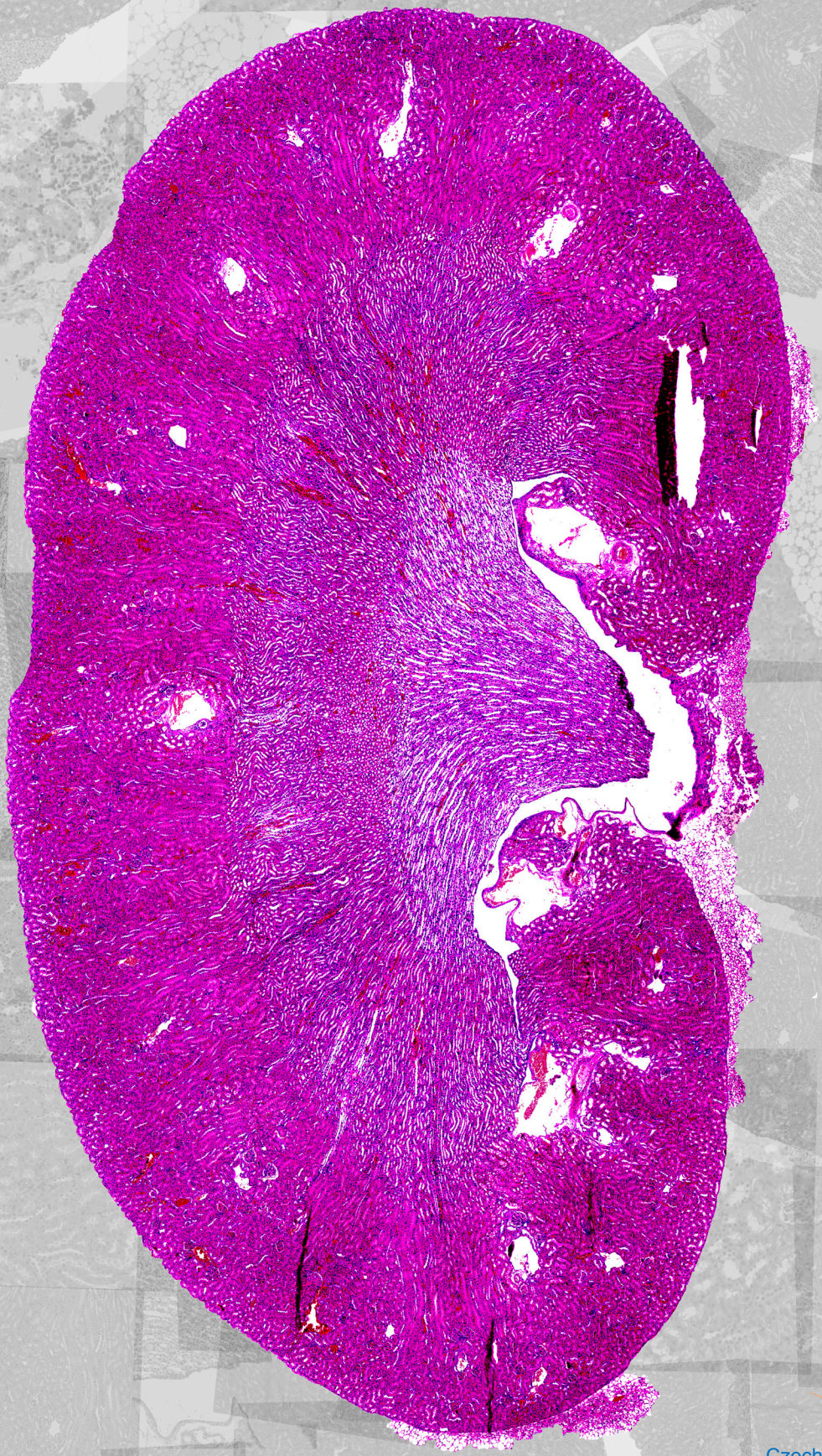
Volume 2, Issue 3 2018

PHENOGENOMICS
NEWSLETTER



Czech Centre for Phenogenomics

The Czech Centre for Phenogenomics is hosted by the Institute for Molecular Genetics AVCR v.v.i.

