NON-INVASIVE ANALYSIS OF CELLS AND TISSUES

RESEARCH, ANALYSIS, CHARACTERIZATION, VALIDATION AND DOCUMENTATION
The isolation and culture of cells from primary material (various tissues and species), differentiation and proliferation of pluripotent stem cells, construction of three-dimensional cell culture systems and the fields of tissue engineering and regenerative medicine are the expertise of the Fraunhofer IGB. We characterize cells with classical invasive methods such as histological and immuno-histological staining, but we have developed and adopted numerous non-invasive methods that allow for the continuous monitoring of cell culture and tissue on the molecular and morphological level. With over 20 years experience in cell culture, we are your partner for complex questions in regenerative medicine, tissue engineering and the development of cell-based assays for toxicology and biocompatibility.

Our specialists provide you with standardized analytics or develop new analytical methods to meet your requirements.

**We offer**
- contract analysis
- quality control
- new method development “from bench to market”
- leadership and partnerships in research projects

**New applications in Raman spectroscopy**

Raman spectroscopy has been a standard tool for pharmaceutical quality control for a number of decades. Recently, Raman has seen a rebirth in the field of biomedical technology. The ability to non-invasively create a molecular fingerprint of cell and tissue without altering their state is of great value for both scientists and clinicians. At the Fraunhofer IGB, we have developed a well-established and published expertise in the Raman analysis of cells and extracellular matrix proteins, as well as other biologicals such as bacteria and cytotoxicity.

**State of the art confocal microscopy**

Powerful laser technologies have enabled the development of new non-invasive analysis techniques such as second harmonic generation, fluorescence lifetime imaging, imaging flow cytometry and single photon counting. Our scientists have developed confocal laser methodologies that are considered the gold standard in the pharmaceutical industry. With our partner lab at the University Hospital Tübingen, we offer cutting edge cell analysis services for both industry and academic projects.

**New opportunities using flow cytometry and laser microdissection**

Flow cytometry and imaging flow cytometry allow for the analysis and sorting of heterogeneous cell populations with antibody-coupled fluorescence dyes. Areas of application range from blood diagnostics, via cell transfection for the generation of cell lines and clone isolation to the identification of stem and precursor cell populations from a great variety of tissue types. Automated microdissection allows the cutting of the smallest areas out of a matrix-immobilized cell layer. Even individual cell nuclei can be isolated for further molecular analysis.
FACILITIES, EQUIPMENT AND SERVICES

**Flow cytometry**
- Immunofluorescence measurements of cell surface and intracellular markers
- Single- and multi-color analysis
- Cell cycle analysis
- Apoptosis detection, viability and proliferation testing (e.g. for testing of biocompatibility)
- GFP analysis for determination of transfection efficiency or establishment of stable clones
- Hoechst-Efflux for side population analysis
- Antibody binding studies
- Determination of growth factors in cell culture supernatants by Cytometric Bead Array
- Imaging flow cytometry for cell signaling, cycles and mitosis as well as internalization, morphology, cell-cell interaction and co-localization

**Establishment of further methods on demand**
- Single cell deposition
- Determination of activation antigens (e.g. on platelets for biocompatibility testing)
- Detection and enumeration of residual leucocytes in blood preparations such as erythrocyte or thrombocyte concentrates
- Allergy diagnostics (activation of basophilic cells)
- Kinetic measurements (calcium flux)

**Microscopy**
- Light microscopy
- Phase contrast microscopy
- Differential interphase contrast
- 3D imaging, time series
- Fluorescence microscopy/with confocal resolution (ApOTome)
- FLIM (fluorescence lifetime imaging) and SHG (second harmonic generation)

**Microdissection**
- Automated dissection of cell nuclei or matrix fragments from fixed tissue preparations for the molecular-biological analysis (DNA, RNA, protein expression)
- Catapulting of single cells and cell colonies for further cell cultivation (e.g. single cell deposition of transfected cells or purification of primary cell cultures)

**Raman spectroscopy**
- Non-invasive spectral detection of biological samples, also in combination with fluorescence microscopy and FLIM
- Active agent efficacy detection on single cell level (prospective)
- Characterization of carrier materials
- Cell characterization
Non-destructive characterization

Raman spectroscopy is based on an optical effect, which was first described in 1928 by the Indian physicist and Nobel laureate C. V. Raman. Raman observed that a small fraction of scattered light undergoes a frequency shift. Molecular vibrations are the cause of this effect. This inelastic light scattering can be used to detect molecules in a sample and to characterize their structure. The spectral bands detected in the Raman spectrum are specific to each molecular bonding. With the help of Raman spectroscopy complex mixtures can be identified by their banding pattern. Raman spectroscopy is contact-free, requires no sample preparation and can be used in liquid solutions. These advantages make the technology for biological and biomedical applications particularly interesting.

