TURKU BIOIMAGING

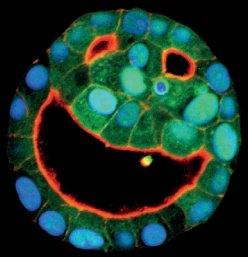
{ More than you can imagine }

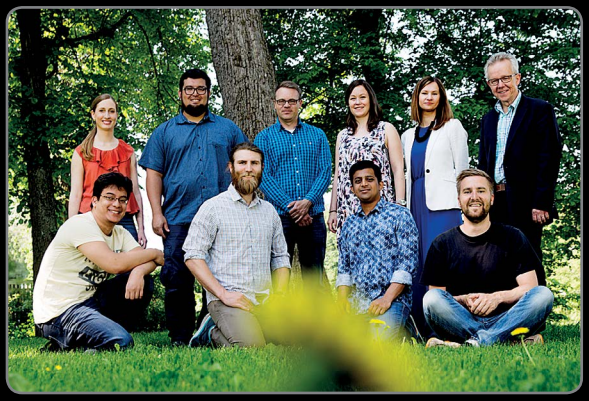
Preface: from atoms to anatomy

This is the third edition of the already rather iconic Turku BioImaging (TBI) “Black Book”, and this time it’s all new!

TBI is an umbrella organization for biological and medical imaging in Turku, and this book presents most of what TBI has to offer to scientists in need of imaging. TBI covers the whole spectrum of imaging, from nanoscale and cellular imaging to small animal and medical imaging – “from atoms to anatomy” – not forgetting aspects such as teaching and image analysis.

The chapters in this book form a continuum from very high resolution to gradually lower resolution, all the way from “atoms” to “anatomy”. The idea has been to illustrate the various imaging techniques along the way, so that each technology has its own spread. The chapters provide concise technology overviews, including application areas, how individual technologies work, and what needs to be considered when using them. For each technique, you will also find a dedicated contact email that can be used to reach local specialists for further details and practical matters. At the end of the book, there is an instrument appendix listing all TBI instruments in the alphabetical order according to the technique names. The appendix also includes information about the locations and contact persons of the respective technologies and instruments, and a map of the campus area to help in locating the instruments.





TBI team in summer 2016.

Bioimage informatics, as a new field of science, has its own chapter at the end of the book, as does the TBI MSc programme in biomedical imaging.

Notably, TBI technologies always operate on open-access principles, some are even available on a large international scale through Euro-BioImaging.

We hope you enjoy this little exploration into the amazing world of imaging!

The Turku BioImaging Team

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1. Turku BioImaging

Introduction

Turku BioImaging (TBI) is an umbrella organisation for "all things imaging" in Turku, coordinating numerous imaging activities ranging from light microscopy to medical PET/MRI-imaging. TBI also trains scientists in modern imaging technologies and hosts a MSc programme in biomedical imaging. Many experts in the field are convinced that the future of bioimaging lies in strong global networking, and TBI has a central role in building up the pan-European imaging network, Euro-BioImaging (EuBI) and its worldwide extension, Global BioImaging. TBI is also very active in Finnish and Nordic imaging networks. TBI has a highly experienced staff that takes care of for instance administration, coordination, web tool development, public relations and funding acquisition, and also actively participates in teaching. The core facilities and other service providers of TBI have their own dedicated staff.

www.bioimaging.fi

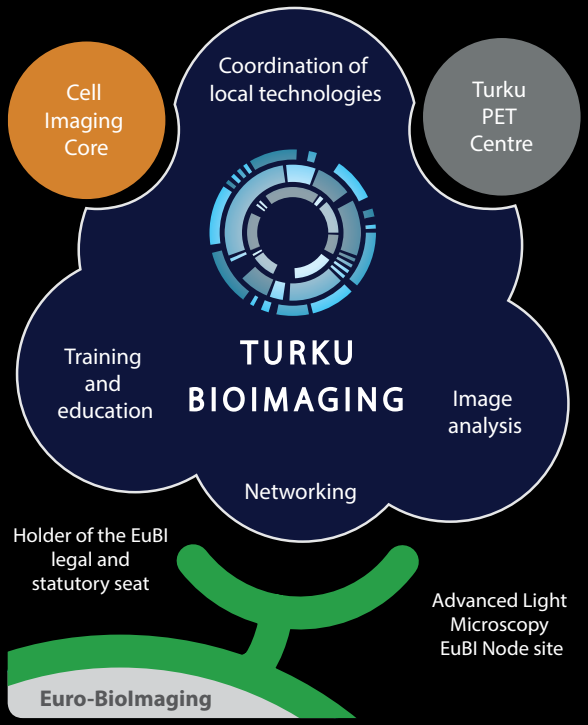
Local technology units

TBI services are offered by two main core units: Cell Imaging Core (CIC) and Turku PET Centre. In addition, numerous other smaller units, such as the Laboratory of Biophysics and the Laboratory of Electron Microscopy, provide important imaging modalities. Affiliated facilities, such as Turku Centre for Disease Modelling, Auria Biobank and the Turku University Hospital, offer supporting and related services.

The Turku CIC Turku CIC is a centralized imaging facility that offers support and instrumentation for both conventional and advanced light microscopy and flow cytometry, with additional capabilities for nanoscale imaging using techniques, such as super-resolution and atomic force microscopy. CIC is one of the core facilities of the Turku Centre for Biotechnology, a joint organization of Åbo Akademi University and the University of Turku. CIC serves approximately 200 users every year, with services open to both national and international academic and industrial users. In addition to state-of-the-art instrumentation, CIC offers theoretical and hands-on training, experiment design consultation and image analysis services.

www.btk.fi/cell-imaging

Turku PET Centre employs short-lived positron emitting isotopes for a number of *in vivo* imaging techniques that are in high demand in clinical diagnostics and practice as well as in biomedical research. The centre, which is a Finnish National Research Institute, provides high-end research, diagnostics, and clinical *in vivo* imaging services for the whole country. Currently, Turku PET Centre employs nearly 200 staff members and investigators with expertise in radionuclide production, radiochemistry, and preclinical and clinical imaging. Apart from PET tracer and drug discovery



TBI is an umbrella organisation connecting local and international imaging facilities. TBI offers both training and education for life scientists.

and development, image analyses and modelling, Turku PET Centre has research expertise especially in the fields of translational, cardiovascular and metabolic research, neuroscience, and oncology. The research modalities include tissue autoradiography, PET, PET/CT, PET/MRIand MRI (3T) imaging and digital ultrasound. All researchis performed in compliance with guidelines and quality requirements, e.g. EU directives and current GMP, GLP and GCP guidelines. *www.turkupetcentre.fi*

TBI as partner in Euro-BioImaging (EuBI)

In recent years, the tremendous development of imaging techniques has revolutionized biomedical research. However, access to cutting-edge techniques is often a bottleneck for the interested user. EuBI aims to overcome this problem by establishing a coordinated open-access network of imaging infrastructure in Europe, as outlined *www.eurobioimaging.eu*

Through EuBI, scientists from Europe and beyond can gain access to any of the imaging technologies offered by the EuBI Nodes, i.e. distinct ratified imaging centers across Europe. The Nodes offer a wide portfolio of techniques, ranging from imaging of cells and tissues to imaging of animal models to clinical imaging of humans. EuBI also provides training, technical support, image analysis, and public image repository services.

TBI has a central role in EuBI, as the EuBI headquarters (Hub) will be located in Turku, in partnership with European Molecular Biology Laboratory (EMBL) in Heidelberg (providing expertise in biological imaging) and University of Torino, Italy (providing expertise in medical imaging). TBI, therefore, has a central role in EuBI, ensuring excellence in bioimaging expertise and infrastructures not only in Turku, but keeping TBI at the very forefront of imaging globally. In addition to the Hub, Finland also hosts one of the EuBI Nodes. This Finnish Node is a multi-sited advanced light microscopy node consisting of three partners: Turku, Oulu and Helsinki. If you would like to use EuBI in your research, getting open access to quality-assured, state-of-the-art imaging services across the continent, submit an application at:
www.eurobioimaging-interim.eu

Hallmarks of Turku BioImaging

2007

TBI was established as a joint organization of University of Turku and Åbo Akademi University.

2009

Ministry of education recognized TBI as a significant national research infrastructure when TBI is accepted to the National Infrastructure Roadmap.

2010

Master's degree programme in biomedical imaging welcomed its first students.

2010

TBI became one of the leading partners building up the EuBI network.

2013

EuBI is accepted to the National Infrastructure Roadmap

2015

Finland and TBI were chosen as the legal and statutory seat of EuBI, and TBI started setting up the EuBI Hub together with partners from EMBL and Torino.

2016

EuBI started its interim operation, with access procedures developed and maintained by TBI. The Finnish Advanced Light Microscopy Node, coordinated by TBI, started its operation.

Any questions or comments? Please contact the TBI office:

tbi-office@bioimaging.fi

2. Nanoscale imaging

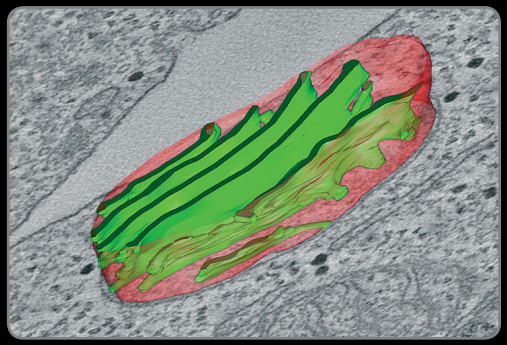
Electron microscopy

What is it for?

Electron microscopy is the method of choice if detailed structural information at nanometre resolution is required from samples of macromolecules, nanoparticles, viruses, bacteria, cells, tissues and other related materials. The Laboratory of Electron Microscopy at the University of Turku is a core facility that provides electron microscopes and related services for investigators within the Turku area and also for scientists from all over Finland. Provided techniques include conventional Transmission and Scanning Electron Microscopy (TEM/SEM), Correlated Light and Electron Microscopy (CLEM) and Electron Tomography.

How does it work?

Electron microscopy is an imaging technique that relies on an electron beam to visualise the sample. Use of electrons, instead of photons, potentially allows imaging at the nanometre scale. The interactions of electrons with a specimen are also used to provide information on the fine structure in electron diffraction experiments. In TEM, the interactions of electrons transmitted through an ultrathin (60 nm) section of a specimen are imaged. In SEM, a focused electron beam is scanned over the sample surface and an image is derived from secondary or backscattered electrons emitted from the specimen.



Electron tomographic reconstruction of mitochondria.

JEOL JEM-1400Plus transmission electron microscope.

Things to consider

For TEM, biological samples must usually be fixed with aldehyde, dehydrated and embedded in epoxy or another appropriate resin before being cut into thin sections. In addition, cellular components need to be stained with heavy metals for a reasonable contrast. For SEM, samples are dehydrated and often coated with a thin layer of conductive material, such as gold or carbon. Macromolecules or nanoparticles can be imaged even without fixing or staining, but it should be kept in mind that the ultra-high vacuum and intense electron beam may affect unstained samples.

- High resolution imaging for a wide range of samples.
- Transmitted or backscattered electrons used for image formation.
- Sample preparation and harsh imaging conditions may affect the sample.

Contact: em@bioimaging.fi

Atomic force microscopy

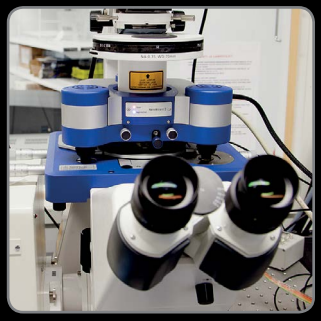
What is it for?

Atomic Force Microscopy (AFM) is primarily used to image surface topography. With functionalised probes, it can also be used to measure specific binding forces between molecules such as receptors and ligands, or between cells and a substrate. AFM imaging is suitable when the resolution of optical microscopy is not sufficient, when a sample needs to be imaged under native physiological conditions, or when the type of sample preparation required for electron microscopy is not possible. In addition, AFM can be used to manipulate objects, e.g. to study the effects of specific mechanical stimuli on cells.

How does it work?

AFM is a type of scanning probe microscopy technique, in which a sharp tip on a flexible spring-like cantilever mechanically scans over a specimen's surface and senses the forces between the tip and the surface. Such forces cause the cantilever to bend, and the deflection is measured using a laser beam reflected from the cantilever. The AFM can be operated in liquid and under physiological conditions, making it possible to study living biological specimens. The technique is label-free, making sample preparation straightforward. Biological AFM is often coupled to optical microscopy.

Collagen fibrils imaged with AFM.



Nanowizard II AFM-scanner incorporated with Zeiss 510 LSM confocal microscope.

Things to consider

AFM acquires the surface topography of specimens. Thus, AFM is limited in studying the internal features of the specimen. Selecting a proper probe (spring constant, tip shape, resonant frequency, and surface functionality) is critical.

AFM utilises mechanical contacts between probes and sample surfaces; therefore, it is prone to noise and vibrations coming from the surroundings. Care must also be taken that these mechanical contacts do not damage the specimen. AFM also requires relatively long image acquisition times (typically several minutes to tens of minutes) and its field of view (scan area) is often smaller than with commonly used optical microscopes.

- Mechanical probing can be used for visualising the topography of a surface, its mechanical properties and biological affinity.
- Possible to image biological samples in liquid in their native state.
- Correlative studies combining confocal light microscopy with AFM are possible.

Contact: afm@bioimaging.fi

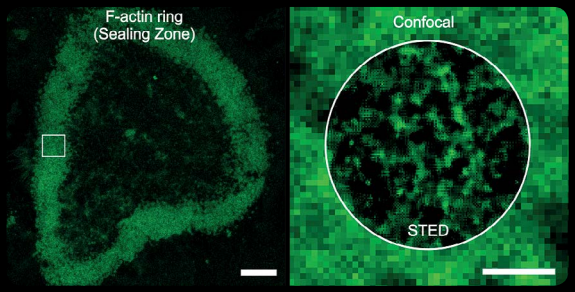
STED microscopy

What is it for?

