mouse embryos using micromanipulators equipped with micropipettes.

The mouse is the most important model organism for analyzing human genetic disease. Engineering the mouse genome is thus a key technology in biomedical research. In "knock-out" mice, genes become inactivated, which allows their essential function to be tested. In "knock-in" mice, on the other hand, gene sequences are replaced or inserted, enabling researchers to mimic human disease mutations or study how specific genes are expressed in the cell.

TARGETING MOUSE GENES TO ELUCIDATE HUMAN DISEASE PROCESSES

We use a powerful enzymatic technique, known as CRISPR-Cas9, to generate new, genetically modified mouse lines. The approach was named "2015 Breakthrough of the Year" by Science magazine and earned a Nobel Prize in Chemistry in 2020. The CRISPR-Cas9 method allows the genome to be easily cut and altered at highly specific locations. To achieve this, CRISPR-Cas9 reagents are injected into single-cell mouse embryos, and gene editing is then achieved through natural DNA repair mechanisms, leading either to gene inactivation (with loss of short genetic sequences) or to precise sequence modifications (with an artificially constructed DNA molecule serving as a template for repair).

The Transgenic Platform offers advice on the preparation of suitable CRISPR-Cas reagents, and performs the embryo isolation and microinjection as well as their transfer into foster females. Of the pups derived from single-cell embryos microinjected with CRISPR-Cas9 reagents, we usually achieve success rates of about 50 percent for sequence deletions and of about 15 percent for sequence replacements.

Our services also include reproductive techniques for the preservation of precious mouse lines. One important approach is the cryopreservation of sperm and embryos in liquid nitrogen. This helps to save animal housing resources when strains are not actively being used for research. It also prevents gene drifting and excludes loss of colonies in case of infection. When required, thawed sperm samples can be used for in vitro fertilization of mouse eggs, with the resulting single-cell embryos being transferred into foster females. The same in vitro technique, which enables hygienic rederivation of specific strains, is also deployed for the pathogen-free import of mouse lines from external repositories.

ENGINEERING A UNIVERSAL TRANSGENIC CONSTRUCT

Many mouse genes are active only in some specialized cells, organs, or developmental stages. A notable exception is a genetic locus (a specific location on a chromosome) known as Rosa26, which is active in all tissues, whatever the subject's age. Transgenic constructs integrated into Rosa26 are universally active, which makes it highly valuable for medical research. More than 500 Rosa26 knock-in mouse lines have been generated so far.

The Transgenic Core Facility, in collaboration with another group at the Max Delbrück Center, was first to demonstrate the insertion of transgenes into Rosa26 using the CRISPR-Cas9 technology. The resulting strain facilitated gene editing in somatic cells, thereby aiding the analysis of somatic cell disorders. Today, this transgenic mouse line (known as Rosa26-Cas9) is distributed via the Jackson Laboratory, a non-profit biomedical research institution in the United States, which is one of the largest sources of genetically defined mice.

OUR TOOLS

- Microinjection of single-cell embryos
- Microinjection of embryonic stem cells into blastocysts
- Embryo transfer
- Sperm freezing
- Embryo freezing
- In vitro fertilization

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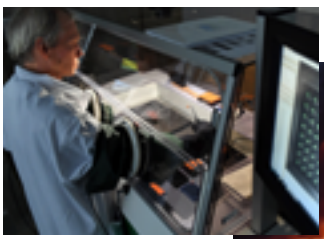
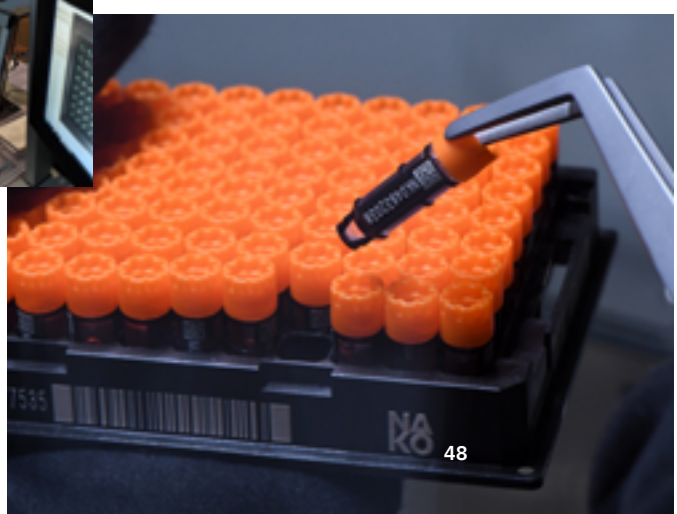
BIOBANK