IMG

PRE KLINIKY

Centre for Preclinical Testing

New services on preclinical testing

After obtaining the certificate of Good Laboratory Practice (GLP) in January 2017, the Centre for Preclinical Testing (CPT) commenced activities with the support of the Czech Academy of Science (CAS). The primary mission of the CPT is to perform preclinical testing of substances that have successfully passed through basic research, and thus to contribute towards the development of new pharmaceuticals to combat life-threatening diseases, including those currently difficult to cure.
The Institute of Physiology CAS performs a coordination role for the CPT, and testing is also performed by the Institute of Molecular Genetics CAS including National infrastructure CCP, the Institute of Animal Physiology and Genetics CAS, and the Institute of Biotechnology CAS
The CPT offers a broad portfolio of tests under Good Laboratory Practice (GLP).

Core services:

- **Toxicity studies**, including toxicokinetic studies of promising chemical or biological agents on model animals – rodents and non-rodents (test systems: mouse, nude mouse, rat, guinea pig, rabbit, minipig) in compliance with ICH and OECD guidelines.
- **Bioanalytical, hematological and biochemical testing** of samples taken from animals during toxicity studies (determination of active substance in plasma or other biological matrices.
- **Development and validation of bioanalytical methods** for various test systems and biological matrices**.**
- **Determination of metabolites** in tissues and biological matrices (blood, plasma, urine)
- **Histopathological evaluation** of tissues from animals used in toxicity studies
- **Pharmacological studies on xenografts (nude mice)** with various cancerous cell lines, including Patient Derived Xenografts (PDX)
- **Cardiology diagnostic tests on animal models** – electrocardiogram (ECG), blood pressure measurement, cardiac imaging (Echo)
- **Synthesis, characterization and certification** of chemical substances with therapeutic potential, development of formulations for drug application

CPT facilities working under GLP regulations have established quality system, which is regularly inspected internally by Quality assurance unit and by National Authority and by our customers. The quality of our services is top priority for all our team members

The CPT offers its services to customers from both the academic and commercial sectors. Testing is carried out by recognised experts with long-term experience. The great advantages are the coordinated approach, flexibility, and the comprehensive nature of the offered services. You can find out more about the scope of CPT activities on the website: www.prekliniky.cz.

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Cover Image: Scanned image of kidney section taken using ZEISS Axio Scan.Z1.

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Note to customers: As valued customers, we welcome your articles and feedback on the service you received. Please send all correspondence to ccc@phenogenomics.cz

The editorial team would like to thank the authors in this issue for their contribution.

INFRAFRONTIER
mouse disease models

REGISTRATION OPEN

NEW INNOVATIVE TECHNOLOGY IN RODENT BODY TEMPERATURE TELEMETRY WORKSHOP

FOCUS ON BROWN FAT METABOLISM

23RD - 24TH JANUARY 2018

This workshop will be held at

The Czech Centre for Phenogenomics, Czech Republic

GUEST SPEAKERS

Prof. Dr. Martin Klingenspor
TU Munich

Dr. Marcel Scheideler
Helmholtz Zentrum München

Includes hands on surgical training

Contact training@infrafrontier.eu to apply

News In Brief

Seahorse Workshop Report

Nicole Chambers & Karel Chalupsky
Metabolism & Clinical Chemistry Units

On the 23rd and 24th April, the Czech Centre for Phenogenomics hosted the 'Metabolism, from cells to mouse' workshop in collaboration with HPST, Agilent distributor. This workshop focused on combining 3 metabolic techniques; Seahorse XF Technology, QTOF-LC/MS and Indirect calorimetry to highlight the strength of all 3 technologies and also promote the power of combining all 3 technologies when tackling complex metabolic studies.

The workshop was attended by 27 participants from Czech Republic and Germany. Presentations from Agilent specialists Shaun Bilsbrough and Svetoslav Kalaydjiev focused on the technology of Quadrupole Time-of-Flight Mass Spectrometry (LC/MS QTOF) and Seahorse and were supported by hands on demonstrations of both technologies lead by Karel Chalupsky and Michaela Pluskalová. During the workshop, a guided tour of CCP's phenotyping facility was organized for participants which focuses on the indirect calorimetry system and also included microCT, Lung function and others.

The workshop ended with a 'fun game' of guess the inhibitor. Here participants were shown data obtained from seahorse, indirect calorimetry and LC/MS QTOF and had to guess which pathway had been affected and which compound had been used. This playful 'game' showed that addressing the same question using different techniques can give you a detailed view of whole body metabolic disturbances.