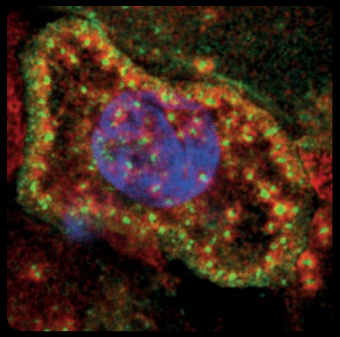
Stimulated Emission Depletion (STED) microscopes provide improved optical resolution (super-resolution) and are the instrument of choice when the resolution of normal confocal microscopy is insufficient and electron microscopy is not suitable. Compared with conventional confocal microscopy, STED microscopy provides 3 – 10 fold improvement in lateral (x-y) resolution and in some instruments axial resolution (z-resolution) as well. A STED instrument can be used in a similar manner to a confocal fluorescence microscope, and thus can provide non-invasive imaging of (living) specimens in three dimensions (3D).

How does it work?

STED microscopy is a type of confocal fluorescence microscopy. Compared with the diffraction-limited resolution by conventional wide field and confocal fluorescence microscopes (180 – 300 nm), STED can provide a resolution beyond the limit, down to 20 nm in biological samples. In STED microscopy, a doughnut-shaped high-power laser, called a STED beam, is overlaid on an excitation laser spot. Under the doughnut, spontaneous fluorescence emission is suppressed and the size of an effective emission



A thick actin ring specific to bone-resorbing osteoclasts is expressed (left). Zoomed-in STED image reveals interconnected F-actin mesh-like structures, which are not visible in a confocal image. Scale 10 µm (left) and 1 µm (right).



F-actin (green), vinculin (red), and nucleus (blue) are shown in human osteoclasts cultured on a glass surface. At STED resolution, structures of vinculin surrounding F-actin podosomes are revealed.

spot is reduced. This is how the resolution is improved in STED microscopy.

Things to consider

Samples for STED are prepared similar to those for confocal microscopy. The Abberior Instruments STED is compatible with fluorescent proteins, e.g. GFP, YFP, mCherry as well as organic dyes, such as Star488, Star520SXP and Star635P. In STED, very good staining is essential and we recommend higher concentration of antibodies and/or dyes than those used for confocal microscopy.

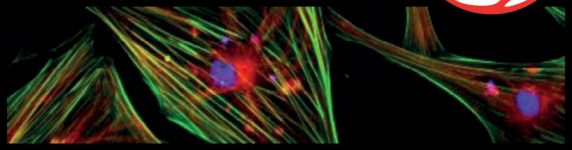
For the best possible resolution, specific mounting media, TDE and Mowiol, are highly recommended; water can be used as well. In addition, thickness-corrected glass coverslips should be employed, if available.

- Super-resolution technique for specimens stained fluorescently.
- Based on the stimulated emission depletion-phenomenon.
- Requires high density of labelling fluorophores in the sample.

Contact: sted@bioimaging.fi

misvik biology

Technology services for HTS



3. Cellular imaging

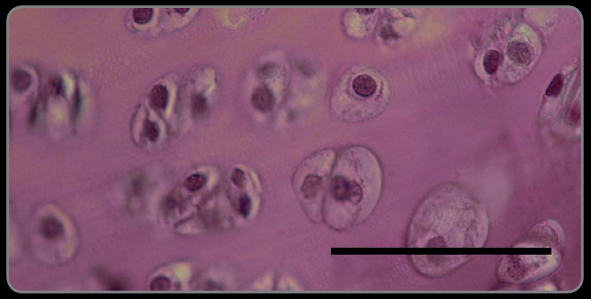
Widefield microscopy

What is it for?

A widefield microscope is an optical microscope that can use fluorescence and phosphorescence in addition to reflected and transmitted light to image and study cells, tissues, and a large variety of other samples. Most common studies in widefield microscopy are of fixed cells or tissue sections either labelled or expressing fluorescent reporters. Histologically stained samples, plant sections and living cells can also be studied. Widefield microscopy is a fast and efficient method to produce images for standard use such as optimisation of fluorescent reporters and the study of cell morphology. Additional contrasting methods, such as differential interference contrast (DIC), Hoffman modulation contrast (HMC) and phase contrast can be used to better visualise morphologies.

How does it work?

In widefield microscopy, the whole field of view in the sample is illuminated simultaneously. The source of light is usually a mercury lamp producing high intensity light with a broad spectrum of wavelengths. In fluorescence microscopy optical filters are used in order to select the wavelength of excitation light that is directed to the sample via a dichroic mirror. The fluorescent light is usually detected with a CCD camera or by eye. Multi-colour images of several types of fluorophores can be composed by combining several single-colour images or by using dual filter blocks. In brightfield microscopy, transmitted light can be simultaneously detected by attaching a black and white (b/w) or a colour camera. Fluorescence and brightfield images can then be overlaid.



Widefield microscopy image of mouse cartilage tissue,showing chondrocytes embedded in specialized extracellular matrix. Haematoxylin and Eosin staining. Scale bar 50 µm.

Leica DM IBRE widefield microscope.

Things to consider

Sample preparation is as important for the experiment as is the imaging step. It is important to know which filter sets and objectives are installed on the chosen instrument to choose the correct fluorophores, coverslips and mounting media. Also, illumination has to be optimised, as the applied light during imaging can bleach fluorophores. If the experiment requires live-cell imaging, it is necessary to use an inverted microscope. Cells should be grown on a special imaging dish and light exposure should be minimised as phototoxicity can damage the cells and alter cellular functions.

- Optical microscope for fluorescent and brightfield imaging.
- Ideal to study fixed cells or thin tissue samples.
- For best results, it is important to know which filter sets and objectives are installed on the chosen instrument.

Contact: widefield@bioimaging.fi

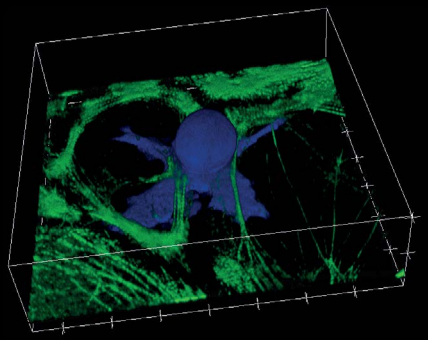
Confocal microscopy

What is it for?

Confocal microscopy is currently the most commonly used fluorescence microscopy technique to study the 3D distribution of proteins and cellular components in cells and tissues. It can be used to image fixed and living cells, and when combined with advanced software, it gives rise to a large variety of additional techniques. With confocal microscopy one can follow dynamic processes, such as the uptake of labelled molecules or the kinetics of intracellular trafficking. In addition, diffusion properties of labelled molecules can be followed using FRAP and fluorescence correlation spectroscopy techniques. Finally, molecular interactions at short distances can be detected by fluorescence resonance energy transfer (FRET) experiments.

How does it work?

Unlike widefield fluorescence microscopy techniques, confocal microscopy has the ability to collect fluorescence from a thin layer of the sample, enabling precise focusing on the region of interest. The confocal microscope uses a pinhole in front of the detector to confine the detection of emitted light to a very thin layer in the sample, often referred to as the focal plane. A highly focused high-speed laser beam is scanned across the specimen causing excitation of fluorophores. The subsequent emission can be detected through the pinhole. The specimen is scanned in several focal planes creating a 3D-image stack. Data from complete optical sectioning can then be used to construct a real 3D-image that can be visualised and analysed with suitable software such as BioImageXD.



An invasive breast cancer cell (blue) pushing its way between two endothelial cells (green). Image acquired with Zeiss LSM780.

Zeiss LSM 780 laser scanning confocal microscope.

Things to consider

Sample preparation and selection of a suitable instrument are as important as the imaging for the success of an experiment. Before choosing suitable fluorophores and mounting media, it is important to know which laser lines, filters and objectives are available in the selected instrument. Additionally, when conducting a live-cell experiment, special equipment should be used and turned on before the experiment. More advanced instrumentation, such as spinning disk microscopy, TIRF and two-photon microscopy are available at TBI. Please contact the CIC to help you select the correct instrument for your specific purpose.

- Enables imaging of cells and tissues in 3D with good resolution.
- Both fixed and living cells can be studied.
- Advanced functional light microscopy techniques (FRET, FRAP etc.) are available.

Contact: confocal@bioimaging.fi

Spinning disk confocal microscopy

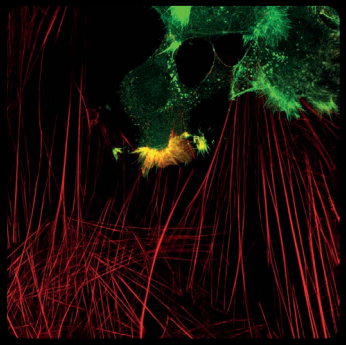
What is it for?

Spinning disk confocal microscopy offers high imaging speed with low phototoxic effects, which is ideal for live-cell imaging. However, the system is also a practical tool for all cell biology experiments, where fast 3D imaging is needed including: tile imaging, optical sections of thick samples etc. Due to the high imaging speed, the system allows imaging of multiple positions in 3D in live-cell imaging experiments with reasonable time intervals.

Emission detection of a spinning disk system is based on cameras with a low noise level compared to laser scanning systems, which produce beautiful images with a high signal to noise ratio. One of the special features of the setup is a near infrared (NIR) excitation laser, which now makes it possible to use five labels at a time. This system has also the Vector FRAP module for photomanipulation assays. All the laser lines are available for photomanipulation.

How does it work?

Spinning disk confocal microscopy is based on laser beams directed through the Nipkow disk with thousands of small pinholes, which produce thousands of excitation beams that are swept across the specimen as the disk spins. Thus, a whole field of view can be illuminated very fast. The resulting emission signal from the sample is transferred through the spinning disk to cameras, which are used for detection instead of photon detectors used in laser scanning systems.



Breast cancer cells expressing lifeact-mCherry (to specifically visualise their actin cytoskeleton, green) were left to invade through 3D collagen in the presence of primary mammary fibroblasts. All cells were stained with phalloidin to visualise the actin cytoskeleton (red). Spinning disk 100x

The spinning disk microscope at CIC: 3i Marianas imaging system with CSU-W1.

Things to consider

Data storage and image analysis steps must be planned carefully; the sCMOS camera produces large sized RAW files when full frame is used. For example, the size of a full 3D stack of a cell is around 1GB (4 channels, 30 optical sections). The photomanipulation module is easy to use, and the fast imaging speed makes the system attractive for performing FRAP experiments. Photomanipulation can also be set up and executed on the fly, which is useful e.g. in photo-activation experiments.

- For fast and minimally invasive 3D/4D imaging of fluorescently labelled specimen.
- Camera detector allows high signal to noise ratio.
- FRAP photomanipulation experiments available.

Contact: spinning@bioimaging.fi

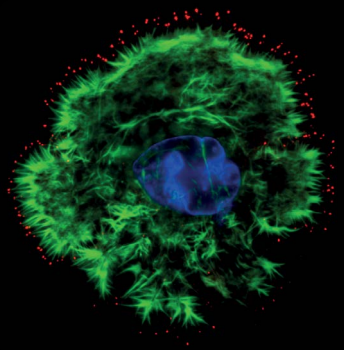
TIRF microscopy

What is it for?

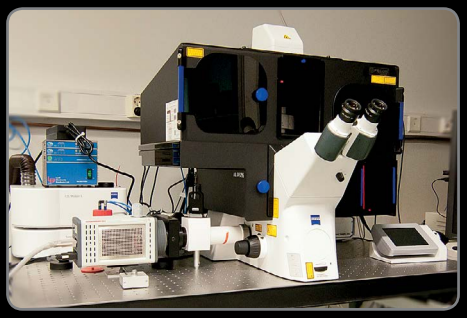
Total Internal Reflection Fluorescence microscope (TIRF) is a tool to image structures and molecular events at the cell membrane with a high z-resolution - usually less than 200 nm. Typical applications are cell adhesion, ligand binding and endosomal trafficking studies of fixed and live cell samples. TIRF microscopes have sensitive components in common, which make them good tools for all kind of widefield microscopy.

How does it work?

In a TIRF microscope, an excitation laser is directed to a sample from a specific angle, so that instead of penetrating the sample, the laser beam exhibits a total reflection at the interface of a cover glass and sample medium. This is possible only in the case of high NA objectives and a sufficient difference in refractive indices of the two materials at the interface (i.e. cover glass/water). Although the laser beam is reflected at the cover glass-sample interface, a small proportion of the light travels parallel along the cover glass, which creates a physical phenomenon called an evanescent field in a very restricted volume in aqueous phase. The evanescent field diminishes logarithmically as it enters deeper into the medium, and only the fluorophores near the interface are excited resulting in a z-resolution of 70-300 nm.



An osteosarcoma cancer cell expressing myosin-X (red) was stained with phalloidin to visualise the actin cytoskeleton (green) and with dapi to observe the nuclei (blue). TIRF 160x



Zeiss TIRF-3 at CIC

Things to consider

The sample must be in PBS or in cell imaging medium to achieve total internal reflection.

The whole cell volume can be imaged in widefield, but TIRF resolution is restricted only to the cell membrane facing the cover glass. TIRF is very sensitive to all variables affecting the light path, and a user must pay extra attention to a sample, glass-bottom sample dishes, immersion oil, objectives, sample holders, etc.

- Usefull technique for studying cellular membranes and their function.
- Total reflection confines fluorophore excitation in a very thin layer facing the cover glass.
- Requires high optical grade materials for sample preparation.

Contact: tirf@bioimaging.fi

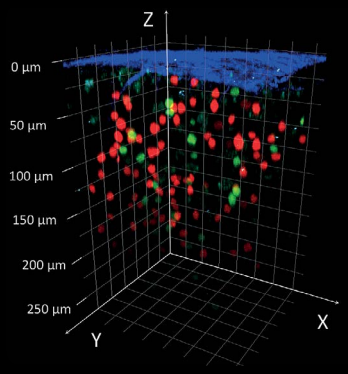
Two-photon microscopy

What is it for?