Raman spectroscopy for biological applications

Microorganisms, cells and tissues can be analyzed with Raman spectroscopy under physiological conditions. The molecules of biological samples found in the Raman spectrum’s characteristic pattern is similar to a fingerprint. The system used at the Fraunhofer IGB is equipped with a 785 nm diode laser. Laser light in the near-infrared range does not damage biological samples even after prolonged exposure and allows studies on living cells. The laser is focused through the objective of a fluorescence microscope on the sample. Spatially resolved spectra representing the molecular composition of the sample are produced. In order to examine targeted tissue structures or specific cell populations, fluorescent labeling can be performed prior to the Raman spectroscopy. The method allows the detection of spectral levels and dynamic cellular processes such as cell death and differentiation in real time.

Raman spectral database as cell and tissue archive

In order to easily compare Raman spectra within another, spectra of different types of cells, such as stem cells, cell lines and primary isolated cells, as well as spectra of extracellular tissue components such as collagen, elastin and proteoglycans are stored and archived in a database. The Raman spectrum of an unknown sample can be compared with the total spectral database quickly identifying, for example, a special cell within a heterogeneous population.

Applications

- Marker-free discrimination of cell types [1–4, 6]
- Analysis of stem cell differentiation [2]
- Detection of pathological cells [3, 4]
- Analysis of cell viability [4]
- Cytotoxic assays and substance testing
- Real-time monitoring of cell death [4]
- Identification and degradation of collagen [6]
- Detection of elastin [7]
- Analysis of proteoglycans and hydroxyapatite [8]
- Discrimination of healthy and cancerous tissues [3]
- Detection of calcified tissues
- Identification of bacterial infections

1 Raman microscope at the Fraunhofer IGB.
2 Raman spectra of stem cells.
3 Principle of Raman spectroscopy.
4 Tissue isolation before Raman analysis.
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References

Laser-induced autofluorescence microscopy

Laser-induced autofluorescence microscopy is an optical method for analyzing the quality of cells and tissues in their natural state. The sample is excited with a powerful near-infrared laser and after a few nano to microseconds, the sample begins to emit a light, or fluorescence, at a wavelength different than the excitation wavelength. Because the light emitted in all directions, it is possible to derive two and three-dimensional images of the sample.

At the Fraunhofer IGB, laser-induced autofluorescence is a standard optical imaging technique used in many of our projects and we have a very broad expertise on this method.

Second harmonic generation

Second harmonic generation (SHG) is a non-linear optical process where photons with the same frequency interact with a sample and their combination generates new photons with double the energy, but at twice the frequency and half the wavelength of the initial photons. In biomedical technologies, SHG is a tool for high-resolution optical microscopy of non-centrosymmetric structures, such as collagen or myosin.

At the Fraunhofer IGB, our researchers have used SHG to analyze pathological damage to tissue in the cardiovascular [1, 2, 3] and musculoskeletal systems [4], as well as identified extracellular matrix (ECM) deterioration as a character pathogenesis in Sjögren’s syndrome [6]. We have uncovered unknown mechanisms of heart valve disease due to cardiac inefficiency that have significant clinical impact [1] and we have developed this technology into a quality control tool for tissue implants [5].

Fluorescence lifetime imaging using Time Correlated Single Photon Counting (TCSPC)

The Department of Cell and Tissue Engineering and its partner lab at the University Hospital Tübingen offers FLIM services with a unique 5D laser system that includes a 360 degree rotatable arm, which is ideal for in vitro and in vivo studies. This system is capable of simultaneously performing multiphoton and SHG imaging of samples in both time and dimensional based assays (time course and z-stack imaging). This system is additionally equipped with state-of the art single photon counting boards which allow extensive analysis of the photons being emitted from any material due to excitation by a specific wavelength and provides a photon distribution analysis of the materials. Notably, this facilitates fluorescence lifetime imaging (FLIM), which produces images based on the differences in the exponential decay rate of the fluorescence from a fluorescent sample, where the lifetime, not the intensity, of a fluorophore signal is used to create the image. This method reduces the effect of photon scattering in thick samples. This method also avoids sample bleaching and photo-induced toxicity. Investigated fluorophores can be naturally present in the cell (e.g. NAD(P)H, which is indicative of cellular metabolism), or fluorophores that we can introduce externally to understand cell pathways and signaling.

Additionally, the TCSPC mode can build up photon distributions over the time after the excitation pulse, and a number of additional parameters. These can be the wavelength of the photons, coordinates along the dimensions of a detector array, the coordinates of a scanning area, the time from the start of an experiment. Any parameter that describes the photons or the state of the system under investigation can be used. Multi-dimensional TCSPC also includes excitation wavelength multiplexing by a number of multiplexed lasers or...
laser wavelengths. Depending on the operation mode and the configuration of the optical system single optical waveforms (decay curves), multi-wavelength decay patterns, sequences of single decay curves or multi-wavelength decay patterns, FLIM images, sequences of FLIM images, multi-spectral FLIM images, or time-resolved spectra can be recorded. We are currently using FLIM to study the dynamics of pluripotent stem cell differentiation and cardiogenesis.

