New regulations on animal experimentation

Norway’s new regulation on animal experimentation will most likely be implemented during the summer of 2015. A new instruction for the animal research authorities will be implemented at the same time.

The new regulation is a result of the new EU directive 2010/63/EU. The new system involves several important changes for researchers and staff involved in animal research.

These are the changes that are most important for NTNU:

• All applications will be handled by The Food Safety Authority (regulation, § 6). They have a maximum processing time of 40 working days, with the possibility of adding 15 more days for especially complicated projects (instruction, § 7). All researchers need to prepare for longer processing times, and plan their projects well in advance. The Food Safety Authority recommends that all applications are evaluated by a local person, to improve the quality of the application, thereby decreasing the processing time, and also to secure that all the necessary approvals and equipment are available in the facility. This will most likely be the case at NTNU. The local evaluation will be performed by the person responsible for animal welfare, in cooperation with the animal welfare body (see below).

• All applications must include a non-technical project summary that will be made public by the authorities (regulation, § 8).

• All changes of the project that may have negative impact on animal welfare must be approved by the authority. For changes without a negative impact, a notification is enough. All changes that affect the project summary must be followed by a revised non-technical project summary.

• The authorities shall secure that the applications are evaluated by persons with the necessary competence within the field in question (instruction, § 9). This can be achieved by involving different experts outside the Food Safety Authority.

• All projects shall be classified as non-recovery, mild, moderate or severe (regulation, appendix A. Examples are found in appendix B).

• As a minimum, all projects classified as severe shall undergo a retrospective assessment. This is performed by the authorities, on the basis of the necessary documentation submitted by the user. The authorities shall evaluate whether the objectives of the project were achieved, the harm inflicted on the animals, the severity of the procedures and any elements that might contribute to improving the 3Rs (replacement, reduction, refinement).

• All facilities shall appoint a person responsible for animal welfare, and an animal welfare body (regulation, § 25 and 26). The animal welfare body shall give advice to researchers and staff about animal welfare, establish and review SOPs in relation to animal welfare, follow the development and outcome of projects and give advice on further implementation of the 3Rs. Any advice given by the animal welfare body shall be kept for at least 3 years, and made available for the competent authority upon request.

(Continues on next page)
• All procedures causing a level of distress equivalent to, or higher than, the introduction of a needle is covered by the regulation, and must be applied for. According to the old regulation, simple blood samples were an exemption, but this is no longer the case (regulation, § 4).

• Everyone involved in animal research must demonstrate the necessary competence before being allowed to participate in projects and perform new procedures. Documentation of continuous training and education is mandatory (regulation, § 24).

The Food Safety Authority will require confirmation that all researchers and staff have read the new regulation before it is implemented. We advise all researchers to read the outlines as soon as possible, to be prepared for the changes to come.

If you have questions, please contact:
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New managers

Two of the core facilities have new managers:

**Bjørnar Sporsheim** is the new manager of Cellular and Molecular Imaging Core Facility (CMIC). He has a background in cell- and molecular biology from the department of biology and the department of physics at NTNU. During his PhD in Molecular Imaging he has been working on quantitative confocal laser scanning microscopy with a focus on instrument optimization for *in vivo* and *in vitro* analysis of intracellular transport. He has more than 8 years’ experience in advanced microscopy and image processing and analysis, as well as a broad university teaching experience.

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**Davi de Miranda Fonseca** has replaced Lars Hagen as general manager of the Proteomics and Metabolomics Core Facility (PROMEC). He has a background in Soft Matter Physics and Structural Biology. He has previously managed the X-ray Crystallography Laboratory of the DNA Repair group at NTNU where he established new techniques, such as the introduction of microfluidics in the crystallization of proteins. He has 12 years’ experience in advanced scientific equipment; including maintenance of instruments, design of experiments and sample environment controllers, as well as training users.

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New instruments at the Cellular and Molecular Imaging Core Facility (CMIC)

STED principles

Stimulated emission depletion (STED) is one of the so-called superresolution microscopy techniques available today. STED is an optical way of increasing the resolution beyond what is obtainable with conventional light microscopy. In short, the STED resolution improvements come from a reduction of the physical dimensions in the focal spot from where the fluorescence can be emitted. As the spot gets smaller the resolution increases. This is achieved by introducing a so-called depletion-“donut” mask overlapping the normal (diffraction-limited) spot in the focus plane. By increasing the laser power of the depletion laser the “donut” gets bigger, and consequently the spot gets smaller. In our new STED 3X system yet another laser beam is added to produces an additional Z-“donut”, which also increases the axial (Z-direction) resolution. By combining the two “donuts” with the gating functionality of the system, high resolution 3-D data can be generated, down to a resolution less than 50 nm. The resolution is dependent on the sample, the fluorophores used, and the intensity of the depletion laser.