Two-photon microscopy is a laser-scanning microscopic technique typically used for the acquisition of 3D time-lapse images of living samples. As a major advantage, it allows a greater penetration depth into tissues than what is possible with conventional confocal microscopy (several hundred µm versus ~80 µm). Moreover, it induces less phototoxicity effects in living samples. This technique is thus ideally suited for intravital imaging in living, anaesthetised animals or living, explanted tissues.

How does it work?

Like confocal microscopy, two-photon microscopy allows optical sectioning of fluorescently-labelled samples, although on the basis of a different principle. In conventional fluorescence, excitation of a single photon (emitted by a visible-light or UV continuous-wave laser) absorbed by a fluorophore molecule is sufficient for its fluorescence. In contrast, two-photon excitation occurs after the near-simultaneous absorption of two photons (emitted by an infrared, femtosecond-pulsed laser), each of which carries only half of the energy needed. Since two-photon excitation events are rare, they only occur at places with a high density of photons, i.e. at the focal point. The result is fluorescent excitation occurring exclusively in the focal plane, not above or beneath it. Thus, optical sectioning is an inherent property of the technique, and no pinholes are needed.



A 3D image of an intact mouse lymph node demonstrating the potential imaging depth of a two-photon microscope. Red and green fluorescent lymphocytes are visible as deep as 250 µm below the capsule (blue) at the surface of the lymph node.

Leica TCS SP5 MP with multiphoton system

Signal detection is achieved by using close-coupled, highly sensitive non-descanned (external) PMT detectors. The utilisation of high excitation wavelengths results in greater tissue penetration, and due to confined excitation within the focal plane phototoxicity is significantly reduced.

Things to consider

While the technical setup is simple and the instrument is easy to operate, imaging experiments on living tissue require a lot of practice. If cells or tissues are labelled with a vital dye, e.g. CFSE, labelling conditions have to be carefully optimised and the combination of dyes (in the case of multi-colour experiments) has to be carefully planned. In the case of ***in vivo*** imaging experiments, the microsurgical procedures usually require a lot of practice as well.

- Good penetration and low phototoxicity for live tissue imaging.
- Simultaneous absorption of two low energy photons excites the fluorophore.
- For intravital microscopy of living animals.

Contact: 2-photon@bioimaging.fi

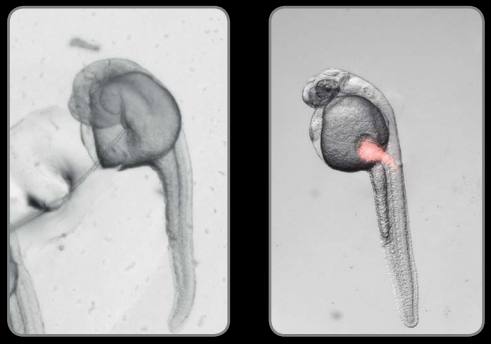
Stereo microscopy with microinjection

What is it for?

A stereo microscope is an optical microscope suitable for studying several kinds of samples, for example cell cultures, animal and plant tissues, whole plants, zebrafish, eggs (CAM model) and insects. The stereo microscope at TBI is equipped with 0.8x and 1.2x long working distance objectives and an Eppendorf InjectMan NI2 semi-automatic microinjection device permitting microinjection of any material into various samples. During the injection the sample can be visualised through objective lenses with a maximal magnification of 120x. The most frequently injected materials are DNA, proteins, tracers and chemicals with or without attached dyes.

How does it work?

Stereo microscopy is designed for imaging various samples at a low magnification. It typically uses two separate optical paths with two objectives and eyepieces to provide slightly different viewing angles to the left and right eyes. This arrangement produces a possibility for 3D visualisation of light reflected from the surface of the sample. Since



Zebrafish embryos immobilised in gel. Left: Brightfield image with injection tip penetrating the yolk sack. Right: A one-day-old zebrafish embryo transplanted with fluorescent breast cancer cells (in red).

Zeiss SteREO Lumar with Eppendorf InjectMan NI2 semi-automatic microinjection device.

the microscope is intended for low magnifications, long working distance objectives can be used for observation, leaving space between the objective lens and the sample. These characteristics enable detailed sample manipulation under the microscope. Additionally, stereo microscopes can typically be combined with fluorescence for more advanced applications.

Things to consider

The 3D effect is significantly dependent on the structure of the sample, such as thickness, opacity and contrast. In addition to the reflected light, most fluorescent material can also be imaged. The instrument at TBI is equipped with both b/w and colour CCD cameras to visualise fluorescent and non-fluorescent samples, respectively. For microinjection, commercial or self-made needles can be used. If the sample is alive, suitable living conditions have to be ensured for optimal results.

- Low magnification observation with availability of 3D vision.
- Can be combined with fluorescence imaging.
- Enables microinjection of material into various samples.

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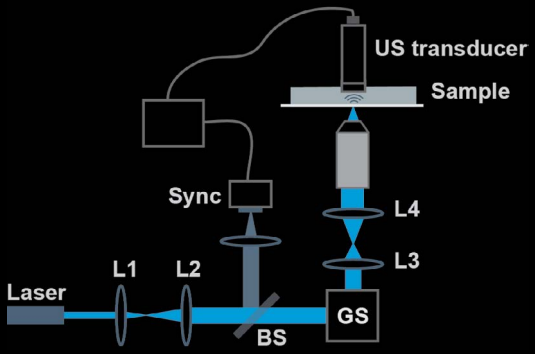
Photoacoustic microscopy

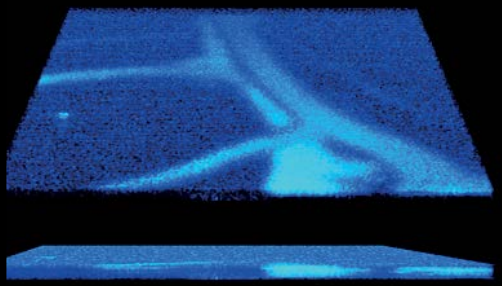
What is it for?

Photoacoustic microscopy (PAM) is an ***in vivo*** imaging technique that can generate high-resolution (µm-scale) images at depths of up to a few millimetres. PAM provides absorption contrast, similar to fluorescence techniques, but it does not require specific labels, which makes it a label-free technique. Labelling is possible as well, if the molecule of interest does not naturally absorb light.

PAM can be used for ***in vivo*** imaging; microvasculature, in particular, is a popular target due to the good endogenous absorption contrast of haemoglobin. Several other molecules can be imaged as well with visible laser wavelengths, e.g. lipids, water, collagen, DNA/RNA, bilirubin, cytochrome C and glucose provide intrinsic contrast for PAM in biological tissue. These endogenous contrast agents allow the visualisation of anatomical structures and the monitoring of functional indicators, such as blood oxygenation and temperature.



A photoacoustic microscope: The galvanometric scanner (GS) together with lenses L3 & L4 enable raster scanning of the focused laser beam. Lenses L1 & L2 expand the laser beam diameter to suit the microscope optics. Beamsplitter (BS) directs a small fraction of the excitation light to the (Sync) photodiode for electronics synchronisation.



Example of an image obtained by PAM. Vasculature of the chick chorioallantoic membrane is visualised.

How does it work?

PAM is based on "listening" to the broadband ultrasonic signal that is generated by absorption of pulsed excitation light in the sample due to the photoacoustic effect. Such combination of optical excitation with acoustical detection preserves the diffraction limited spatial resolution, whilst extending the penetration depth to mm range, well beyond the reach of purely optical imaging techniques. In PAM, an optical resolution point-illumination-based method is utilised, when a focused laser spot is scanned through the field-of-view and for each pixel ultrasonic signal is recorded.

Things to consider

The PAM system in Turku is an in-house built experimental system. It can be flexibly configured for different applications and at the moment, it can be used in collaboration with our local experts. Our PAM system currently only has a 532 nm laser excitation source, and thus multi-wave length imaging is not currently supported.

- Label free *in vivo* imaging technique offers mm penetration depth with µm resolution
- Based on measuring the photoacoustic effect where light absorption within the sample triggers mechanical sound wave propagation to the medium

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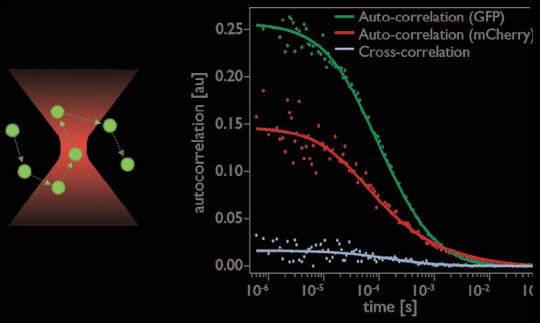
Fluorescence Correlation Spectroscopy

What is it for?

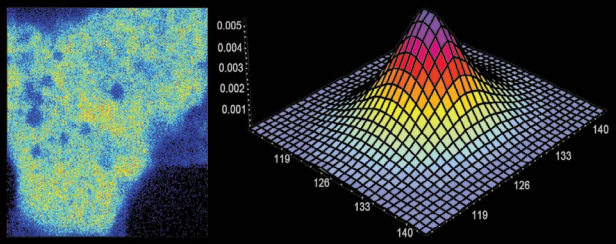
Fluorescence Correlation Spectroscopy (FCS), Fluorescence Cross-Correlation Spectroscopy (FCCS) and Raster Image Correlation Spectroscopy (RICS) are three techniques based on the analysis of fluorescence fluctuations in a femtolitre focal volume. These techniques can be applied in either a confocal or in a super-resolution STED microscope to provide information on parameters such as diffusion and concentration of labelled molecules. FCS, FCCS and RICS can also be used to identify and describe binding events, or by using variations of the focal volume, to follow plasma membrane dynamics in living cells.

How does it work?

Fluorescence spectroscopy techniques are normally implemented by counting photons over time on sensitive detectors such as avalanche photodiode detectors. Photons are collected and a correlation analysis of the fluorescence events is performed. Next, the correlation function is fitted to known mathematical models considering all processes that may contribute to the signal fluctuation to finally obtain quantitative information on parameters such as the diffusion coefficient and concentration.



Graphical illustration depicting the principles of FCS and FCCS (left) and plot presenting sample curves of auto-correlation and cross-correlation functions (right) used to determine diffusion and binding parameters.



RICS experiment showing a sample cell (left) and the resulting fit of the image correlation function (right) used to determine diffusion parameters.

In the special case of FCCS, a two-colour signal is recorded in separate channels and in addition to the two individual autocorrelation functions, a third cross-correlation function between the two colours is calculated. The cross-correlation function is used to identify binding events between molecules labelled with the two different tags of choice.

In RICS the illumination is not over a fixed local volume, as in FCS and FCCS, but instead the volume is moved to scan a larger region of interest. Laser scanning has to be done at such speed that the molecules will not move much before the laser can scan them again.

Things to consider

Fluorescence spectroscopy is used when quantifiable information about molecular dynamics is needed and not to create images. These techniques provide information about diffusion, concentration and binding events. Additionally RICS can generate diffusion and concentration maps containing information about changes in the dynamics from one location to another.

- Provides information about diffusion, concentration and binding events of labelled molecular species.

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Live cell imaging

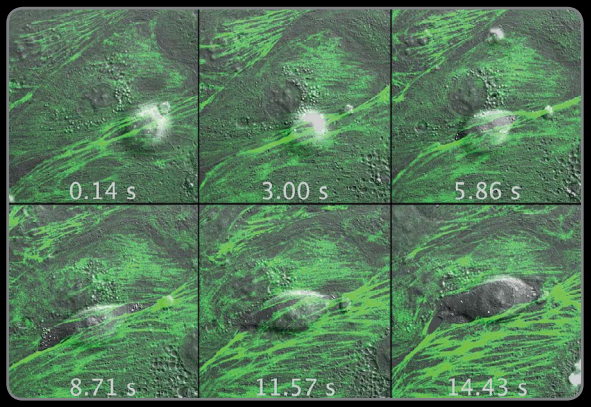
What is it for?

Live-cell microscopy is a versatile group of techniques allowing imaging of a living specimen. It is a preferred technique when the research question requires information about different phases of the cell life span rather than endpoint information.

Possible applications for live-cell imaging could be studies of fluorescent protein behaviour in the cell and cellular processes such as endocytosis, apoptosis, cell division, protein diffusion and translocation of proteins. The effects of abnormal conditions, such as hypoxia or high or low temperatures can also be studied.

How does it work?

TBI offers several live-cell imaging platforms on which the cells could be visualised in real time. These instruments offer traditional transmitted light and fluorescence imaging as well as advanced methods from TIRF and high-content widefield fluorescence microscopy to high-throughput confocal microscopy. Functional cell imaging techniques, such as fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP), photoactivation and FCS can also be conducted.



Invasive breast cancer cells pushing between two endothelial cells (green) during intravasation. DIC contrasting was used for the transmitted light. Image was captured with Nikon A1 confocal microscope.

Things to consider

The primary requirement for live-cell imaging is the establishment of suitable conditions for the cells (CO_2 level, humidity, and temperature) to protect normal cell function during image acquisition. Additionally, cells should be grown in a suitable dish for live-cell imaging. Cell culture media should preferably not contain a pH-indicator and, if the used instrument does not have a CO_2 controller, it should be CO_2 buffered.

Exposing living cells to stressful environments can reduce their biological functioning or even lead to cell death. Living cells are very light sensitive, thus the minimisation of laser power and acquisition time during imaging is crucial. Additionally, high cellular concentration of fluorescent reporters can be toxic to the cell and should be optimised. Live cell imaging is always a compromise between achieving the best image quality and preserving the health of the cell.

- Live cell imaging enables the study of dynamic processes in living cells.
- Living cells are sensitive to surrounding conditions.
- Live-cell Imaging experiments have to be well planned.