Attendees found the workshop useful and also a time to establish and strengthen collaborations within the field.

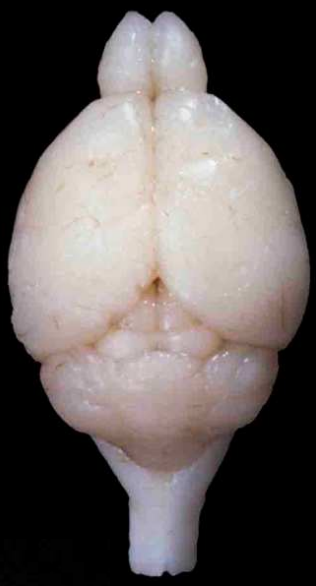
Neuron Impulse Under 33 Award for Petr Kašpárek

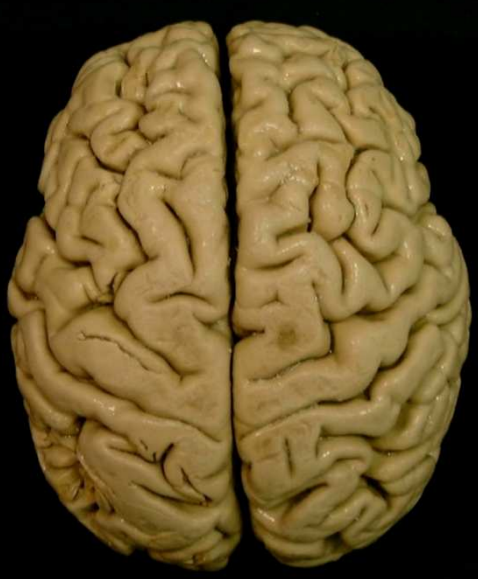
The Czech Centre for Phenogenomics would like to congratulate Petr Kašpárek who was recently awarded Neuron Impulse under 33.

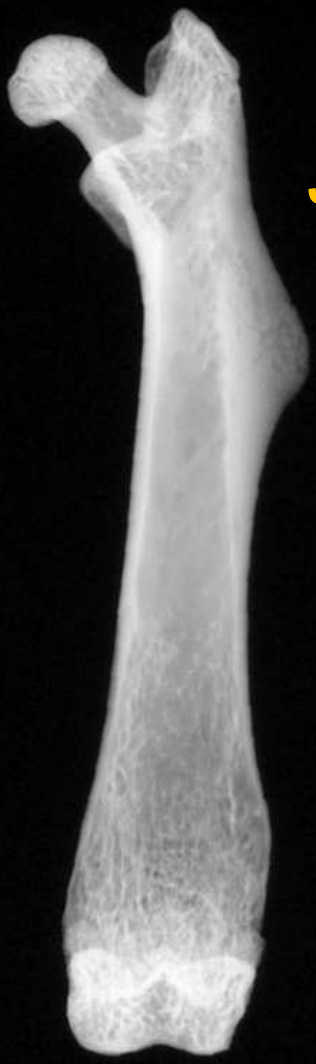
Petr previously discovered that genetically modified mice with deregulated skin proteases showed severe hair growth disorders. An overview of this work can be found in Volume 2 Issue 1 2017 edition of Phenogenomics Newsletter. The goal of the Neuron Impuls project is to investigate the origin of these hair defects and try to describe the role of proteases in hair growth and recovery for the first time.

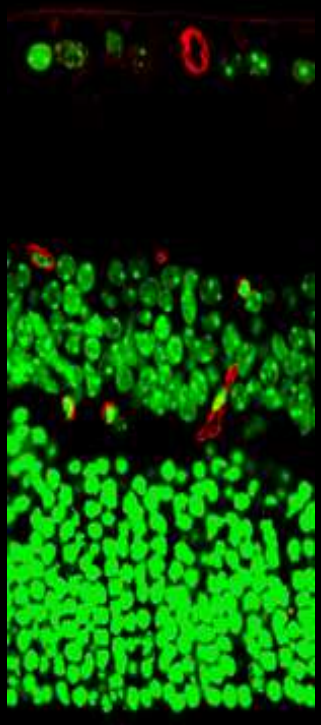
Mouse vs Human Comparative Morphology

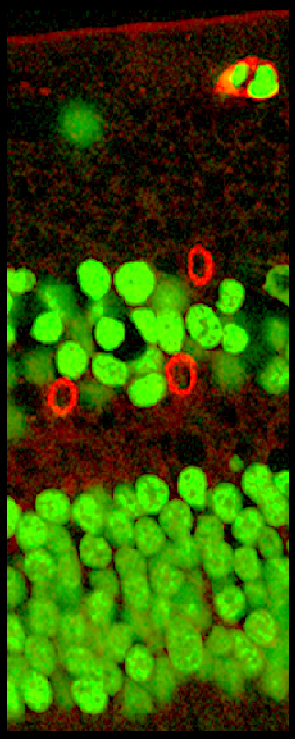
Essentials for accurate interpretation of Precision Medicine models