We offer scientists a fully automated platform for efficiently storing and retrieving liquid biological samples. In addition, our group provides planning advice for research projects with biospecimens, and takes part in major clinical and epidemiological studies.





This storage rack contains automation-friendly sample tubes with unique bar-codes (right). The continuous cooling of samples is also guaranteed during manual handling by using a cryo-workbench (above).

Many medical research projects make use of biological specimens such as blood, urine, tissue samples, cells, or DNA, which have to be stored over time. For example, clinical and epidemiological studies may aim at exploring long-term correlations between specific genetic and metabolic characteristics (biomarkers) and the development of disease. This requires an infrastructure – a biobank – to reliably preserve and retrieve probes when necessary. Biobanks allow researchers to archive biological information for planned as well as unforeseen future use.

HIGH-QUALITY STORAGE AT ULTRALOW TEMPERATURES

The biobank can support any type of study that requires state-of-the-art storage of liquid biological samples. This includes cohort studies and clinical trials, but also experimental research projects involving, for instance, stem cells. Specimens are stored at temperatures below -160°C in the vapor phase of liquid nitrogen in a fully automated robotic system. This allows high-quality storage as well as swift identification and efficient retrieval of samples for analysis.

Our platform currently encompasses four large-dimension liquid nitrogen tanks (plus one emergency reserve tank) with a capacity for 5 million 250-µL tubes as well as tubes of 700 µL, 1000 µL, and 2 ml. The handling procedures guarantee that the cold chain is not interrupted when transferring samples into the biobank. A laboratory information management system provides full documentation, including sample characteristics, storage conditions, project information, and analysis results.

In addition, our group offers advice for projects using biosamples when it comes to study design, standard operating procedures, data protection, and ethical issues. We also offer DNA or RNA extraction. Scientists can request the biosamples stored in the biobank for research projects.

IDENTIFYING BIOMARKERS OF HUMAN DISEASE

Among others, the biobank stores samples from 30,000 persons in Berlin and Brandenburg who are taking part in the German National Cohort, a large nationwide epidemiological study that aims at elucidating the mechanisms of major chronic diseases. In addition, we store samples from a cohort of patients with high cardiovascular risks for the Berlin Long-term Observation of Vascular Events (BeLOVE) study. It aims to improve short- and long-term prediction of cardiovascular dieases as well as the mechanistic understanding of progression and outcomes. These studies will enable researchers to explore the associations between biomarkers and the risk of incident and recurrent health problems. Stored samples from the BioCog study (Biomarker Development for Postoperative Cognitive Impairment in the Elderly) allow to identify biomarkers for postoperative delirium and cognitive dysfunction. The platform also supports other studies of the Max Delbrück Center, the **Berlin Institute of Health at Charité (BIH)**, and the **Charité – Universitätsmedizin Berlin.**

OUR TOOLS

- Cryo-workbench for manually handling and scanning samples at -80°C
- Fully automated storage in the vapor phase of liquid nitrogen
- Laboratory information management system for sample tracking, history, and data connection