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4. High-content techniques

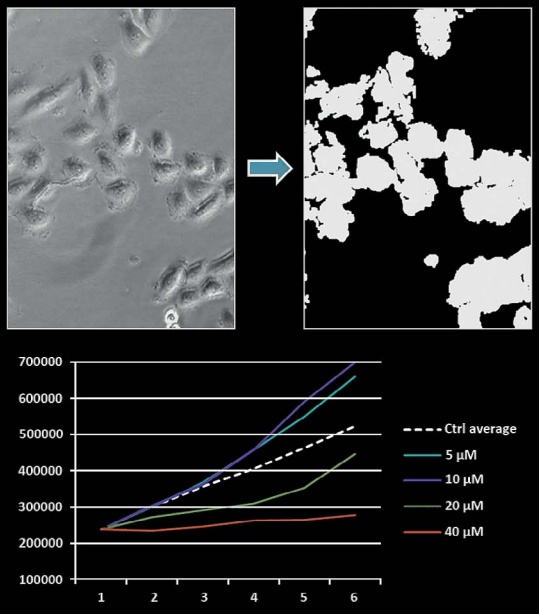
Basic high-content imaging

What is it for?

In addition to more advanced high-content imaging devices, such as those based on confocal microscopy, there are simpler devices for basic high-content brightfield and fluorescence imaging. Typical examples are Cell-IQ and IncuCyte. The former has its own built-in incubator, whereas the latter is placed inside a normal cell culture incubator. These devices are typically rather easy to use, and can be used to image multiwell plates stably over several days. Typical studies include cell proliferation and migration assays, used for instance in drug screening.

How does it work?

Cells are cultured on standard plastic multiwell plates and placed in the imaging device. Imaging patterns are then set up (imaging frequency, positions and fluorescence settings



Segmentation and analysis of cell proliferation under different drug concentrations using Cell-IQ and the BioImageXD software.

Cell-IQ high-content imaging device.

etc.), after which the imaging goes on automatically, often with automatic focusing. The cells are kept under constant temperature and CO_2 during imaging. When the experiment is finished, the images are analysed, using either software provided by the device manufacturers, or by third party software. In some cases, multiple images from a single well are stitched together during post-processing.

Things to consider

High-content experiments take a long time and produce a lot of data. Proper experimental design, timing and quantitative image analysis are important. When planning an experiment, one should also consider the time it takes for the device to capture one set of images. This can be rather long, and it limits how short the time interval between successive time-points can be. As with all live-cell imaging, experiments are often a compromise between image quality, resolution and sample viability.

- High-content imaging techniques based on widefield microscopy can be used for migration and proliferation essays.
- Instrumentation consists of an incubator and an imaging device allowing long follow-up studies of e.g. cultured cells.

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High-content microscopy

What is it for?

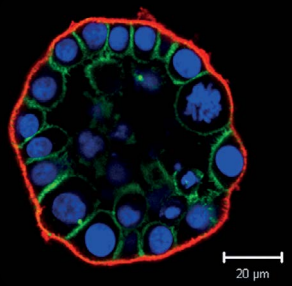
High-content imaging is a rapidly growing field of microscopy with a broad spectrum of applications that range from cell biology and basic research to pharmaceutical drug discovery. High-content imaging is now widely used in drug testing, systems biology, siRNA screening, and functional genomics.

How does it work?

High-content imaging typically relies on fluorescence microscopy experiments in which the biology has been adapted to multiwell plates, and in which image acquisition and subsequent quantitative analysis have been more or less automated. Often, two or more "multiplexed" assay formats are combined based on, for example, immune staining, reactive dyes, or traceable cells and cell lines that stably express fluorescent proteins.

Many morphological properties can be analysed in parallel, including the expression or localisation of fluorescent labels within cells, changes in intensity over time, alteration in cell morphology and movement, quantification of cell proliferation versus cell death, as well as the characteristics of organelles, nuclei or the cytoskeleton.

Simultaneous imaging of multiple cellular targets allows the analysis of numerous cellular features within a single experiment. With increased scale and speed of image acquisition and analysis, the demand for sophisticated instrumentation platforms is increasing. The hardware (the microscope) and software in high-content imaging systems are typically closely integrated, with the goal to standardise



Acinar differentiation of prostate epithelial cells, as imaged in the Axiovert 200M spinning disk microscope. Immunofluorescence staining of actin (green), laminin (red), and DNA counterstain (Hoechst).

Perkin Elmer Operetta. A compact spinning disk confocal microscope, particularly designed for rapid analyses of fixed and live cells in microtiter plates.

image acquisition, speed up image analysis and support data visualisation.

Things to consider

The high-content imaging process is typically divided into 4 stages: cell biology, image acquisition, image analysis, followed by data analysis and visualisation. Different high content microscopes provide diverse advantages that need to be carefully considered for each experiment. This depends on the experimental throughput, the desired image resolution, the inherent nature of the cells, and the matching assays utilised.

- High-content imaging uses integrated instrumentation for fast imaging of multiple samples and automated image analysis.
- Often used for fluorescence microscopy experiments.

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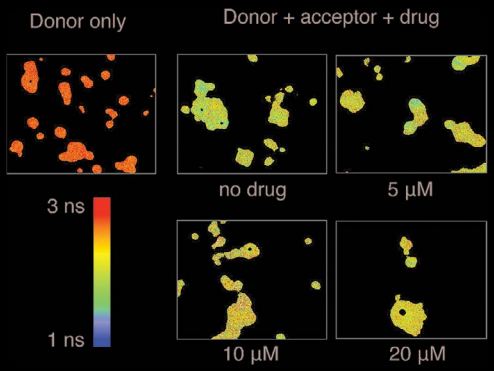
FLIM-FRET microscopy

What is it for?

The automated high-throughput FLIM-FRET system enables high-content FRET measurements in a fast and precise way representing an ideal method for drug discovery and target validation. Fluorescence lifetime imaging microscopy (FLIM) is a sensitive and precise technique to create microscope images based on the differences in the fluorescence lifetime of a fluorophore, or in other words, based on changes in the exponential decay rate of fluorescence from a fluorescent sample. Given that the fluorescence lifetime of a molecule changes depending on environmental factors, like pH, temperature or fluorescence resonance energy transfer (FRET), FLIM can be used as a precise method to detect changes in such environmental factors. In particular, detecting FRET, an energy transfer phenomena caused by the close proximity of another fluorophore (typically less than 10 nm away) can be done precisely by FLIM. FLIM-FRET is useful to report on protein conformation changes and on protein-protein, protein-nucleic acid, and protein-small molecule interactions.

How does it work?

FLIM-FRET measurements are performed on a conventional inverted microscope with a frequency domain FLIM-FRET attachment that serves to excite the donor fluorophore and detect its fluorescent signal. The LI-FLIM software connected to the system then calculates the fluorescence lifetimes for each of the pixels in the image. For high-content applications, the FLIM-FRET system is equipped with an automated stage and focus drive that move and position the sample according to the specified locations. The stage can be used to acquire a sequence of images in a grid pattern on a microscopic slide, or to scan all the different wells of a 24-, 48- or 96-well plate. The system is also equipped with a focus compensation system that can correct changes in the z-direction.



Sample lifetime images showing changes in dynamics occurring due to FRET under different conditions, e.g. in the absence of protein-protein interaction (donor only / no FRET) or in the presence of protein-protein interactions (donor + acceptor + drug at different concentrations).

A1	2	3	4	5	6	7	8	9	10	11	A12
B	2,563	2,245	2,250	2,256	2,277	2,296	2,323	2,338	2,370	2,371	B
C	2,553	2,250	2,251	2,263	2,266	2,290	2,313	2,345	2,368	2,366	C
D	2,565	2,248	2,259	2,263	2,266	2,283	2,319	2,335	2,359	2,364	D
E	2,556	2,243	2,248	2,251	2,282	2,297	2,321	2,362	2,365	2,373	E
F	2,564	2,232	2,251	2,278	2,279	2,293	2,323	2,343	2,367	2,369	F
G	2,557	2,239	2,258	2,275	2,277	2,304	2,328	2,341	2,374	2,370	G
H1	2	3	4	5	6	7	8	9	10	11	H12

Min	50%	Max
2,2317	2,3983	2,565

Colour-coded table displaying the fluorescence lifetimes [ns] values obtained in a 96-well plate where each column corresponds to six repeats of the same condition and each well represent an average of four images taken in the same well.

Things to consider

FLIM-FRET has advantages over intensity-based FRET measurements because it is insensitive to the concentration of fluorophores and it does not suffer from cross-talk issues. However, as already mentioned, fluctuations in pH and temperature can affect the lifetime and therefore they should be kept as constant as possible.

- Useful for reporting molecular events such as protein-protein interactions in nm scale.
- Automated high-throughput ideal for fast drug discovery and target validation.

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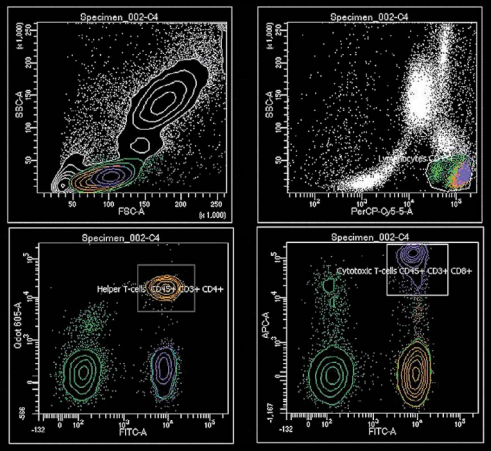
Flow cytometry and cell sorting

What is it for?

Flow cytometry is a laser-based technology for biomarker detection in cells and other particles, and is also used for sorting specific sample populations. It is routinely used in diagnosing blood cancers and other health disorders. It has many applications in basic research and clinical practice. Flow cytometric cell sorting is an efficient way to enrich cell populations quickly and with high purity. There are clear advantages of using cell sorting instead of, for example, magnetic cell enrichment. For instance, one can use several labels to choose exactly which cell population to isolate. There are no alternative methods for some enrichment procedures, such as sorting of GFP-positive cells.

How does it work?

First, the sample is pressurised and then analysable particles (cells) are suspended in a sheath fluid stream. The particles pass a laser in a queue, advancing at great speed (from 500 to >70000/s). When the particle passes the laser, its size, granularity and fluorescent properties are quickly measured and registered. In cell sorting, particles or cells are similarly



Multicolour flow cytometry detection of cytotoxic and helper T-cells from human peripheral blood.

The LSR Fortessa flow cytometer with laser lines visible.

run in a sheath fluid stream. The stream is converted to droplets by a vibrating nozzle. Each droplet optimally contains only a single cell or no cell at all. If one wants to collect a specific droplet, it will be given an electric charge. Then, high voltage deflection plates will guide the droplet into the collection tube or a multiwellell plate.

The results of flow cytometry experiments can be analysed with specialised software, such as locally made Flowing Software:
www.flowingsoftware.com.

Things to consider

Prior discussion with CIC personnel is vital. The cells have to be in a single cell solution. The fluorochromes have to match the lasers and detectors.

- Laser-based technology for cell/particle sorting and biomarker detection.
- Suspended cells or particles are individually registered or sorted.
- Several fluorescent labels can be measured simultaneously.

Contact: flow@bioimaging.fi

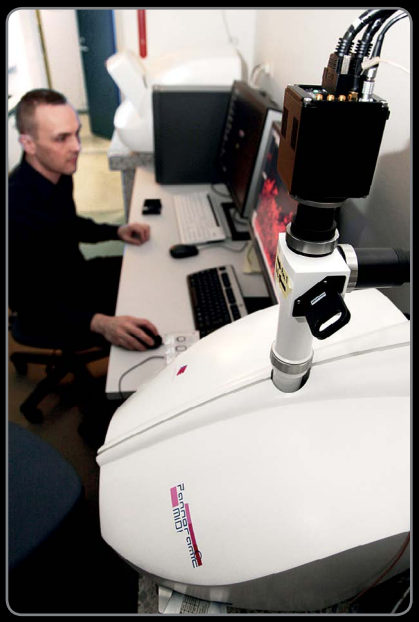
Microscope slide scanners

What is it for?

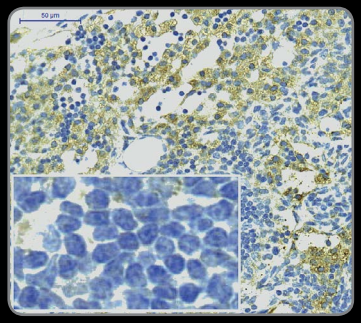
Slide scanners are used to automatically digitise entire microscope slides. Earlier, microscope-mounted cameras were used to capture histological details of interest, but modern whole slide imaging allows storage and sharing of all the histological information present on a microscope slide. This allows, for example, the visualisation of large digital datasets with so called virtual microscopes, and in this way slides can be evaluated by multiple persons at the same time. Moreover, advanced image analysis can be used for e.g. automatic tissue recognition. Two slide scanners are available: Pannoramic 250 Flash for brightfield imaging and Pannoramic Midi for fluorescence and brightfield imaging.

How does it work?

From the imaging point of view the slide scanners work in the same way as ordinary brightfield and fluorescence microscopes. In addition, they have fully automated scanning systems to produce digital slides from the whole slide area. The brightfield mode can image all stains that produce contrast in ordinary brightfield imaging. See the instrument specification list in this book for the available fluorescence wavelengths.



Pannoramic Midi fluorescent slide scanner in the front. Pannoramic 250 Flash visible in the back.



Digital slide scanner with brightfield slide scanner Pannoramic 250 Flash.

Things to consider

Care should be taken in preparing the slides to ensure good quality scanned images. Slides must be essentially free of any flaws such as cracks, crooked coverslips, bubbles in mounting medium, etc. Not all slide brands are compatible with the Pannoramic Midi because of their thickness variance, so contact responsible personnel for advice before preparing slides to be scanned in the Midi, especially fluorescence samples.

- Slide scanners digitise whole slides of tissue samples.
- Virtual microscopes can be used to visualise data in a convenient way or image analysis can be used for e.g. automated tissue recognition.

Contact: scanners@bioimaging.fi

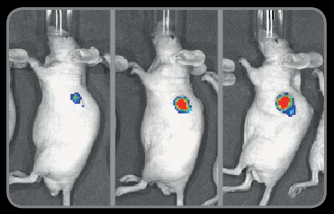
5. Small animal and preclinical imaging

Optical *in vivo* imaging

What is it for?

