Technologies at the Molecular Imaging Center

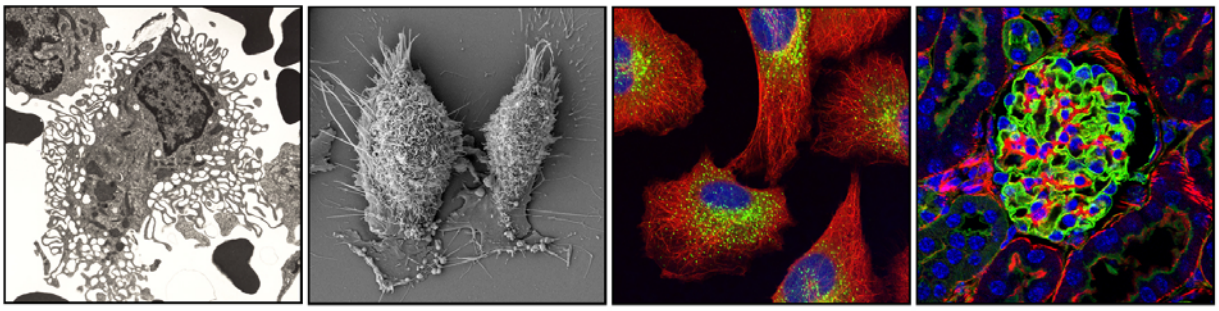
A short and simple summary

http://www.uib.no/rg/mic/facilities/equipment

Seeing is believing – make us believe what you see!

Cell and Tissue Imaging

MIC has a well-equipped instrument park for cell and tissue imaging. The technique you choose highly depends on what you want to see and the resolution you need to see it. With electron microscopy it's only possible to look at dead material. Within the fluorescent microcopy techniques it's possible to image live cells in addition to fixed cells and tissue, and even fixed and live small organisms like *C. elegans* and fish larvae.



TEM SEM Confocal

Electron microscopy (EM) gives images of very high resolution and we are able to see structures down to 10 nm. An electron beam is used to illuminate the specimen and because of the very short wavelengths of the electrons the resolving power of EM is a lot better than for light microscopy.

Transmission electron microscopy will give you detailed information about the structures inside your cells/tissue. Cells and tissue that have been prepared for TEM, is sliced into ultrathin sections (60 nm). The slices are mounted on grids that are placed in the vacuumed column of the microscope. The preparation process is crucial for the image quality and is the biggest challenge in TEM. MIC has an EM-

preparation lab where our specialist technician will perform all the necessary steps to bring forward the sample (from fixation to end product). Different techniques are applied to different samples and applications.

Scanning electron microscopy (SEM) will give you detailed images of the surface of the sample, and in addition to investigate cell and tissue samples it's widely used in nanotechnology. The preparation for SEM is less time consuming than for TEM and consists basically of dehydration and coating with a metal. This can also be performed at MIC.

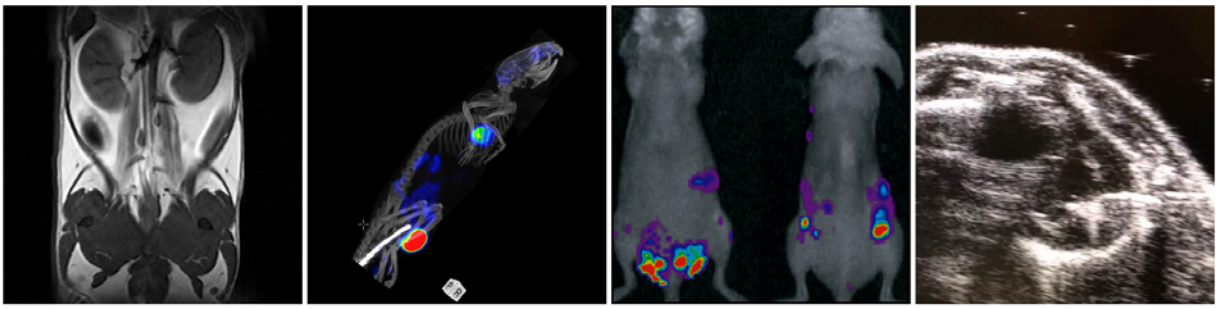
Fluorescence microscopy is light microscopy where the signal that creates the image is coming from the light of fluorescence. The smallest object you possibly can see under ideal conditions (rarely the case) is 200 nm. Fluorescence is an event that only occurs in molecules that have the ability to become excited by certain wavelengths of light followed by their emission of light with longer wavelengths. This means that you need to stain you cell/tissue with fluorescent molecules, which most commonly are attached to antibodies or organelle specific probes. Since different fluorescent molecules have different excitation and emission spectra we are able to easily combine four different fluorescent stains on a sample simultaneously. Some cells/tissue contains intrinsic molecules that produce fluorescence, this is called autofluorescence and can cause background signal in an image.