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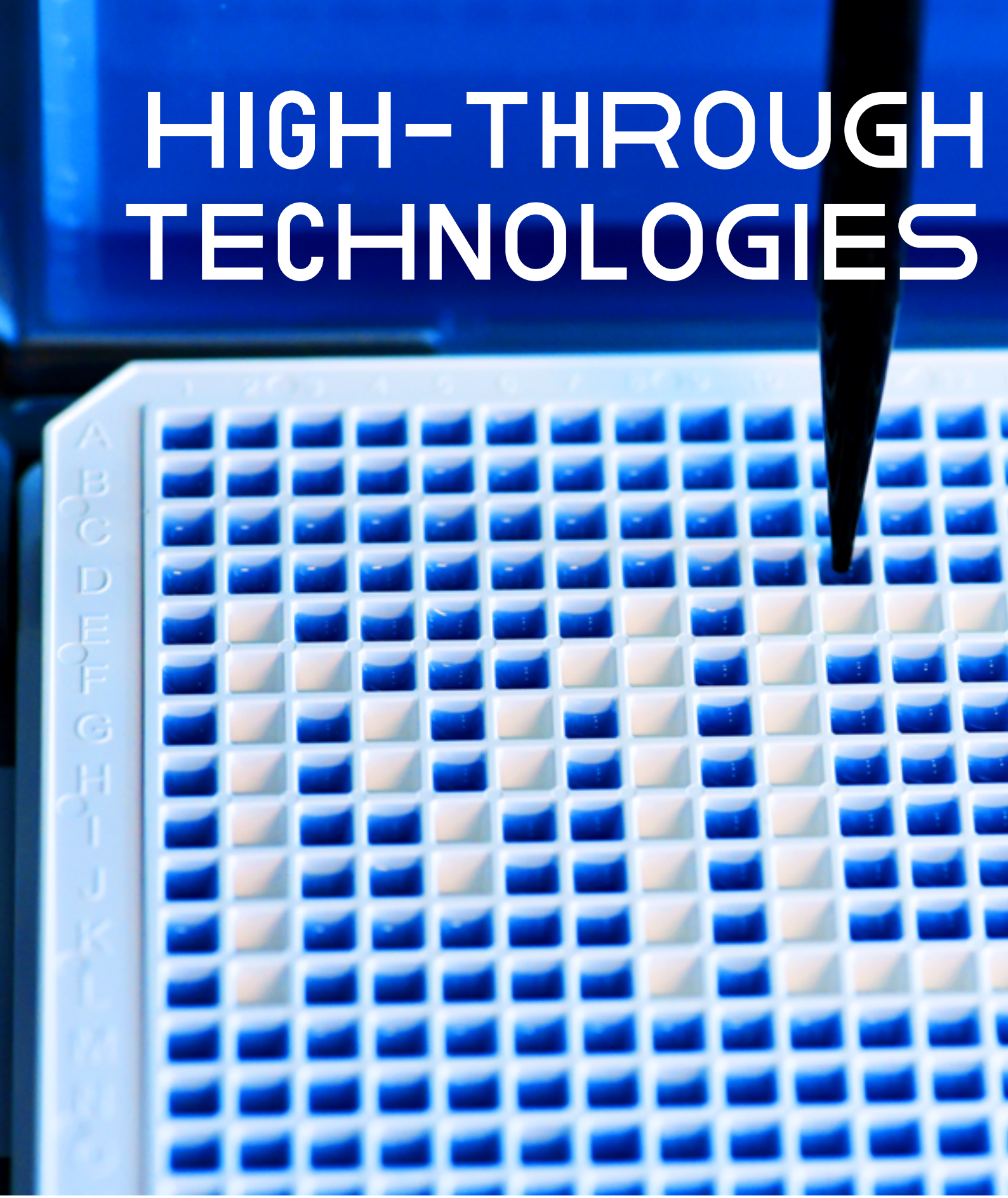
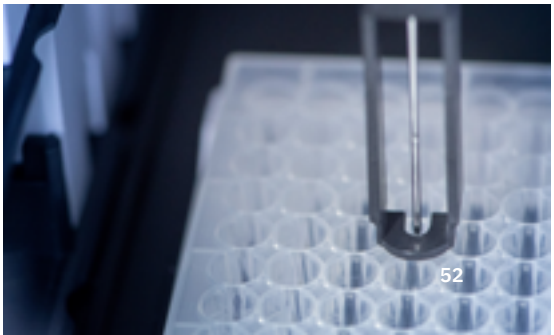
HIGH-THROUGH TECHNOLOGIES



PUT

PROTEIN PRODUCTION AND CHARACTERIZATION

Proteins are key to many research projects in life sciences. Our platform has a strong technical expertise in protein biochemistry and offers services for customized protein production and the biophysical characterization of proteins.



Our laboratory utilizes intact protein mass analysis on an Agilent 6230B time-of-flight LC/MS for quality control of purified proteins.