The IVIS optical imaging system provides quantitative fluorescence and bioluminescence imaging data from mice or rats ***in vivo***. Cells expressing luciferase or labelled with a suitable fluorescent molecular species can be visualised with the IVIS Spectrum or IVIS Lumina II system. Macroscopic ***in vivo*** imaging enables, for example, phenotyping of transgenic mice, observation of tumour growth in a living animal, follow-up of treatment responses, visualisation of inflammation and infections and monitoring of nanoparticle delivery to designated targets. A principal application area is the study of oncological xenografts, in which cancer cells with bioluminescent/fluorescent reporters are inoculated into immunodeficient mice/rats. These oncology models can be used to assess anti-cancer therapies over the course of treatment ***in vivo***. Non-invasive imaging of tumour growth and metastasis allows longitudinal evaluation of tumour development before, during, and after treatment, offering an excellent preclinical strategy for assessment of tumour response and recurrence.

Caliper LS (Perkin Elmer), IVIS Spectrum instrument in the small animal imaging lab.



Three week follow-up of a mouse with subcutaneously inoculated PC-3M prostate cancer cells carrying a red fluorescent reporter imaged with IVIS Spectrum.

How does it work?

The system employs a light-tight imaging chamber. Inside the chamber, a heated stage with built-in anaesthesia masks is available for five animals. The system has a CCD camera thermoelectrically (Peltier system) cooled to -90°C ensuring a low dark current and very little noise. The camera has high quantum efficiency over the entire visible-to-near-infrared spectrum. The stage is height-adjustable, allowing for a field of view of 5-12.5 cm. LED illumination is provided for reference photography, and a halogen lamp is available for fluorescence imaging. The system has a multi-position excitation filter wheel, and replaceable filter wheels to handle emissions at various wavelengths. All of these refinements are motor-controlled via IVIS Living Image software.

Things to consider

IVIS Spectrum or IVIS Lumina II is appropriate when fast or repeated imaging is required and a large number of animals are to be screened.

- Optical *in vivo* imaging of fluorescence and bioluminescence in small animals.
- Xenograft imaging, response follow-up and visualisation of inflammation and infection.

Contact: ivis@bioimaging.fi

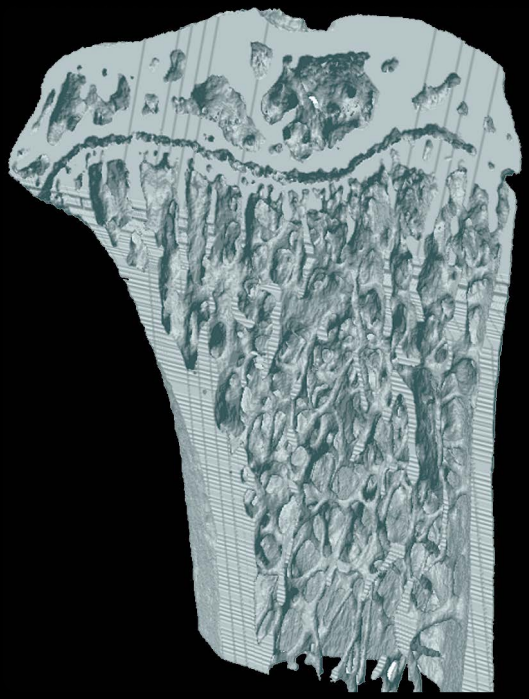
Micro-CT Imaging

What is it for?

Micro-Computed Tomography (micro-CT) is a method whereby X-ray computed tomography affords resolution on a micrometre scale. Micro-CT images can be used for volumetric analysis of scanned samples and 3D image reconstruction.

How does it work?

In micro-CT, hundreds of digital X-ray images are obtained from a single sample, but with slight variation in the rotational angle. The spatial location of every voxel (a 3D pixel) can be geometrically calculated from the images. Resolution of such images is, at best, on the micrometre scale, depending on sample size; the smaller the sample, the better the resolution.



Reconstruction of a mouse tibia.

Things to consider

Micro-CT is strictly an ***ex vivo*** technique. Micro-CT is principally used for analysis of bone samples as part of basic research in bone biology, and in the study of various implant materials developed to correct defects in bone. In addition, micro-CT can be applied to soft tissues, which, naturally, are of varying densities. Vasculature can be visualised after perfusion of a radio contrasting agent after sacrifice of the animal. The use of micro-CT is somewhat limited because of the need for small sample sizes (maximally that of a thumb tip), and the relatively long times required for sample scanning (about 45 min) and reconstruction (approximately 10 min/sample).

Analysis of sample density calls for proper machine calibration. It is preferable to try to scan the samples within a short time period as the X-ray source wears out upon time. If your study requires analysis of samples at time points very distant to each other you have to use a calibration according to the date of the scan.

- *Ex vivo* imaging technique for studying both biological and inorganic samples.
- 3D data reconstruction.
- Mainly used for studying rodent bone structure.

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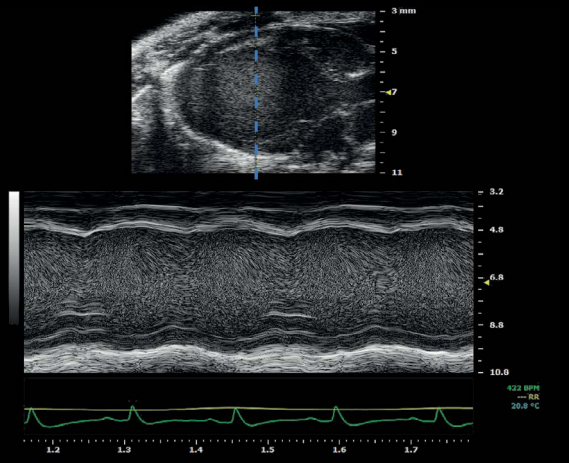
Ultrasound imaging

What is it for?

The high-frequency, high-resolution digital imaging platform with linear array technology and Colour Doppler Mode can be used non-invasively in a variety of animal models. This setting is especially effective in investigating cardiovascular function and structure, cancer and inflammatory-related processes.

How does it work?

A handheld transducer with a linear array of piezoelectric elements is used to produce acoustic pulses above 20 kHz that deliver sound waves into the animal's body. Diverse tissues, organs and disease lesions absorb and reflect sound waves differently depending on their density. High-resolution grayscale images are produced when the partially reflected sound waves return to the transducer. The resulting image is instantly viewable and can be captured as a still photograph or movie.



B-mode image of a mouse heart (top). M-mode image is acquired along the blue dashed line and shows the change in heart volume as it beats (bottom).

Although B-Mode, which displays a 2D cross-section of tissue, is the most common imaging mode with ultrasound, other image types can also be produced for studying physiological properties such as localisation and direction of blood flow, tissue stiffness and cardiac strain, vascularity, tissue motion over time and the presence of molecules and biomarkers.

Things to consider

As the apparatus is rather expensive and complicated to use, proper training is needed before one can use this equipment. The tip of the transducer is especially sensitive, avoid touching it with sharp objects or cleaning it with rough materials such as a hand towel paper.

Although ultrasound imaging is a very versatile technique for ***in vivo*** imaging, not all questions can be addressed by using it. Ask advice from experienced users regarding your study design. This might improve your research.

- Ultrasound is a non-invasive technique for studying e.g. tumour xenografts and cardiovascular function and structure in animal models.
- Several imaging modes for different purposes exist, such as, B-mode, M-mode and the Colour Doppler mode.

Contact: ultrasound@bioimaging.fi

PET imaging

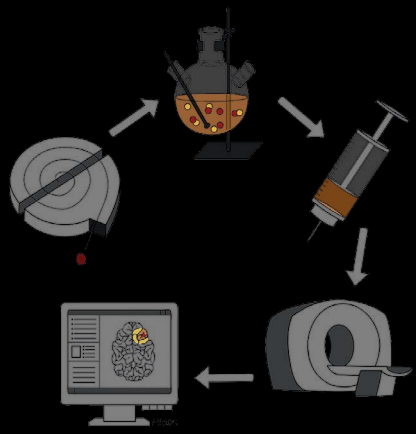
What is it for?

Positron Emission Tomography (PET) is a non-invasive method for imaging biochemical and physiological processes ***in vivo***. Minute amounts of biologically active compounds are labelled with positron emitting radionuclides and then administered to the subjects. The temporal and spatial distribution of these tracers within the body is visualised and measured with PET.

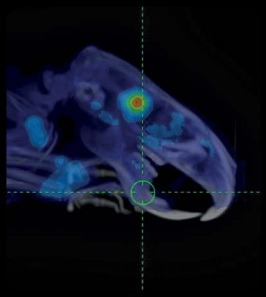
With PET it is possible to study tissue function and metabolism, the function of neurotransmitters and receptors, gene expression and drug pharmacokinetic/ pharmacodynamics profiles.

How does it work?

Positron emitting radionuclides are usually produced with cyclotrons. Short-lived (T½= 2-110 min) radionuclides are then incorporated into molecules of interest using sophisticated radiochemical syntheses. Due to the short half-life of the positron emitters, these syntheses have to be performed in a very rapid process. The tracers are then injected into the subject and a PET scan is performed. The images are reconstructed as tomographic images by mathematical processing of the data.



PET methodology from radionuclide production to image analysis.



Binding of the PET tracer [^{18}F]CFT, (a cocaine analogue) to the monoamine transporters in the rat striatum.

Things to consider

PET methodology always involves radioactivity and availability of suitable tracers is crucial for studying any phenomenon with PET. Time slots for radiosynthesis and PET scans should be planned carefully as projects are scheduled four times a year. Connecting with the expertise in existing groups is highly encouraged.

- Non-invasive method for imaging biochemical and physiological processes *in vivo*.
- Requires radiotracers suitable for highlighting physiological events.

Contact: pet@bioimaging.fi

6. Medical imaging

PET, PET/CT and PET/MRI

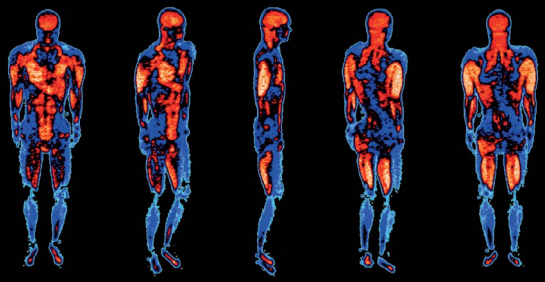
What is it for?

Positron Emission Tomography (PET) is an unsurpassed method for imaging biochemical and physiological processes *in vivo*. Combined with Magnetic Resonance Imaging (MRI) or Computed Tomography (CT), PET findings are localised anatomically. PET is used in patients with cancer and in cardiovascular, neurological and inflammatory diseases in clinical medicine. PET combined with tracer kinetic models measures blood flow, membrane transport, metabolism and ligand-receptor interactions noninvasively and quantitatively.

In drug development, PET characterises drug candidates in the early stages of development, including studies for pharmacological mechanism of action, pharmacokinetics, therapeutic dose range, subject selection and stratification.

How does it work?

Minute amounts of biologically active compounds are labelled with positron-emitting radionuclides (^{11}C, ^{15}O, ^{18}F, ^{68}Ga) and administered to subjects. The temporal and spatial distribution of the tracers within the body is measured with PET cameras. The data is reconstructed as tomographic images by mathematical processing. CT or MRI images are overlaid with PET images. Data is analysed mathematically and visually. See also section for the Carimas-software.



Skilful use of PET creates vast amounts of quantitative data for research and diagnostics.

Modern PET/CT cameras offer a non-invasive, versatile method of imaging physiological phenomena. Picture: PET/MRI camera at Turku PET Centre.

Things to consider

PET is a quantitative method. For the full use of the data, imaging, modelling and analyses should be carefully planned with PET experts.

While Turku PET Centre offers ~40 PET tracers for human use, the availability of a suitable PET tracer needs to be checked well in advance. A new tracer requires strictly regulated set-up and validation for human use (GMP). The authorities consider PET tracers as drugs but a “microdosing” concept is applied.

PET involves the use of radiation and Ethics Committees require calculations of radiation burden to subjects. Usually, the amounts of radiation remain low.

- A quantitative, non-invasive method for imaging physiological processes in humans.
- PET can offer cost-effective data for drug development .

Contact: pet@bioimaging.fi

PET tracer development

What is it for?

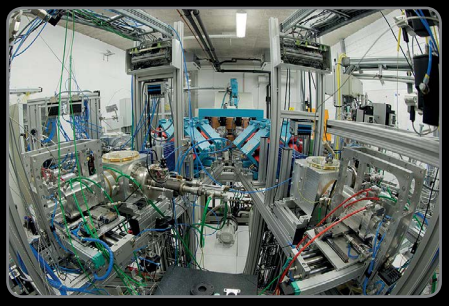
PET tracer development is a pre-designed, well organised and documented package of validated work stages that leads to a high-quality radiotracer (or radiopharmaceutical) to be used as a diagnostic tool in PET. Radiotracers are labelled with short-lived radionuclides, such as ^{11}C, ^{15}O, ^{18}F and ^{68}Ga that decay with positron emission. The goal is to develop a robust and reproducible production procedure for the radiotracer, the characteristics of which can be reliably analysed and can be safely used in both preclinical and clinical studies.

How does it work?

The following methods are developed and optimised during radiotracer development:

- Production of the radionuclide with a cyclotron using an appropriate nuclear reaction or a suitable radionuclide generator
- The chemical reactions with which the radionuclide is attached to the unlabelled molecule in order to obtain the desired radiotracer
- Procedures to isolate/purify the desired radiotracer from side-products
- Suitable formulation medium for the radiotracer, applicable for i.v. injection

Hot-cell for radiopharmaceutical production and a radiochemist at work.



CC 18/9 cyclotron for radionuclide production.

- Sterilisation of the radiotracer
- Quality assurance of the radiotracer

The entire development process is validated through a Process Verification procedure, a series of three consecutive production runs with full quality control.

Things to consider

Radiotracers are administrated as “trace” amounts, typically less than 1 µg, that do not induce any pharmacological effects. Hence, the specific radioactivity (SA, proportion of radioactivity to the tracer mass) is very high. A good radiotracer binds to its intended biological target with good selectivity and specificity and has a suitable metabolic profile for the study in question. EU guidelines of GMP (adequate facilities, qualification of devices, validation of methods, and training of personnel) have to be applied throughout the PET tracer development.