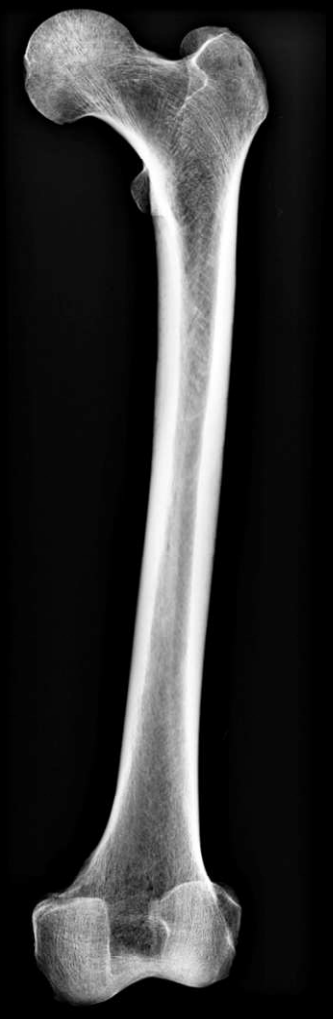












Course Coordinators

J. Ruberte and R. Sedláček

BARCELONA and PRAGUE 2018

INFRAFRONTIER

Czech Centre for Phenogenomics

Sharing Knowledge with the Science Community in Czech Republic and Beyond

Nicole Chambers
Editor

The Czech Centre for Phenogenomics is dedicated to the goal of sharing knowledge with the scientific community both in the Czech Republic and Europe. To this end the centre is focused on delivering workshops and seminars aimed to showcase new technology, new techniques and new insights in various fields including transgenic research, anatomy and morphology etc.

These courses and seminars are open to researchers, students and technical staff interested in the specific topic, or with an aim to expand their research into a new field of interest. Together with internationally respected professionals, CCP staff share their experience and knowledge with the aim to increase the quality and impact of research in the Czech Republic.

In 2018, the Czech Centre for Phenogenomics will host 5 courses, spread throughout the year. Details of each course are listed in Table 1 and more information is available on CCP's website www.phenogenomics.cz

Course	Date of Course	Description	Registration
New Innovative technology in rodent body temperature telemetry workshop	January 2018	The workshop will involve presentations on energy and brown fat metabolism from the level of molecular and cellular functions, metabolomics up to whole body energy balance regulation, an overview of the TSE Stellar technology and state-of-the-art implantation surgery including analgesia and animal welfare aspects.	training@infrafrontier.eu
3rd Programmable Nuclease Course	April 2018	This course is designed to give practical, hands-on training in current technologies and methods employing programmable nucleases (CRISPR/Cas9) - mediated genome manipulations in the generation of mice mutants. Participants will gain theoretical knowledge from our expert instructors and have the opportunity for discussion during our evening social events including the opportunity for expert insight for your own individual project.	ccp-tam@img.cas.cz
Mouse vs Human Comparative Morphology (Module I)	June 2018 (Barcelona, Spain)	The aim of this training course is to capacitate participants for interpreting mouse morphology and to provide them the essential knowledge to understand similarities and differences with human. Module I is devoted to anatomy and imaging that will be held in Barcelona	victor.nacher@uab.cat
Mouse vs Human Comparative Morphology (Module II)	September 2018	The aim of this training course is to capacitate participants for interpreting mouse morphology and to provide them the essential knowledge to understand similarities and differences with human. Module II dedicated to histology and advanced imaging.	Registration will open in April 2018
R for Biologists	Winter 2018	The aim of this course is to give scientists an introduction to R, describe data handling and advanced statistics and data visualization possible in R.	Registration will open in September 2018

Table 1. Courses and workshops available at CCP in 2018.