Proteins are used for a wide range of applications in biomedical research. Some proteins serve as research tools, for example certain enzymes that are necessary for genome engineering and sequencing. The production of such "tool proteins" is particularly important for the technology development together with other platforms or research groups. However, proteins are not only tools but also the topic of many research projects as they are key players in health and disease. By producing samples of specific proteins of interest, we support researchers who want to uncover their biological function.

TAILOR-MADE PROTEINS FOR COLLABORATIVE RESEARCH

Our platform offers services along the entire protein production pipeline, in close cooperation and active exchange of ideas with our research partners. After initial project consultation, we analyze the amino acid sequence of the protein of interest with the help of various bioinformatics programs. We then propose a series of protein fragments, so-called constructs, and test whether they can be produced in host organisms such as E. coli or mammalian cells using recombinant techniques. Based on the resulting expression and solubility levels, we select suitable constructs for large-scale production. Finally, to purify proteins, we apply a variety of chromatographic techniques that exploit distinct protein properties such as size and overall charge.

Besides the customized production of proteins, the platform is also an ideal partner in joint research projects on topics such as protein engineering and protein characterization. Our group has great expertise in studying the physicochemical properties and the structure-function relationship of proteins. More specifically, we analyze how proteins fold and assemble, we assess and optimize protein stability, study the interaction of proteins with binding partners (e.g. other proteins, DNA, RNA, or small molecules) and determine their three-dimensional structure.

Internationally, our group is involved in the P4EU community, a European network of research technology platforms focusing on various aspects of protein production and characterization. Within this framework, we promote the exchange of expertise and participate in benchmarking studies.

LEVERAGING EUKARYOTIC CELLS AS PRODUCTION SYSTEMS

Protein function in higher organisms often depends on biochemical surface modifications such as glycosylation and phosphorylation. Currently, we offer to purify such modified proteins using HEK and CHO cells as mammalian production hosts. We are also able to produce monoclonal antibodies at large-scale by culturing provided hybridoma cell lines.

In addition, our group set up multi-user access to equipment for protein production and biophysical characterization of proteins. We offer individual on-site training courses for researchers (e.g. intact protein mass determination, ÄKTA usage, dynamic and static light scattering applications and thermal shift assays) and help with data evaluation. Other labs of the Max Delbrück Center thus have the opportunity to use the centralized equipment to answer specific research questions. As a long-term goal, we plan to establish a central freezer collection for our tool proteins as well as for plasmids, cells and antibodies.

OUR TOOLS

- Ultra-high throughput benchtop bioreactor (Epiphyte3) for large-scale bacterial expressions up to 48 liters
- Advanced cell culture lab for cultivation of mammalian cells
- Protein purification systems supporting various chromatographic techniques
- TOF LC/MS (Agilent) for intact protein mass determination
- DynaPro Plate Reader III (Wyatt Technology) for high-throughput and automated dynamic and static light scattering analyses
- Real-time PCR detection system (Bio-Rad) for thermal shift assays
- MicroCal PEAQ-ITC (Malvern Panalytical) for ligand binding studies
- SEC-RALS (Malvern Panalytical) for protein oligomerization studies
- Chirascan CD spectrophotometer (Applied Photophysics) for protein folding analysis
- Access to synchrotron (Bessy II, HZB) for protein structure determination by X-ray crystallography

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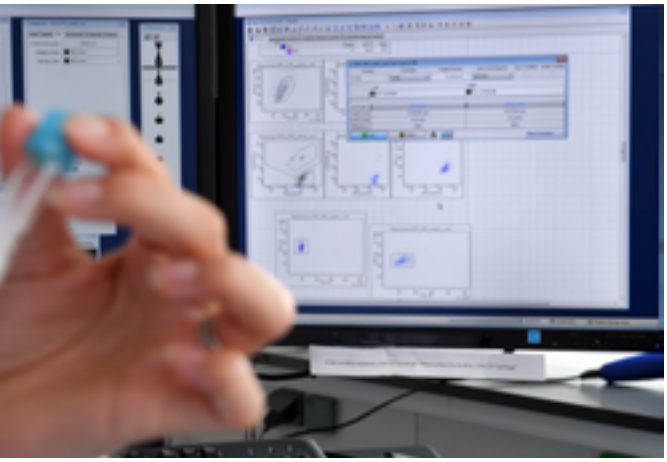
FLOW CYTOMETRY

Our platform provides technology for analyzing and separating cellular populations into distinct subsets based on their physical and chemical characteristics. This is the basis for studying and enriching specific cell types in both health and disease.