Applications

- Marker-free discrimination of cell types
- Analysis of stem cell differentiation
- Detection of pathological cells
- Analysis of cell viability
- Extracellular matrix remodeling [1 – 5]
- Protein-protein interactions
- Intracellular physiology
- Metabolic state of cells and tissue
- Real-time monitoring of cell death
- Identification and degradation of collagen and elastin [1 – 5]
- Analysis of proteoglycans and hydroxyapatite [4]

References


1 SD confocal microscope.
2 ECM imaging of an embryonic body.
3 3D projection of tissue-engineered elastic fibers. Green represents elastic fibers and white cell nuclei.
Flow cytometry

At the Fraunhofer IGB, we supplement classical cell characterization methods with flow cytometric analysis on behalf of our customers. The well-established and standardized FACS technique allows the characterization of relevant cell populations. Various immunofluorescent dyes or antibodies can be coupled with the antigen or protein that is of interest. Thus, a very wide variety of cell populations can be detected and sorted for further analysis.

Advanced FACS technology

The Fraunhofer IGB runs a flow cytometry service unit with a high-performance and dynamic cell analyzer (FACS Verse™ equipped with a three laser compact optical deck, and numerous detectors). Up to ten parameters can be simultaneously determined (Forward scatter FSC, side scatter SSC, six colors, time). A set of various optical filters allows the use of different fluorochromes. It is designed to reduce light loss and increase resolution for multicolor applications. The system includes a patented automated laser alignment, smart filter-mirror units for the detector arrays, and a stainless steel flow cell, which are designed to maximize reliability and improve system performance.

Our performance – your competitive edge

The FACS service unit of the Fraunhofer IGB has already supported numerous companies and research institutes at home and abroad. We perform the analysis of relevant cell populations according to your requirements. Furthermore, kinetic studies (e.g. calcium flux), analysis of cell cycle and proliferation, apoptosis as well as detection of fluorescent markers such as green fluorescence protein (GFP) are included in our range of services.

Documented quality

Our FACS analyses quality is regularly assessed in various inter-laboratory tests for flow cytometry at external institutions. The inter-laboratory test of the RfB determines the immune status of human donor blood samples. Here, six parameters are determined, such as the absolute number of lymphocytes, the number of entire T cells, T-helper / T-suppressor cells, B cells and NK cells as a percentage of the lymphocytes measured by means of a four-color analysis. With the inter-laboratory test CD34+ enumeration of the INSTAND e.V., the absolute number of CD34+ stem and progenitor cells, as well as the CD34+ percentage of the stem and progenitor cells of all leukocytes in human donor blood is determined using flow cytometry.
**ImageStream®X Mark II**

Imaging flow cytometry is a radical advancement in flow cytometry allowing for the in-depth imagery analysis of every cell. It provides elucidating data for the study of cell-cell analysis, DNA damage and repair as well as cell morphology. The machine provides both bright field and dark field images, and 10 fluorescent markers with sensitivity exceeding standard flow cytometers.

The Department of Cell and Tissue Engineering’s partner lab at the University Women’s Hospital Tübingen leads the University Core Facility for Imaging Flow Cytometry.

**Applications**

- Cell death and autophagy
- Cell signaling
- Cell cycle and mitosis
- Cell-cell interaction
- Co-localization
- DNA damage and repair
- Internalization
- Morphology analysis
- Spot counting

1. **FACS Analysis service performed at the Fraunhofer IGB.**
2. **Imaging FACS Analysis services performed at Fraunhofer IGB partner lab.**
LASER CAPTURE MICRODISSECTION

The microdissection unit consists of a microscope, combined with a laser beam. With this unit, the smallest samples (1–1000 µm), i.e. individual cells, but also cell nuclei, can be dissected from a tissue section for post-processing. For example tumor cells from the adjacent healthy tissue can be isolated and examined.

Mode of operation

A digital video camera files the pictures of a sample to a computer. For the microdissection, the elements marked before at the screen, e.g. certain cell nuclei from a certain tissue or also individual living cells, are dissected from the surrounding tissue with a software-controlled laser beam. Afterwards they are extracted by single laser pulse counter-gravity into a sampling container.

Benefits: contact-free and contamination-free

The 355 nm solid laser has an operation accuracy of distinctly less than 1 µm. This procedure guarantees that no unwanted sample elements can get into the container. As the laser reacts for only about a nanosecond during this process, the dissected sample cannot warm up. The procedure is thus totally contact- and contamination-free. Moreover, it ensures the greatest possible protection of the material.

References


Dissecting development for regeneration

As part of the Fraunhofer Attract Grant, our researchers have used laser microdissection to isolate developing fetal heart valves to better understand the process of heart valve development as a strategy to apply these mechanisms into the design of a biological and growing heart valve replacement [1, 2]. We are also isolating fetal cardiac progenitor cells from their developing niches to elucidate how these cells proliferate and differentiate with the aim of translating the knowledge into bioreactor systems designed to increase the yield of induced pluripotent stem cell derived cardiac progenitor cells.

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1 Tissues are dissected from heart samples.
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Fraunhofer IGB
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