How to Collaborate with a Statistician

Petr Simecek

Bioinformatics Specialist

Have you ever stared into the email of your colleague with a feeling that not only you do not understand the overall meaning but also need to look up most of the words? In the rapidly changing field of Genetics, our collaborators might have very different backgrounds. The goal of this article is to give you a few hints what to do if you run into a Statistician.

I still remember what happened when I first left the gates of Faculty of Mathematics and Physics, experimenting with data from eye-tracking camera. I have been estimating what was the minimal time of a focused look. I developed a complex Bayesian model of Gaussian mixtures. I tried to introduce it to my supervisor. He was smiling. It was the kind of smile of Bunny Scientists in the well-known YouTube video "Always Use Three Patients"[1]. Finally, he took his plastic ruler, put it on the monitor, approximated the distance and said: "Well, good enough for me."

To avoid misunderstandings like that, this is what to do Before, During and After the project.

Before

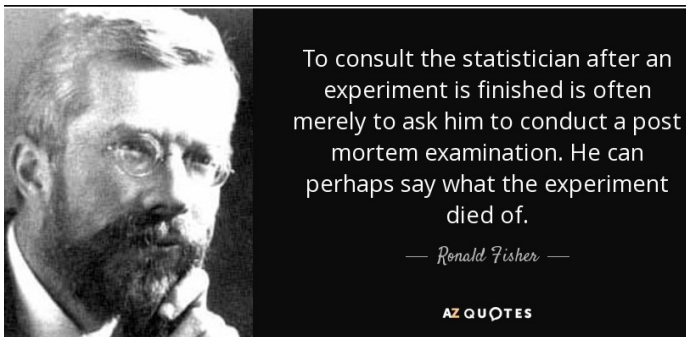
There is the whole subfield of Statistics devoted to a design of experiments and sample size determination. In practice, the following formula is often used

Number of samples =Money available/Cost of one sample

Occasionally, Statistician can identify that number of animals is clearly insufficient, stop the experiment before it even started and avoid wasting the money.

However, you should contact the statistician for randomization and stratification of your samples because of a danger of confounding (see Karl Broman: Experimental design and sample size determination[2]. In the simplest case, it means processing your samples in a random order. If your data are structured and processed in batches, better procedures can be applied.

Before data are being collected, you should also formulate your hypothesis and write down what statistical methods are



you going to use. This process is quite formal in case of clinical trials but is helpful in other fields as well. I have never witness a Statistician rejecting a request for a consultation before the experiment. On contrary, I have seen many experiments buried by a bad design (classical mistake: running the control group first, the treatment group afterwards while a batch effect is known to be present).

During

Make a life of your Statistician easier and organize your datasheets properly[3,4]:

Each variable you measure should be in one column

Each observation of that variable should be in a different row

Don't use font colour or highlighting as data

No empty lines or columns, make your data "rectangular"

No spaces, brackets or special characters in column names

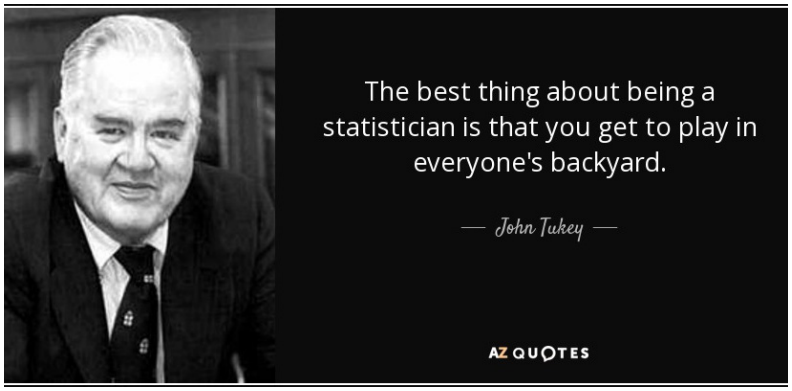
Be consistent (do not use "Male", "male" and "M", choose one, and stick to it)

Include data dictionary (the long variable names, units, expected minimum and maximum values...)

Statisticians are little curious creatures. Educate them! Feed them your excitement! They will do better job if they understand why the problem is important. Teach them your jargon (or try to avoid it). Send them introductory texts and reviews. If all else fail, draw pictures[5].

Many statisticians are sensitive about visual representations of data. The scope of grammar of graphics goes far beyond this short text but simple rules include...