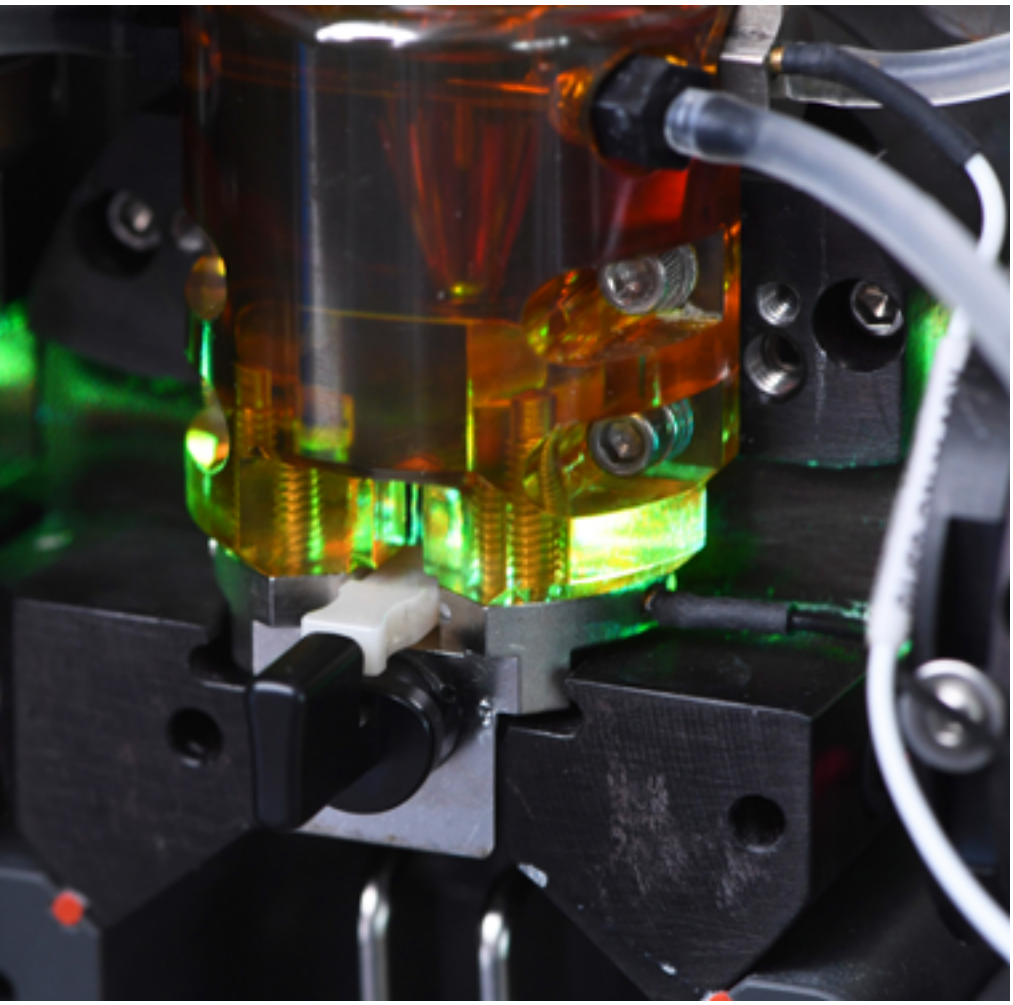
This software (BD FACS Diva) controls the fluorescence-activated cell sorting and records the experiments.

In multiple areas of molecular medicine, researchers are interested in finding out the specific properties of cells based on their genetic make-up, surface proteins, or other markers. These kinds of experiments can be performed by using flow cytometry to sort cell populations. Modern flow cytometers continuously analyze thousands of cells per second in a liquid stream, based on light scattering and cell surface characteristics. In particular, an advanced laser-based technology known as fluorescence-activated cell sorting (FACS, a BD trademark) allows the enrichment of highly specific cells of interest for subsequent investigations.