- In PET, radiotracers are diagnostic tools that can be used for highlighting physiological or metabolic details.
- In radiotracer development, radionuclides are synthesised into suitable tracer molecules.

Contact: pet@bioimaging.fi

7. Bioimage informatics

Bioimage informatics is a new field of science, covering the processing, analysis, visualisation, storing and handling of image data produced by biological and medical imaging devices.

Bioimage informatics is based on specialised software, designed for scientific work with imaging data.

Bioimage informatics is a complex and rapidly growing field, predicted to become revolutionary, but made challenging by the large number of software solutions, algorithms and settings available. Life scientists are encouraged to seek professional guidance to ensure fast and reliable results.

In this chapter, bioimage informatics is divided into three sections: image processing, image analysis, and image visualisation. Often, all three are needed.

Three major software packages, all developed mainly at TBI, are also presented: BioImageXD for biological imaging Carimas for medical imaging and AMIDA for high-content imaging.

Processing

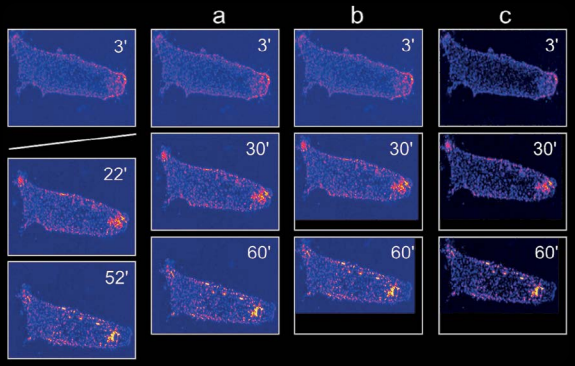
What is it for?

Bioimaging devices often produce images plagued with noise, unwanted movement, focus drift or out-of-focus haze or images that simply require some pre-processing before they can be used. Today, raw bioimages are seldom usable as they come out from an imaging instrument – image processing is needed before the images can be properly visualised or quantitatively analysed.

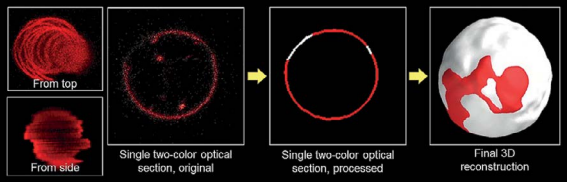
Image processing is used, for instance, to remove different types of noise, register images so that unwanted movements over time are compensated for, or to perform arithmetic or morphological operations. Image processing also covers areas such as reconstructing 3D datasets from tomographic data, or using deconvolution to improve image quality. Image processing also entails such basic adjustments as brightness and contrast, or inverting images or changing their pseudo colour palette.

How does it work?

Image processing is most often performed with specialised software, although generic image processing software can be used in some cases. The desired software is first identified, downloaded and installed, and then the correct algorithms and settings are determined. Image processing can also be obtained as a service, guaranteeing fast and reliable results but enabling scientists to be involved as much as they want to be.



Typical processing steps of live-cell imaging data: a) concatenating time points with correct time stamps, b) compensating for unwanted downwards cell movement, c) noise and background reduction.



An example of advanced image processing: compensation of vesicle "shaking" in a 3D confocal image, noise removal, improved quality and finally whole vesicle 3D reconstruction.

Things to consider

For basic adjustments, such as brightness and contrast, software with graphically represented algorithms is recommended to follow the norms of scientific publications..

- Image processing should be kept to a minimum, especially before quantitative analyses.
- •Image processing cannot "create" new resolution or data, but it can destort it and introduce errors.
- •Apply with care and consult experts whenever possible.

Contact: analysis@bioimaging.fi

Analysis

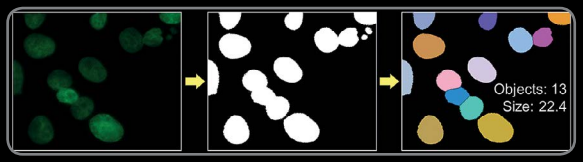
What is it for?

Today, displaying bioimages is not sufficient for reliable scientific conclusions or proper publications- quantification of images is a requirement. Within the last 15 years, bioimaging has transformed from a qualitative to a quantitative science. Without image analysis, images are only data, not yet information. Image analysis is needed to convert the data into valuable and understandable information.

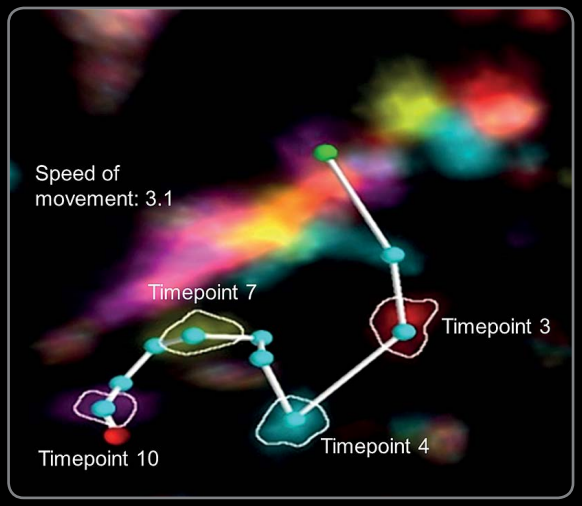
Image analysis can be divided into two main groups. In voxel-based analyses each voxel of the image is analysed separately, for instance to quantify colocalisation between two different markers. In segmentation-based analyses the image data is first segmented, meaning that it is divided into objects that the computer can identify. After segmentation, the number, size, shape and distribution of the objects can be quantified. Before image analysis, image processing (see previous section) is often needed.

How does it work?

Biomedical image analysis requires specialised open source software. The desired software is first identified, downloaded and installed, and then the appropriate workflows are determined (intermediate steps, algorithms and their settings, statistical analyses, etc.). In some cases tailored modifications and programming are required. Image analysis can also be obtained as a service, guaranteeing fast and reliable results but enabling scientists to be involved as much as they want to be.



In segmentation-based analysis the image is divided into identifiable objects that can be quantified. Many different algorithms are available.



Motion tracking is one of the most complex image analysis procedures, requiring identification of segmented objects in successive time points.

Things to consider

Image analysis offers endless possibilities and can revolutionise bioimaging.

- One should know exactly what each step does and how/why.
- Use with care and consult experts whenever possible.

Contact: analysis@bioimaging.fi

Visualisation

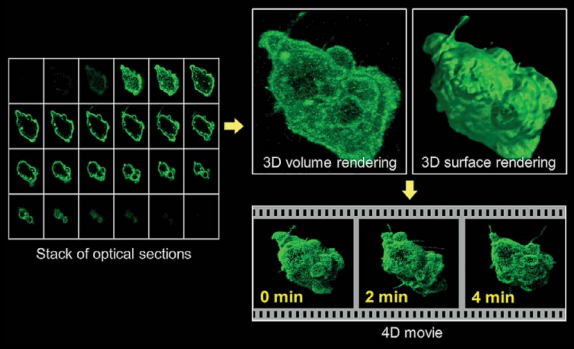
What is it for?

While quantitative analyses nowadays produce the actual results of bioimaging experiments (see previous section), generating visual representations that illustrate the quantitative results is often needed for publications and presentations. Visualisations are also frequently used as an aid when image processing and analysis workflows are being set up.

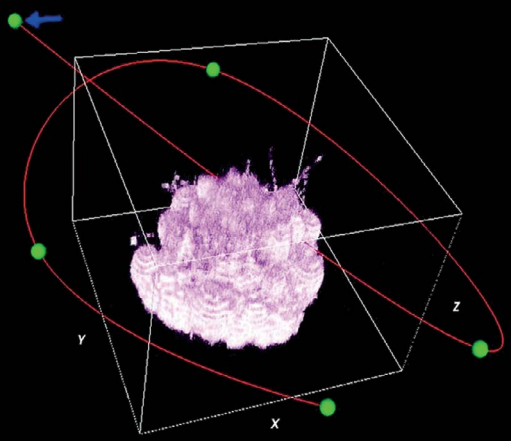
With 2D image data, visualisation is simple and regular photo processing software can sometimes be used. With 3D image data, special software is needed to either project the 3D data onto a 2D plane (e.g. maximum intensity projection) or to create 3D renderings. The latter can be either translucent volume renderings or surface renderings based on tiny geometric primitives. Stereo/3D images and movies can also be created and they are often the best method to convey 3D structural information.

How does it work?

In most cases special 3D rendering software is used to create either volume or surface renderings, or combinations of both. After identifying, downloading and installing the required software, each data channel is set up separately, and data channels must not be confused with different colour or opacity channels.



Two main classes of 3D rendering are volume and surface rendering. 3D renderings can be assembled into 4D movies.



Movies can be created by specifying a flight path (red curve) for a virtual camera.

Things to consider

Compared to image processing and analysis, there is more freedom in visualising, because the visualisations are not used for scientific conclusions. Consult experts if creating suitable visualisations or videos seems challenging.

- Avoid red-green superimposed images as these are often unclear especially for colour-blind people.
- Quantitative analyses must be done on original image data, never on visualisations such as projections or 3D renderings.
- Colours should only be used if they are essential for conveying the information being visualised.

Contact: analysis@bioimaging.fi

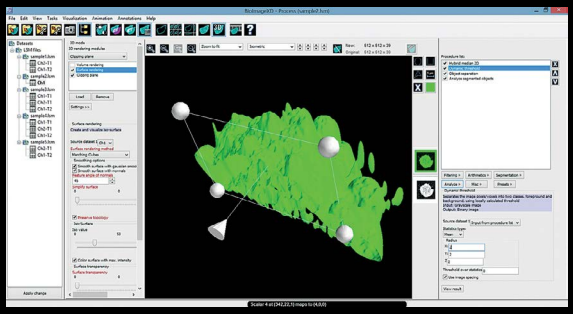
BioImageXD-software

What is it for?

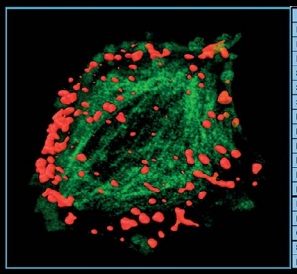
BioImageXD (Kankaanpää et al. Nature Methods 2012) is an open source software package for processing, analysing and visualising multidimensional biomedical image data. It is especially suitable for 3D and 4D cellular images, but can be used with any type of image data. BioImageXD is among the most versatile bioimage informatics tools available, capable of doing most of the things described in the preceding three sections. This covers tasks such as noise reduction, morphological operations, colocalisation analyses, segmentation-based analyses, motion tracking, deconvolution, 3D rendering and 4D animation. BioImageXD can also be used to automatically process large amounts of data, such as high-content imaging results. BioImageXD has been referenced hundreds of times, and it is used worldwide in numerous research projects, from neuroscience and parasitology to cancer medicine and virology.

How does it work?

BioImageXD was developed based on six leading principles: open source code, extensive feature set, usability, full adjustability, applicability for present-day imaging needs and easy extendibility. The software can be downloaded free of charge, and it reads common microscopy and image file formats directly. Images are read into memory only when needed, not automatically upon loading, enabling quick loading and processing of large amounts of data. The user interface of the software is based on a single, large window



BioImageXD user interface.



	Bis	Fil	Gen	L-NA	Saf	Sta
Intensity 5'		-			+	-
Intensity 45'				+	-	
Intensity Δ					- - -	
Size 5'		- -				- -
Size 45'		-			-	-
Size Δ					-	
Number / area 5'					+ + +	- - -
Number / area 45'			+	+	+ + +	-
Number / area Δ		- -	- - -	- - -	+ + +	- - -
Dispersion / area 5'		+		+	+	
Dispersion / area 45'		+ +		+	+	+
Dispersion / area Δ			+ + +			
Internalization	- - -	- - -	- - -	- - -		
Distance to cell center	-	-	-	- -		
Colocalization with cav-1		- - -	- -	- - -		
Speed	-	-	- -	+		
Directional persistence	+	+ + +	+	+ +		-

BioImageXD can be used to quantitatively analyse complex phenomena, such as cluster formation and internalisations of molecules in cells.

and colour-coded settings.

Nearly all features of BioImageXD can be run through its Batch Processor, which enables the set-up of complex workflows without any need for programming, and allows the processing of hundreds or thousands of images in one go.

Things to consider

For bioimage informatics, it is nowadays recommended that open source software be used, due to the transparency required for scientific work ("you have to know what your algorithms do"). As any advanced bioimage informatics software, BioImageXD is not always easy to use, but in scientific image analysis there are no shortcuts or "magic buttons". Consult expert help if needed.

www.bioimagexd.net/

- BioImageXD-software is especially suitable for processing, analysing and visualising 3D and 4D cellular images.
- Free and open source software supporting several file formats.
- Easy-to-use Batch Processor for complex work flows.

Contact: bioimagexd@bioimaging.fi

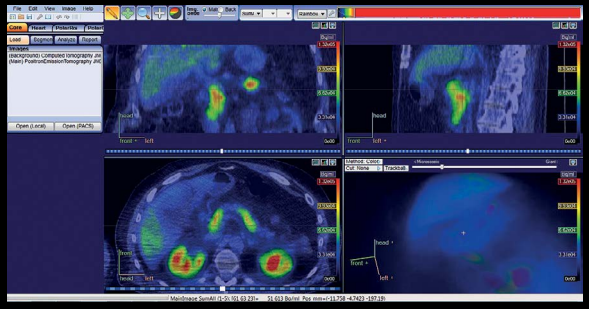
Carimas-software

What is it for?

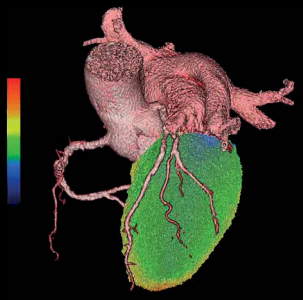
Carimas is a software platform for processing medical images generated by the most commonly used modalities, such as PET, SPECT, CT and MRI. It is a very useful analysis tool for PET imaging data. A large number of visualisation tools, ROI/VOI(region/volume of interest), numeric analyses and modelling methods are included.

How does it work?