Never ever use pie charts (human eye doesn't excel at comparing areas



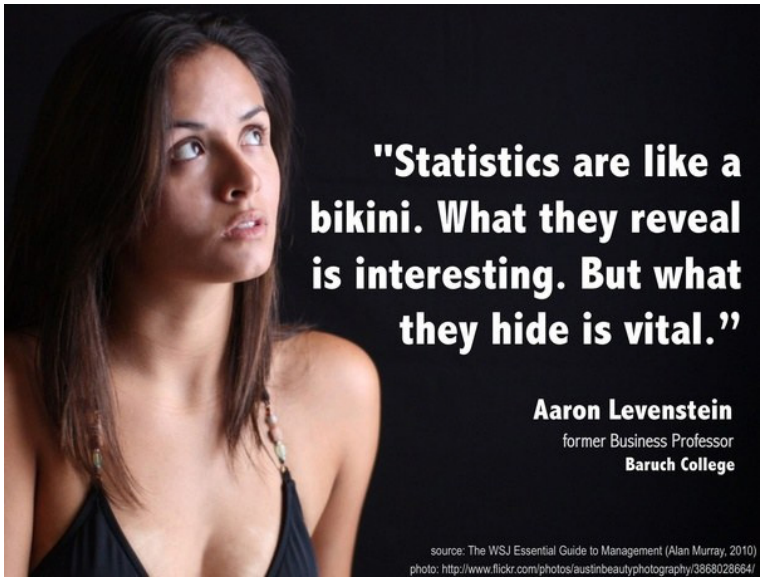
Avoid using 3D figures (like 3D barplots) if the third dimension does not carry information

Do your best to represent variability of your data (the bars and little antennae are wrong choice in case of 3 observations per group or a distribution far from Gaussian bell curve)

MS Excel is not a good tool for a publication level graphics, either purchase something better or learn to use free tools (like ggplot2)

Google "Top ten worst graphs" and learn from the mistakes of others

Many statisticians can help you to make you a beautiful



graphics, but others miss the necessary artistic talent. For high profile publications, it might be better to engage your graphical department or to consult a professional.

AFTER

If your experiment is designed properly, it contains positive and negative controls to warn you if something goes wrong. Your technicians are mere humans and if they process hundreds of samples, you can be sure they will occasionally make a mistake.

Your ability to detect and correct such mistakes might be crucial for success of the project[6] .

We have noted above that it is important your statistician understands the problem and devotes some time to orient herself or himself in the field. However, it is a two-way street and it is also very useful if researchers get at least a basic orientation in Biostatistics as well. In the times of internet, free online resources and MOOC courses, everybody can acquire knowledge if sacrifices time.

You can start with:

Software Carpentry[7]: a volunteer non-profit organization dedicated to teaching basic computing and statistical skills to researchers, all course materials are available and free to use

Coursera MOOC includes Data Science specialization[8] prepared by Jeff Leek's group at John Hopkins University

More deep and mathematical intro Data Analysis for Life Sciences[9] is available at EdX platform by Rafael Irizarri and Michael Love from Harvard university

Modern Dive[10] book from Chester Ismay and Albert Y. Kim, a statistical book that you can not only read but also write

References:

1 http://bit.ly/alwaysuse3patients

2 http://slideplayer.com/slide/6023200/

3 http://kbroman.org/dataorg/

4 https://peerj.com/preprints/3139/

5 https://simplystatistics.org/2013/10/08/the-care-and-feeding-of-the-biostatistician/

6 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4592999/

7 https://software-carpentry.org

8 https://www.coursera.org/specializations/jhu-data-science

9 https://www.edx.org/xseries/data-analysis-life-sciences

10 https://ismayc.github.io/moderndiver-book/index.html

LUNG FUNCTION UNIT - OVERVIEW OF SERVICES

Benoit Piavaux

Lung Function unit

At the lung unit we aim to offer complete and custom services ranging from screening of lung-function phenotypes in naïve (transgenic) animals to the complete screening of lung-pathophysiology in models of pulmonary diseases.

LUNG FUNCTION

For lung function measurements we use the Flexivent systems from Scireq, which are considered the 'golden standard' for lung function assessment. These systems are computer controlled, piston driven, small animal ventilators. They allow us to measure mechanical parameters of the lungs such as resistance to the airflow of the airways and lung tissue and compliance (elasticity related parameter) of the lung tissue. For these measurements we intubate the mice, instead of performing a tracheotomy like most other labs do, which allows us to repeat measurements at different time-points in the same animal.