PROVIDING TECHNOLOGIES TO SORT AND ANALYZE CELLS

Our platform primarily enables researchers to access and operate cell sorters that are based on the FACS technology. These sorters can isolate up to 20,000 cells per second for large-scale cell isolation and even allow for single cell sorting, in which individual cells are precisely spotted into the well of a microtiter plate for analytical purposes. Our group assists in the design and setup of specialized and sophisticated experiments that use additional analytical cytometers or large numbers of fluorescent labeling compounds (fluorochromes), with up to 25 different colors possible. The facility has three flow cytometry analyzers and four high-capacity digital cell sorters.

The specific analytical software of flow cytometers allows researchers to tackle general research questions relating to programmed cell death (apoptosis) and cell viability, cellular signal transduction, cell cycle analysis,



cellular protein production, and the efficiencies of gene modification experiments. Sorted cells are routinely prepared for gene expression analyses. In addition, isolated cells may be prepared for genome editing, DNA sequencing, live-cell imaging, or transfer into animal models of disease.

The Flow Cytometry Platform is a multi-user facility. Numerous groups from the Max Delbrück Center and partner institutions have used the platform for various research projects over the past few years.

ENABLING GENOME EDITING

The possibility to specifically alter and edit the genome with a powerful enzymatic technique (CRISPR-Cas9) has revolutionized the field of genetics, and FACS is an essential tool in utilizing the full potential of this technology. CRISPR-Cas9 allows targeted gene inactivation ("knock-out") or insertion ("knock-in") in virtually every cell type. However, the efficiency of these manipulations varies dramatically between experimental setups. FACS-based cell sorting is therefore used to separate mutated and non-mutated cells. Mutated cells can be tracked by detecting cell surface proteins or the fluorescent dyes that mark manipulated cells. In this way, FACS allows us to create purified mutated cell populations, which is a prerequisite for studying their impact on health and disease.

OUR TOOLS

Flow cytometry analyzers and wcell sorters:

- Symphony A3 (5 ultraviolet – 7 violet – 2 blue – 5 yellow green – 3 red)
- Fortessa (2 ultraviolet – 6 violet – 2 blue – 5 yellow green – 3 red)
- Cytek Aurora (spectral)
- Aria 1 (6 violet – 2 blue – 5 yellow green – 3 red)
- Aria 2 (3 violet – 2 blue – 5 yellow green – 3 red)
- Aria 3 (6 violet – 2 blue – 5 yellow green – 3 red)
- Aria F (2 ultraviolet – 6 violet – 2 blue – 5 yellow green – 3 red)

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Four 384-tip pipetting systems enable us to test up to 160,000 compounds per day for their biological effects.

CHEMICAL BIOLOGY

Our platform enables researchers to identify and generate bioactive compounds via screening, chemical optimization and in silico methods. We thus help to unravel the molecular mechanisms of health and disease, and to pinpoint novel drug targets and therapeutic approaches.

Most therapeutic drugs are small molecules – chemical compounds with a small size and a low molecular weight – that target a biological function. To drive medical progress forward, there is an ongoing need to identify and chemically optimize small molecules that influence cellular target structures in a specific and stable manner. In addition, complex analysis procedures for large data sets collected from cellular systems are needed to identify exactly how and where such compounds are active, and to allow researchers to unravel the molecular mechanisms of health and disease. Today, such investigations in the field of chemical biology deploy various high-throughput approaches as well as computational and modeling techniques, enabling researchers to map the activity of small molecules, even at the level of a single cell within millions of cells analyzed.

IDENTIFYING DRUG CANDIDATES AND MOLECULES FOR RESEARCH

The Chemical Biology Platform is part of the Infrastructure of the neighboring Institute, the **Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)**. The platform offers open access to screening technologies for novel drug candidates (about 180,000 drug-like compounds) as well as genome-wide gene function studies through interfering RNA molecules or by gene-editing via CRISPR-Cas9. In addition, our platform is working to generate and optimize chemical compounds as molecular tools for scientific research.