(Bjørnar Sporsheim & Kjartan W. Egeberg)


Huygens Professional software

As part of the Leica SP8 package we also have Huygens Pro for deconvolution of 3-D STED data, as well as other imaging processing and analysis. In short, deconvolution is a computational method that is capable of reassigning out of focus blur back to its original focus plane, to produce higher resolution and lower noise levels than in the original datasets. This software is installed on the Leica SP8 STED computer.

http://www.svi.nl/HuygensProfessional
http://www.svi.nl/HuygensDeconvolution

Pen tablet (Wacom Intuos Pro medium)

This pen acts like a mouse pointer and can be used for image segmentation and measurements, painting masks, circling ROI's, and of course for teaching purpose. The Wacom is connected to the image processing computer “Imaris-PC” at room 432.03.014. For more information and training please contact Bjørnar Sporsheim.

Good year for microscopy

2015 is the International Year of Light and Light-Based Technologies – a good year for microscopy! More information on www.light2015.org/
New LC-MS/MS instrumentation

A new LC-MS/MS triple quadrupole instrument will be installed during summer 2015. This will significantly enhance our analytic capability of metabolites, especially modified nucleosides in DNA and RNA. Currently we have established quantitative assays against more than 50 modified nucleosides, including 5-methylcytosine (5-mC), 5-hydroxymethylcytosine (5-hmC) and uracil in DNA. 5-mC and 5-hmC are verified epigenetic regulators, while several other candidate modifications are receiving increasing attention for their potential role in epigenetic regulation.

Selected publications from PROMEC

*Off-target responses in the HeLa proteome subsequent to transient plasmid-based transfection* (Hagen et al., 2015, [http://www.ncbi.nlm.nih.gov/pubmed/25448019](http://www.ncbi.nlm.nih.gov/pubmed/25448019)). Transient overexpression of novel genes in mammalian cells is widely employed to study downstream biological effects. Here we demonstrate that chemical transfection of plasmid vectors *per se* induce an off-target response at the proteome level, resembling viral infection. Such responses may result in misinterpretation of experimental results, especially in studies of innate immune responses, expression of viral proteins etc. The response appears to be cell-type specific and thus appropriate cell models should be employed for the experiments in question.

*AID expression in B-cell lymphomas causes accumulation of genomic uracil and a distinct AID mutational signature.* (Pettersen et al., 2015, [www.ncbi.nlm.nih.gov/pubmed/25486549](http://www.ncbi.nlm.nih.gov/pubmed/25486549)). AID/APOBEC enzymes deaminate genomic cytosine to uracil as part of adaptive and innate immune responses, respectively. However, several recent studies demonstrate that they also induce off-target mutations and may thus promote cancer. In this study we find that B-cell lymphoma cell lines contain elevated levels of genomic uracil, and that the levels are highly correlated with AID mRNA and protein expression. B-cells stimulated to express endogenous AID and undergo class switch recombination displayed a several-fold increase in total genomic uracil, indicating that B cells may undergo widespread cytosine deamination after stimulation. In line with this, we found that clustered mutations (kataegis) in lymphoma and chronic lymphocytic leukemia predominantly carry AID-hotspot mutational signatures.

*Modulation of cell metabolic pathways and oxidative stress signaling contribute to acquired Melphalan resistance in multiple myeloma cells.* (Zub et al., 2015, [www.ncbi.nlm.nih.gov/pubmed/25769101](http://www.ncbi.nlm.nih.gov/pubmed/25769101)). Melphalan is a DNA alkylator and became one of the first chemotherapeutic drugs used against cancer more than 50 years ago. It is still widely used to treat multiple myeloma, but relapse inevitably occurs due to drug resistance and the mechanisms underlying resistance are poorly understood. We demonstrate by mass-spectrometry based proteome profiling of melphalan sensitive and resistant cells that the resistant cells have elevated aerobic glycolysis (Warburg metabolism) as well as modulated oxidative stress-signaling. The results are verified by specific inhibitors against enzymes in the glycolytic pathway as well as mitochondrial NAD+‐generating steps. Some of these inhibitors are already approved and should be further investigated as adjuvants or replacement drugs in melphalan-resistant multiple myeloma.