Carimas supports Dicom and many other medical image formats (Ecat, MicroPET, analyse, interfile and Nifti). It includes four basic parts: visualisations, segmentation, modelling and reporting. Volumetric and dynamic images can be visualised. In addition to several defined shapes and volumes included within the software, users can manually define ROI/VOI if required. Automatic segmentation is available. Carimas provides the basic curve fitting methods, PET-specific graphic analysis and modelling methods. A report containing patient information, fitted curves and estimated parameters can be saved and printed. A few plugins (specific tools) for cardiac PET studies have been implemented. All analysis statuses and results can be saved. A special connection via intranet with PACs is available for effective data transfer.



Carimas' main panel and visualisation windows.



3D reconstruction of a coronary artery tree based on fused cardiac CT angiography and a PET perfusion study.

Things to consider

All medical imaging data, such as PET/CT, SPECT/CT, PET/MRI and small animal PET/CT, can be visualised and analysed using this platform. It is a very useful tool for PET dynamic studies, since a large number of graphic analyses and modelling methods are included (over 20 validated models). In PET studies, you need to scan patients or animals using conventional or novel radionuclide-labelled tracers that target specific tumour biomarkers, diseased tissues or certain cellular signalling pathways. Unlike clinical imaging data, PET research data usually need to be further analysed using specific computer software as an assistance tool, since most required parameters, such as absolute myocardial perfusion values, glucose uptake index of myocardium or skeletal muscles and ligand-receptor binding can be estimated only after complicated mathematical processing.

www.turkupetcentre.fi/carimas

- Carimas software is developed for processing, analysing and visualising medical images obtained with e.g. PET, SPECT, CT and MRI.
- Supports several medical image formats.
- Offers several validated models for dynamic studies.

Contact: carimas@bioimaging.fi

AMIDA-software

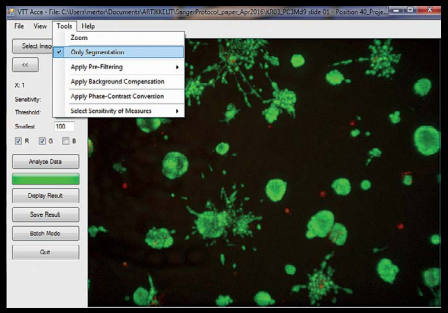
What is it for?

AMIDA (Automated Morphometric Image Data Analysis) is a software package for the rapid analysis of large numbers of confocal microscopy images. It was specifically developed to fill an unmet need in automated image analysis of complex 3D cultures.

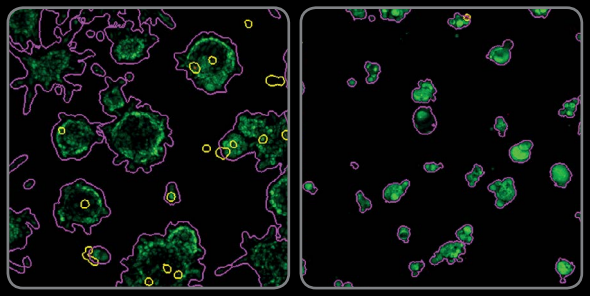
AMIDA can extract a number of morphometric features based on size, shape or texture of multicellular organoids formed in organotypic cultures. As a key component of a phenotypic screening platform, AMIDA is ideally suited for investigating a broad spectrum of defined, biological questions in drug discovery as well as personalised medicine. Technology and screening platforms are applicable for multiple types of research, such as quantitatively measuring the response of primary cancer cells or cell lines to drugs, siRNAs or other perturbations.

How does it work?

AMIDA combines the effective capture of the inherent complexity and dynamics of micro-tissues with high experimental throughput. Instead of analysing single organoids or spheroids in detail, hundreds or thousands of such structures can be analysed in parallel. This approach enables researchers to address many biologically and physiologically relevant features inherent to living tissues. These include aspects such as differentiation and maturation of functional properties, heterogeneity and dynamics;



Graphical user interface of the AMIDA-software.



Overlayed segmentation (purple) on confocal microscopy images of prostate cancer cells (PC3) grown in a 3D-cultrure. Left: Untreated cells. Right: Paclitaxel-treated cells showing inhibited growth, less invasive contour (shape) and smaller number of dead cells (yellow).

cell-cell and cell-matrix interactions and the role of the microenvironment. AMIDA further has a strong focus on quantitatively measuring cell motility and invasion e.g. of cancer cells in 3D matrices.

Things to consider

Different versions of AMIDA exist: the free open source software allows analysis of small numbers of images, such as phase contrast or confocal microscopy images. The proprietary and continuously developed rAMIDA version, in contrast, can handle thousands of complex images (stacks of confocal microscopy images or maximum projections) in batch mode, preferentially in the frame of high-content drug screening campaigns and in collaboration with the University of Turku's HCS Lab.

- Software package for high-throughput automated morphometric analysis of confocal microscopic images.
- A research tool suited ideally for drug discovery and personalised medicine.

Contact: amida@bioimaging.fi

8. MSc programme in Biomedical Imaging

Master´s Degree Programme in Biomedical Imaging (BIMA) is an international programme jointly administered by the two universities in Turku, the University of Turku and the Åbo Akademi University. It is part of the MSc programme in Biomedical Sciences, formed around the spearhead expertise areas of Turku universities. BIMA aims to train professionals that will have a thorough understanding of diverse imaging technologies along with practical skills in a wide range of imaging methods and applications.

Programme strongholds

BIMA has been assembled on the true imaging strongholds of Turku, and comprises a truly interdisciplinary array of prominent research groups and departments. The local collaborators are the national Turku PET Centre, Turku Centre for Biotechnology, Turku Center for Disease Modeling and Turku University Hospital. Additionally, BIMA has collaborators in the Nordic countries, Europe, the USA and Asia, and offers courses given by international experts.

The interdisciplinary curriculum provides graduates with an excellent basis for careers in many different areas of life sciences, both in academic research and industry.

Structure of the BIMA

The curriculum consists of major subject studies and optional studies including advanced microscopy, ***in vivo***-imaging, image analysis, multimedia editing as well as an international lecture series and hands-on courses. BIMA as a part of Biomedical Sciences programme, provides students with good practical skills in imaging methods ranging from molecular and cellular to tissue and clinical imaging.

Who may apply?

BIMA is intended for both Finnish and foreign students who have completed a lower university degree equivalent to a Finnish B.Sc. degree in Life Sciences or relevant areas of biomedical sciences, such as physics, chemistry or engineering. All applicants must prove that they have adequate English skills according to the programme requirements.

www.bioimaging.fi/program

- Two-year MSc programme taught in english, 120 ECTS credits.
- Annual intake max. 20 students.

Contact: bima-office@bioimaging.fi

9. Map of the campus

- Turku BioImaging facilities

1. **BioCity**
2. **PharmaCity**
3. **Medisiina**
4. **Turku University hospital and Turku PET- Center**
5. **Turku University main building**
6. **Arken**
7. **Gadolinia**
8. **Arcanum**
9. **Turku School of Economics**
10. **Dental**
11. **Mikro**
12. **Teutori**
13. **ICT-building**
14. **DataCity**

10. Instrument appendix

Atomic Force Microscopy

JPK Nanowizard II combined with a Carl Zeiss confocal microscope

- For wide scan range (100 x 100 x 15 µm), experiments under physiological conditions, or long Z-range up to 100 µm.
- BioCity, floor-1, staircase A
- Pasi Kankaanpää.

Agilent ILM 5500, combined with Abberior STED

- Magnetic AC mode for soft samples and simultaneous topography and molecular recognition mapping. AFM with super-resolution STED microscopy.
- BioCity, 5th oor, staircase A
- Elnaz Fazeli

Confocal microscopy

Zeiss LSM780

- Serves a work horse of the Cell Imaging Core.
- Should the possibility to perform FRAP, FRET, FC(C)S experiments
- BioCity, 5th floor, room 5149
- Markku Saari and Jouko Sandholm.

Leica TCS SP5 MATRIX (HCS A)

- Used for high throughput and more common multi-well applications.
- BioCity, 2nd floor, room 2209
- Jari Korhonen.

Zeiss LSM510 with atomic force microscopy

- Allows correlated AFM studies with confocal microscopy
- BioCity, floor -1, staircase A
- Pasi Kankaanpää.

Abberior STED

- STED super resolution capabilities
- BioCity, 5th floor, staircase A
- Sami Koho.

Electron microscopy

JEOL JEM-1400Plus Transmission Electron Microscope (TEM)

- 120 kV transmission electron microscope equipped with 11 Mpix Osis Quemesa digital camera
- Medisiina A, 4th floor
- Markus Peurla

Leica Ultracut UCT ultramicrotome

- Ultramicrotome and a selection of diamond knives for TEM sectioning
- Medisiina A, 4th floor
- Markus Peurla

Reichert Lynx Tissue processor

- Automatic tissue processor for EM samples
- Medisiina A, 4th floor
- Markus Peurla

Glow discharge

- Glow discharge unit for making surfaces hydrophilic
- Medisiina A, 4th floor
- Markus Peurla

Balzers CED 010 carbon coater

- Vacuum coater for thin layers of carbon
- Medisiina A, 4th floor
- Markus Peurla

FLIM-FRET microscopy

Lambert FLIM widefield microscope

- The Lambert Instruments LIFA is a fast frequency domain FLIM system, which is attached to an inverted Carl Zeiss AxioImager microscope body. The light source is multi-LED with excitation lines 406, 469 and 533nm. The LIFA system can image lifetimes with a resolution < 100 ps in a range of 0.1 ns- 1 ms.
- BioCity, 5th floor, room 5086
- Camilo Guzmán, Jouko Sandholm and Markku Saari.

Flow cytometry and Cell sorting

BD LSR Fortessa

- Our newest analyzer BD LSRFortessa is equipped with four lasers (405nm, 488nm, 640nm, 561nm).
- It can be used to detect up to 17 colors simultaneously. In addition to the BD™ High Throughput sampler, single tube acquisition is also possible.
- Location: BioCity, 5th floor, room 5126
- Jouko Sandholm

BD LSR II

- The BD LSR II is equipped with three lasers (405, 488 and 635 nm) and can handle advanced multi-color applications. Up to eight different fluorescent probes can be measured simultaneously. In addition, the advanced flow system allows high speed sample runs. This instrument is used for many applications including FRET measurements.
- Lasers:405 nm violet, 488 nm blue, 633 nm red
- Detectors:Cell size, granularity, 2 for violet, 5 for blue, 2 for red
- Sample injection:96 well plate
- Location: BioCity, 5th floor, room 5126
- Jouko Sandholm

BD FACSAria II

- The instrument is designed to isolate pure living cell populations, based on morphological (size, granularity) and fluorescent properties
- Can sort up to 20000 events/s. The cells can be sorted into the tubes or to a well plate. When sorting to tubes, up to 4 populations can be isolated simultaneously. Well plate sorting is an excellent tool for single cell sorting.
- BioCity, floor 5, staircase A, room 5126
- Ketlin Adel

Fluorescence correlation spectroscopy

Zeiss LSM 780 confocal microscope

- Equipped with a GaAsP detector making it suitable for FCS and FCCS. It also includes in-built Zeiss software that provides fitted

results of FCS and FCCS on the fly. For RICS, images are acquired in normal confocal mode and analysed using SimFCS 2.0 software installed on a separate computer.
- BioCity, 5th floor, room 5149
- Camilo Guzmán, Jouko Sandholm and Markku Saari.

Abberior STED microscope with PicoQuant electronics for photon counting.
- PicoQuant expansion of the STED microscope allows detection and quantification of individual photons and therefore use of FCS and FCCS. PicoQuant so ware SimPhoTime 64 is used for data analysis and quantification of FCS and FCCS curves. Same as with the Zeiss confocal, RICS images are acquired on the microscope, exported and analyzed using SimFCS 2.0 software.
- BioCity, 5th floor, room 5086
- Camilo Guzmán and Sami Koho.

High content microscopy

Zeiss Axiovert-200M microscope with Yokogawa CSU22 spinning disc confocal unit
- A fully motorized inverted widefield fluorescence microscope ideal for live-cell imaging applications. Equipped with a small stage incubator for temperature control and CO2 supply. Based on its Nipkow disk technology with microlenses, the Yokogawa CSU unit is a particularly useful tool for high speed, live cell imaging.
- Allows excitation in the range of 488- 640 nm. Its SlideBook software allows control of the motorised filters, shutters and stages to set up time-lapse imaging experiments in multiple channels, and multiple stage positions. Objectives available: 5x, 10x, 20x, 64x
- Pharmacity, HCS Laboratory, 4th floor, staircase B (Room 4052)
- Mervi Toriseva

Perkin Elmer Operetta High Content Imaging System
- A compact spinning disc confocal microscope, particularly designed for rapid analyses of fixed and live cell applications in microtiter plates. The included PE Harmony High Content Imaging and Analysis Software (v3.5) allows quantitative evaluation of morphometric image data and automated image analyses.
- Operetta has a live cell chamber that maintains environmental conditions (temperature, CO2), and provides a spectrum of kinetic capabilities for time course experiments and observation of cellular dynamics. Allows excitation in the range of 360 – 640 nm. Objectives available: 2x WD, 10x WD, 20x WD, 60x NA
- Pharmacity, HCS Laboratory, 4th floor, staircase B (Room 4054)
- Malin Åkerfelt

Essen Bioscience Incucyte FLR live-cell fluorescent imaging device
- An inverted microscope with a camera that fits within a standard cell culture incubator.
- Equipped with a 10x objective for phase-contrast and fluorescence detection in the green spectrum.
- Has inserts to fit multiwell plates, microslides, 10 cm single plate dishes.
- Pharmacity, HCS Laboratory, 4th floor, staircase B (Room 4054)
- Mervi Toriseva

Olympus ScanR
- An imaging cytometer, which allows automated image acquisition coupled with data analysis of a broad spectrum of biological samples. Processes and analyses large numbers of diverse samples and enables their quantitative analysis
- Collects morphological information e.g. from cells and tissue sections. ScanR allows excitation of a broad range of fluorochromes and allows complex analysis and visualisation of imaging data through histograms or dot plots.
- Pharmacity/Biohautomo, Misvik OY, 4th floor, staircase B
- Rami Mäkelä