We established a broad portfolio of high-throughput approaches that allow researchers to explore the cellular effects of specific molecules. These approaches include high-content screening with automated microscopes (confocal and standard), high-speed kinetic imaging and impedance measurement. Our platform supports all kinds of fluorescence and luminescence measurements. It also offers methods for studying the binding properties of specific substances (surface plasmon resonance) as well as protein interactions (capillary electrophoresis). Data documentation and analysis have been automated to allow for effective process control.

Using medicinal chemistry approaches, potential drug candidates identified during screening ("primary hits") can be further optimized, for example, through systematic chemical alterations improving activity, specificity and the secondary profile like solubility or permeability. Such small molecules are then suitable and versatile chemical tools for pharmacological experiments in cellulo and in vivo but also potential leads for drug development. Additionally, they can be further developed for diagnostic means by labeling them with a reporter group such as a fluorophore or converting them into a PROTAC.

Our small molecule druglike screening collection contains of about 80,000 compounds. 40,000 compound comprise a commercial highly diverse screening library derived by the World Drug Index including 3,000 biologically annotated compounds with a subset of U.S. Food and Drug Administration (FDA) approved drugs. 10,000 compounds were collected by different academic chemistry sites and 30,000 compounds are offered for a blind screening of natural products. As partner site in the context of EU-OPENSCREEN an additional screening library of 100,000 highly diverse compounds is available for high-throughput screening.

Not least, the platform also provides support from computational methods, such as virtual ("in silico") screening, similarity searches in existing substance libraries, modeling approaches, and molecular dynamics simulations.

TARGETING TUMORS

Using this set of powerful tools, we have been able to identify various drug candidates that interfere with tumor development and metastasis as well as the malformation of blood vessels in a rare disease. Some projects are already in an advanced stage, with substances being evaluated in preclinical studies or even ready to enter clinical trials. In the future, we will establish morphology profiling of cells under different treatment conditions according to the Cell Painting protocol using confocal microscopy. To do so, we will develop analysis protocols for pattern recognition supported by machine learning routines. We will respond to the evolving methodological demands of future research projects on our campus.

OUR TOOLS

- High content screening and morphology profiling
- High-speed kinetic imaging
- Fluorescence-activated cell scanning
- Capillary electrophoresis
- Surface plasmon resonance spectrometry
- NMR spectrometry
- Liquid chromatography – mass spectrometry
- Medicinal Chemistry optimization, structure-based design, tailored labeled chemical probes for target identification and assay set-up, PROTACs
- Automated compound store with 67,000 small molecules
- Screening libraries: 180, 000 compounds, 60,000 genome-wide probes (human, mice, RNAi, CRISPR-Cas9)