We can do these measurements with or without challenge during the measurement. Measurements without challenge are useful for primary screening but will only detect pathologies which lead to extensive remodeling of the lung tissue or the airways, like fibrosis or emphysema. For lung pathologies without extensive remodeling it is usually useful to apply an aerosol challenge. In asthma models for example, the remodeling is relatively low, but mice develop airway hyper-reactivity, a land-mark feature of asthma. This airway hyper-reactivity can be measured by applying methacholine challenges during the measurement. A more detailed description of the lung function measurement and the measured parameters can be found on our website: http://www.phenogenomics.cz/phenotyping/ in the section 'Lung function'.

MODELS OF RESPIRATORY DISEASES

Asthma

Asthma does not naturally occur in mice, but an asthma like disease can be induced in many different ways. Most protocols are mimicking allergic asthma, which is the most occurring form of asthma in humans. The classic models of allergic asthma use ovalbumin (OVA) as model allergen. The mice are first immunized through IP injection with OVA with or without alum as adjuvant and then challenged with an OVA containing aerosol (Fig 1). These OVA-driven protocols have the advantage of being faster than other models using more natural allergens (cockroach extract, house dust mite extract, etc.) and they replicate most of the land-mark features of the human disease. As they are the oldest and most used models for asthma, they are also the best characterized models. This makes them a good starting point to investigate the role of a gene or a pharmacological intervention in the development of the disease. But as every model the OVA models have some drawbacks. The inflammation is much more pronounced compared to humans with a much larger influx of eosinophils and the airway remodeling is only very weak compared to human allergic asthma. Like all other models of asthma currently available the OVA-driven models do not lead to a chronic disease in mice as more allergen challenges will lead to tolerance towards the allergen. The OVA models have also been adapted to include immunotherapy, the only treatment currently known to really cure asthma instead of fighting the symptoms.

As previously mentioned there are also models with natural aero-allergens, which are also the allergens which trigger asthma in human. These models take longer and are more prone to

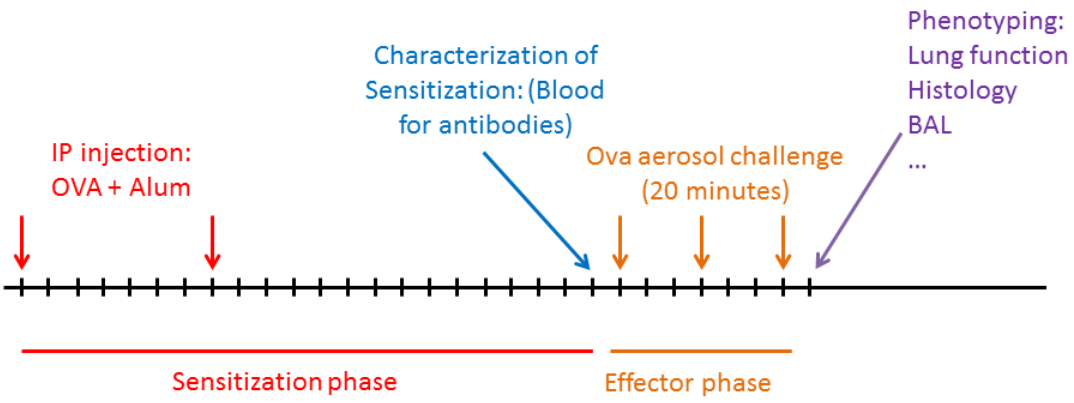
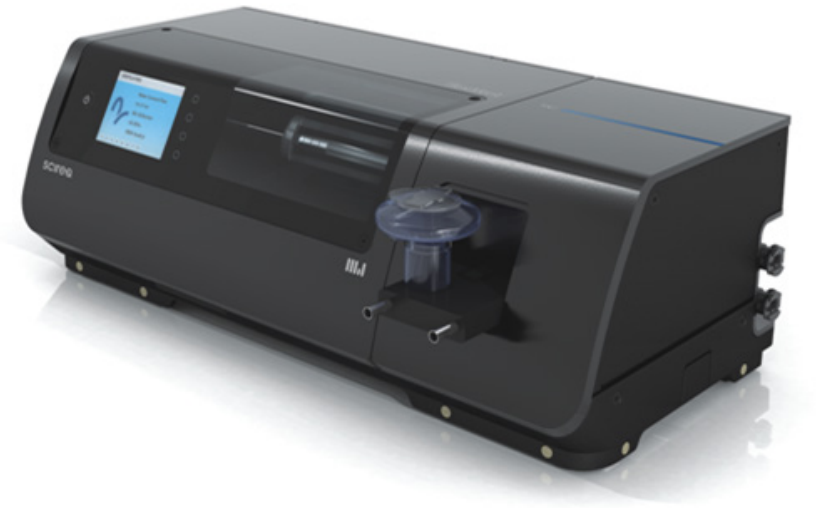