BD Pathway 855 High Content Analyser Integrated System
- The integrated system contains at its core the BD Pathway 855 High Content Analyser. This is an automated multipoint microscope, capturing widefield or spinning-disk confocal images of fixed or live cells and model organisms under CO2 and temperature control.
- Automated plate replenishment is achieved by integrated storage devices with a capacity for 200 plates of cells, tips and reagents feeding the on-stage pipettor with compounds allowing the capture of responses of live cells to stimulation. The three integrated storage devices provide cooled, ambient and cell incubator conditions as needed.
- The integrated external liquid handling table allows scheduling of preparative washing or reagent addition steps prior to imaging each plate, and complements the on-stage pipetting.
- Reproducible quantitative microscopy is ensured by calibratable lamps delivering stable illumination in the millisecond to month time range.
- Objectives, from 1.25x to 40x N.A. 0.95, move under a fixed stage. This permits multipoint imaging of even poorly-adherent or non-adherent cells.
- Spectral requirements of dyes are met by wheels containing 16 excitation filters and 8 emission filters.
- The system is designed for 96, 384 or 1536 well plates (SBS format). An SBS-compatible holder for slides is available.
- Multi-terabyte storage servers connect all imagers to 3

dedicated analysis workstations configured with Attovision segmentation, analysis and data mining software and other tools for batch processing of large datasets that are acquired.
- Pharmacity/Biohautomo, High Content Screening Laboratory, 4th floor, staircase B (Room 4051)
- Michael Courtney

Biomek NXP laboratory automation work station
- Automated benchtop robotics system for liquid handling – including pipetting, dilution, and dispensing of media or assay components. Forms a functional unit together with Cytomat 2 C-LIN automated cell culture incubator and BD Pathway 855 imaging system.
- State of the art, integrated and compact liquid handling system particularly suitable for high content screening purposes.
- Pharmacity/Biohautomo, High Content Screening Laboratory, floor 4, staircase B (Room 4051)
- Michael Courtney

Live cell imaging

- Several technologies and instruments listed on this appendix are suitable for live cell imaging. Special precautions need to be taken to ensure that cells are not seriously affected by the intense light, oxygen deprivation, temperature etc. For more information, please contact local experts: live@bioimaging.fi

Micro-CT

- Skyscan 1072 micro-CT scanner Highest voxel resolution 5 micro meters,only for ex-vivo samples
- Department of Cell Biology and Anatomy, Medisiina 2nd floor room C204
- Jorma Määttä

Microscope slide scanner

Pannoramic 250 Flash
- High capacity bright field microscope slide scanner.
- 20X objective
- 250 slide capacity in one loading
- Medisiina A, 4th floor
- Markus Peurla

Pannoramic Midi
- Fluorescence and bright field microscope slide scanner.
- Medisiina A, floor 4
- Markus Peurla
- 20X and 40X objective
- 12 slide capacity in one loading
- Slide thickness must be exactly 1.0 mm, for example. Menzel Superfrost works well.
- Available fluorescence filters:
- DAPI (excitation 365nm, emission 445/50 nm)
- FITCH (excitation 470/40nm, emission 525/50 nm)
- Rhodamine/TRITC (excitation 550/25nm, emission 605/70 nm)
- Quad band filter for DAPI/FITC/ TRITC/Cy5 (387-440nm, 485-521 nm, 559-607 nm, 649-700 nm)
- Medisiina A, 4th floor
- Markus Peurla

Optical *in vivo* imaging

Caliper LS (Perkin Elmer), IVIS Spectrum
- Quantitative optical imaging in 2D and 3D using fluorescence and bioluminescence, for mice, rats and cells ***in vivo***
- Highly sensitive CCD camera, excitation filters 415- 760 nm (30 nm bw), emission filters 490 - 850 nm (20 nm bw), spectral unmixing, anaesthesia system available
- BioCity, floor-1, room YK136
- Marko Tirri & Heidi Liljenbäck

Caliper LS (Perkin Elmer), IVIS Lumina II
- Quantitative optical imaging in 2D using fluorescence and bioluminescence, for mice, rats and cells ***in vivo***
- Sensitive CCD camera, excitation filters: 430, 465, 500, 535, 570, 605, 640, 675, 710, 745 nm, emission filters: 515-575, 575-650, 660, 695-770, 810-875 nm, anaesthesia sks can be used
- PharmaCity, floor-1, system on a trolley
- BioCity, floor-1
- Marko Tirri & Heidi Liljenbäck

PET Imaging

Siemens Inveon PET/CT camera
- Suitable for small animal imaging
- PET modality resolution 1.4 mm, CT modality resolution max 20 µm
- BioCity, B stair, floor-1
- Tove Grönroos

Fuji BAS 5000 phosphoimager
- Suitable for measuring radioactivity biodistribution in tissues
- Medisiina, 4th floor
- Marko Tirri

Fuji BAS 1800 phosphoimagers
- Suitable for measuring radioactivity biodistribution in tissues
- MediCity, 4th floor
- Marko Vehmanen

Faxitron MultiRad 350
- Suitable for irradiation of cells and rodents
- BioCity, B stair, floor-1
- Marko Tirri

PET, PET-CT and PET/MRI

GE Discovery 690 PET/CT camera
- Suitable for all human PET/CT imaging, including radiotherapy planning
- 64 slice CT, with possibilities for short imaging time and dual-gating (ECG and respiratory)
- PET Centre, Building 14, 2nd floor
- Tuula Tolvanen

GE VCT PET/CT camera
- Suitable for all human PET/CT imaging
- 64 slice CT, with possibilities for dual-gating (ECG and respiratory)
- PET Centre, Building 14, 2nd floor
- Tuula Tolvanen

Philips Ingenuity PET/MRI camera
- Double gantry camera especially for whole-body PET/MRI imaging
- PET Centre, Building 14, 1st floor
- Virva Saunavaara

MRI 3 Tesla
- Siemens/CTI High Resolution Research Tool (HRRT) PET camera
- Suitable for brain PET imaging with head fixation and movement correction possibilities, also small animal imaging. Resolution 2 mm
- PET Centre, Building 14, 2nd floor
- Virva Saunavaara

Photoacoustic imaging

Laboratory of Biophysics custom PAM system
- A laser line – 532 nm
- BioCity, 5th floor, staircase A
- Elena Tcarenkova, Sami Koho.

Spinning disc confocal microscopy

3i Marianas imaging system with Yokogawa CSU-W1 scanner, Intelligent Imaging Innovations
- The spinning disk at CIC is a Marianas imaging system from the company 3i, which is based on Yokogawa CSU-W1 scanner and an inverted Carl Zeiss microscope stand. CSU-W1 has a wide field of field (16mm x 17mm), and its maximal imaging speed is 200 fps (5ms per frame). Our system is equipped with two optional cameras: Hamamatsu sCMOS Orca Flash 4 to capture beautiful images at 2048x2048 frame size, and high-sensitive Photometrics Evolve 512 10MHz Back Illuminated EMCCD Camera for live cell imaging and low light conditions.
- Suitable especially for live cell imaging and situations where fast confocal sectioning is needed.
- Wide field of view
- Photomanipulation possible
- Unique: near infra-red laser (5 channels available)
- BioCity, floor 5, staircase A
- Markku Saari

STED

Abberior STED Instruments 2 channel
- With up to 20 nm lateral resolution. Three excitation lasers (485nm, 532nm, 635nm) and two STED depletion lasers: 590nm and 775nm. Equipped with Time Gating and Rescue Mode to significantly reduce photodamage during STED imaging. Highly customizable for new kinds of experiments.
- BioCity, 5th floor, staircase A
- Sami Koho

Stereo microscopy with microinjection

Zeiss SteREO Lumar with Eppendorf InjectMan NI2 semi-automatic microinjection device.
- The Zeiss SteREO Lumar.V12 fluorescence stereo microscope is a high-end instrument for low-magnification microscopy. Our 0.8x and 1.2x long working distance objectives allow a researcher to perform (for example) dissection using objective lenses permitting a maximal magnification of 120x. The versatility of the instrument permits imaging of (for example) entire mouse brain slices or, alternatively, single fluorescent cells, using the same objective. The instrument is equipped with both b/w and color CCD cameras to visualise fluorescent and non-fluorescent samples, respectively. The instrument has all of the basic fluorescence filter sets and image acquisition is controlled by AxioVision software.
- BioCity, 5th floor, room 5149
- Markku Saari

TIRF Microscopy

Carl Zeiss Laser-TIRF 3
- Carl Zeiss Laser-TIRF 3 Imaging System, with three high power lasers. It has sensitive high-NA TIRF objectives (63x/1.46, and 100x/1.46), which makes the system very powerful also in common widefield imaging. The system has two cameras: sensitive Hamamatsu ImageEM C9100-13 EMCCD, and a fast Hamamatsu Orca Flash 4, CMOS camera with a wide chip (2048x2048). The fast sCMOS camera has been used here for example to image and measure a speed of cilia from human bronchial epithelia cells.
- TIRF lasers: 488/561/640
- Cameras: Hamamatsu CMOS Orca Flash 4, and ImageEM C9100-13 EMCCD
- BioCity, 5th floor, staircase A
- Markku Saari

Two-photon microscopy

Leica TCS SP5 MP with multiphoton system
- Suitable especially for situations where large penetration depth is required, such as imaging living cells deep within their native tissue environment. Also useful if tissue fixation and labeling is not possible. Allows multiphoton excitation in the range of 690-1040 nm
- BioCity, floor -1, staircase A
- Jari Korhonen/Tibor Veres.

Ultrasound

Visualsonics, Vevo 2100.
- A rodent ultrasound imaging station with micro-injector
- With the imaging station, the animals can be mounted on a fully adjustable and heated rail table. The table can also produce physiological monitoring as animal temperature, ECG and heart rate can be measured.
- Biocity B, animal facility
- Contact person: Saku Ruohonen (saku.ruohonen@utu.fi), Juha Koskenvuo (juha.koskenvuo@utu.fi)

Acuson Doppler echocardiography (2 devices)
- Suitable for cardiac and other ultrasound examinations
- PET Centre, Building 14, floor 2
- Juhani Knuuti

Widefield microscopy

Leica M60 stereomicroscope with Leica DFC290 HD digital camera

- Stereo microscope equipped with Leica CLS 1000 LED high-contrast illumination system using flexible goosenecks for easy adjustment.
- BioCity, 2nd floor, room 2060
- Jari Korhonen

Leica DM2500 upright microscope with Leica DFC290 HD digital camera

- Advanced Ergonomic Microscope System for routine brightfield, phase contrast and differential interference contrast microscopy.
- Objectives: 10x/0.25 , 20x0.40, 40x/0,75 and 63x/1.40 Oil
- BioCity, 2nd floor, room 2129
- Jari Korhonen

Leica DM IL Inverted Laboratory Microscope with Leica EC3 camera.

- Ideal for examination of well plates, tissue cultures and observing micromanipulation. Brightfield and Integrated Modulation Contrast (IMC) modalities. Equipped with the Leica EC3 high speed digital colour camera.
- Objectives: 5x, 10x and 20x
- Biocity, 2nd floor, Room 221
- Jari Korhonen

Zeiss Axiovert 200M inverted microscope

- Suitable for brightfield, fluorescence, phase contrast and differential interference contrast imaging.
- Equipped with ORCA 1394 ERG (b/w) and Zeiss AxioCam MRc (colour) cameras. Suitable for live cell imaging and supports high resolution mosaic imaging.
- Objectives: 5x, 10x, 20x, 40x, 63x Oil
- FL filters: UV/DAPI, GFP/FITC/Alexa488/CY2, TRITC/Alexa555/CY3, YFP, (CFP), (CY5) and (Fura-2)
- MediCity
- Jouko Sandholm, Markku Saari

Leica DM RXA upright widefield microscope with the Nikon DS-Fi2 5MP Color Camera.

- This Leica research microscope is optically in mint condition although it has served several years. In addition to fluorescence, it can be used in normal transmitted mode, or in darkfield mode. Interference contrast is possible with 20x, 40x and 100x objectives. On request also phase contrast is possible with each magnification.
- The microscope is equipped with a new easy-to-use Nikon color camera. Camera is operated with a DS L-3 standalone control unit without computer (touch screen). Images are saved on an external USB memory device or a CF card (type I).
- Objectives: 0x/0.30 DRY, 20x/0.60 DRY, 40x/1.00-0.50 OIL, 63x/1.32-0.60 OIL, 100x/1.40-0.70 OIL
- FL filters: DAPI/Hoechst , GFP/FITC/Alexa 488 and RFP/TRITC/Cy2
- Biocity, 2nd floor, Room 2077
- Jari Korhonen

Leica DM IRBE inverted widefield microscope with the Nikon DS-Fi2 5MP Color Camera.

- This microscope body was formerly a part of a confocal microscope, but nowadays servers as a fluorescence microscope equipped with a color CCD camera (Nikon DS-Fi2). Fluorescence, transmitted light and darkfield are possible with all magnifications. Moreover, phase contrast is possible with all objectives.
- The easy-to-use color camera is operated with a DS L-3 standalone control unit without computer (touch screen). The largest image size with a 10x objective is 0.86x0.64mm. Images are saved on an external USB memory device or a CF card .
- Objectives: 10x/0.30 dry, 20x/0.70 dry, 40x/0.70 dry, 63x/0.70 dry, corr
- FL filters: DAPI/Hoechst, GFP/FITC/Alexa 488, RFP/TRITC/Cy2 and Draq5/Cy5/Alexa 633
- Biocity, 2nd floor, Room 2077
- Jari Korhonen

Evosfl cell culture microscope

- EVOSfl is a convenient inverted microscope for cell cultures and quick sample checking. The microscope has a transmitted and fluorescence light options. The illumination system is based on LED cubes, which makes working flexible. The camera and the computer are integrated in to the microscope stand. Images are saved on external USB memory device.
- Objectives: 4x, 10x, 20x and 40x
- FL Filters: Dapi/Hoechst, GFP/Alexa488, RFP/Alexa555, CFP and CY5 /Alexa647
- Biocity 5th floor, room 5073
- Jouko Sandholm, Markku Saari

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