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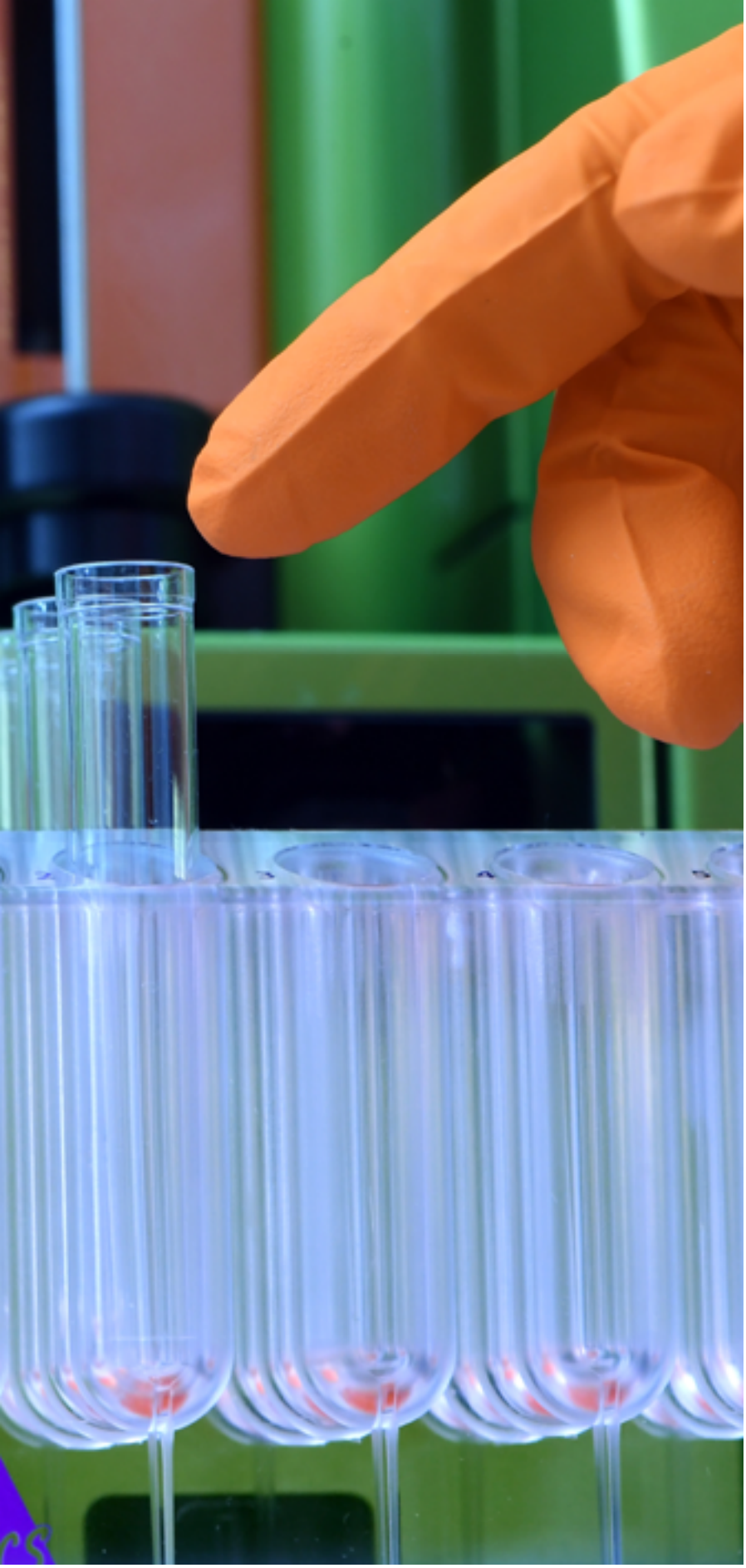
OUR PARTNER

BACKGROUND INFORMATION

The 18 Scientific Technology Platforms presented here are led by established experts and employ approximately 100 people in total. Two of these are run jointly by Berlin Institute for Health at Charité (BIH) & Max Delbrück Center, Cryo-EM is a joint unit with the Charité – Universitätsmedizin Berlin, Chemical Biology a joint unit with the Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP). Two additional platforms (Metabolomics and Translational Bioinformatics) are BIH-funded and complement our portfolio as guest units. All of them generate synergies for the benefit of basic and translational research. They are supported by internal advisory bodies that help recognize trends (e.g., in single-cell analysis) and that pave the way for scientific developments through investments in staff or equipment. Dr. Jutta Steinkötter is Executive Manager of the Technology Platforms, while the scientific strategy is overseen by the Scientific Infrastructure Board, members of which include senior scientists of the Max Delbrück Center.

The Technology Platforms primarily support the work of researchers at the Max Delbrück Center, but also maintain numerous collaborations with scientific partners in Berlin and beyond – particularly under the umbrella of the BIH and in cooperation with Charité, FMP, and the German Center for Cardiovascular Research (DZHK).



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OUR PARTNERS

BIH Berlin Institute of Health @ Charité

DZHK Deutsches Zentrum für Herz-Kreislauf-Forschung e.V.

FMP Leibniz-Forschungsinstitut für Molekulare Pharmakologie

CHARITÉ

HELMHOLTZ IMAGING

MAX DELBRÜCK CENTER

This brochure features 20 overviews that introduce the scientific-technological infrastructure that is available at the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (Max Delbrück Center). The methodological platforms, available to both researchers at the Max Delbrück Center and external project partners, range from imaging technologies and molecular high-throughput analyses to bioinformatics computing, stem cell methods, and transgenic animal models. These customized approaches have been developed with established experts and enable competitive research into molecular medicine.

www.mdc-berlin.de/technology-platforms

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