Confocal microscopy is an advanced fluorescence microscope that also will create images based on fluorescent signal. The advantage a confocal has over a regular fluorescent microscope is the ability to optically section the sample, which means that images of good contrast in is captured from different levels in the depth of the sample. This gives a 3 dimensional dataset (xyz) that can be reconstructed to 3D-representations, giving more information throughout the sample than a regular flat image. The confocal has three essential parts that is the crucial in this technology; lasers for precise excitation of the fluorescent molecules, a scanning unit for scanning the laser light over the sample and a pinhole for rejecting out of focus light.

Live cell imaging can be done at any light or fluorescent/confocal microscope as long as cells can be kept alive on the microscope. For that a heating chamber and a CO_2-controller is needed. But there are several factors that have to be met in order to produce good quality timelapses. For fluorescent timelapses first of all your target of interest needs to be fluorescently marked; this can be done by making a recombinant protein with a fluorescent protein tag and transfect this into the cells or by adding specific fluorescent probes that can be taken up over the cell membrane. Secondly you need to be able to go fast enough without compromising too much on image quality, acquisition speed is especially a challenge if the z-dimension is required. On a regular confocal this would be hard to achieve because of the relatively slow scanning process. The best option is then the **spinning disk confocal** that uses a technology that produces xyz-timelapses fast enough to catch rapid movement inside cells. One crucial component is a fast and sensitive camera instead of the "slow" confocal scanning. Confocality is kept due to the spinning disk that has an array of lenses with corresponding pinholes.

Small Animal Imaging

MIC has available 4 non-invasive *in vivo* imaging devices for small animals, and the animals used are primarily mice and rats. Non-invasive means that the animals are not hurt or damaged by the imaging, and the same animal can undergo several rounds of imaging at different time points throughout an experiment. The same animal can also be imaged on different systems, so called multi-modal imaging, giving powerful results validated by different techniques. Small animal imaging is especially useful in pre-clinical studies of disease development and treatment efficacy, but it may also be applied in other scientific fields. It's also possible to image dead animals, such as fish.



MRI PET/CT OI US

Magnetic resonance imaging (MRI) is based on a high magnetic field aligning the magnetization of hydrogen nuclei within the subject. Harmless radio frequency waves alter the alignment of this magnetization and the nuclei will produce a rotating magnetic field which is detected by the scanner. To enhance contrast in certain applications, contrast agents may be injected. There are various MRI techniques that can be used to follow both pathological and physiological processes like visualization of anatomy (incl. fat distribution), visualization and quantification of diffusion, blood perfusion and blood flow. It's also possible to do MR spectroscopy to quantify lipids and certain metabolites in the specimen.

PET/CT (positron emission tomography/computer tomography) is a technique where the specimen is injected with radioactively labeled biomolecules (tracers) that indirectly emit gamma rays for the system to detect. In combination with computer tomography (x-ray of the animal) the 3 dimensional image of the animal will give information on where the tracers is being taken up in the body. Typical biomolecules are sugars, amino acids, metabolic precursors, hormones etc. One of the most commonly used tracer is FDG (flourodeoxyglucose) which is an analogue of glucose and taken up by living cells via the first stages of the normal glucose pathway. Tumors for instance most often have high metabolic activity and the FDG-tracer will then concentrate in the tumor and highlight these. The radioactive isotopes have relatively short half-lives; the commonly used ^{18}F has a half-life of 110 minutes. For that reason tracers need to be made fresh and at the PET center production of tracers are "next door" to the PET/CT system.

Optical imaging (OI) is based on capturing fluorescence or bioluminescence from inside of the specimen. The target of interest needs to be marked with fluorescence or bioluminescence, and this can either be

done by injecting fluorescent/bioluminescent cells or antibodies/probes into the bloodstream or to use genetically modified (transgenic) animals that express fluorescent/bioluminescent proteins. In order to get information on where the target is in the depth of the animal the lifetime of the fluorescence is measured. Fluorescence lifetime is the average time (nanoseconds) the molecule stays in the excited state before it emits a photon, and it is highly specific for each fluorescent molecule. OI is especially useful for investigating where injected fluorescent cancer cells metastasize and monitoring the further cancer progression over time.

Ultra sound (US) waves are acoustic longitudinal waves that propagate at higher frequencies than the human hearing range. The reflection, transmission and refraction of the incident wave are the bases of the ultrasound image or pulse echo. The physical properties of the pulse echo, such as the pulse length, frequency, and intensity increase or decrease the spatial resolution, which is defined as the ability to distinguish two objects in the image. The higher the frequency, the shorter the pulse, and the better the resolution for distinguishing even blood cells. Ultrasound can be used in a wide range of clinical applications, and because it's a real time imaging modality with a capacity to read up to 1000 frames per second it's especially useful in studies of blood flow and cardiac function.