Figure 1: Schematic representation of the severe OVA-induced asthma model as used by the Czech Centre of Phenogenomics.



variability due variable quality of the allergen preparations, which can contain more or less endotoxin or have variable protease activity. Due to this and the fact that these models were more recently developed, these models are much less well characterized compared to the OVA models. The main advantage of these models is that the inflammation better mimics the inflammation seen in asthma patients and that the airway remodeling is usually stronger compared to the OVA models. These models are therefore usually used when airway remodeling is deemed to be the critical parameter.

Model for pulmonary emphysema

The model of choice for emphysema is to expose the mice to cigarette smoke for up to 6 months as then there is a disease with the same etiology and progression as most human cases of pulmonary emphysema. Unfortunately we are not able to perform these models as we have no equipment for this and we have no possibility to evacuate the smoke filled air efficiently from our phenotyping barrier. As alternative we can offer the elastase model in which elastase is instilled in the lungs. Despite having a different etiology than pulmonary emphysema in humans it still gives rise to a progressive disease which mimics the progression of human emphysema, including the inflammatory part of the disease, relatively well. The model is very fast compared to the smoking model as the mice already develop detectable levels of emphysema at 3 weeks after the instillation of the elastase. The decline of lung function and destruction of the lung tissue persists after these 3 weeks for at least 6 months, which makes the model suitable to investigate strategies to stop the progression of the disease.

Other models of respiratory diseases

The classic model for pulmonary fibrosis is the bleomycin model in which bleomycin is directly instilled in the lung. It gives rise to a severe, but not persistent, fibrosis 3 weeks after instillation. After these 3 weeks a progressive recovery is observed.

Acute lung injury is a common problem in intensive care patients under ventilation, as the mechanical stress of the ventilation exacerbates mild pulmonary infections to a level in which they become a serious health issue for the patient. The disease can be modelled in mice by a signle installation of LPS. It will cause an acute lung injury like disease, which is strongest 24 h after LPS administration and resolves within 1 week.

As our aim is to offer custom services, we can also develop custom models which fit our customer's needs

Phenotyping

For the lung models we can do full necropsy with harvest of the desired organs and samples. The samples can be processed by us or delivered to the customer. For the analysis of the samples, the expertise of all other screens and modules of the Czech Centre for Phenogenomics can and will be used.

Summary of services

- Assessment of lung mechanics without challenge
- Assessment of lung mechanics with challenge
- Mouse Models of Pulmonary Diseases
    - Asthma Models
    - Emphysema Model
    - Pulmonary Fibrosis Model
    - Acute Lung Injury
- Complementary Phenotyping Services
    - Histopathology
    - Clinical biochemistry
    - Immunology

Transgenic rats in TAM:

Expand your research with a new animal model

Irena Jenickova, Inken M. Beck

Transgenic and Archiving Module

The rat is the most widely studied experimental animal model in biomedical research, and rats were also the first domesticated species for scientific research in general (dating to 1850). Rats and mice are both members of the rodent family and therefore share several common features. However, the evolutional separation of rat and mouse by 15-20 million years made them two distinct species with number of functional and behavioral differences. For many reasons, the rat is preferred over the mouse in physiology, neurobiology, pharmacology, and behavior research.

Rats are physiologically more similar to humans than the mice and it is why there are widely used in pre-clinical animal experiments in the development of new medicinal drugs. The larger size of rats makes them a preferable model to study human cardiovascular, neurodegenerative, and metabolic diseases. Several rat strains have been bred for human diseases such as hypertension, autoimmunity, or hepatitis. Certain disease processes in the rat, such as mammary cancer, are generally closer to human pathology in terms of their features and origins than they are in the mouse.

Rats are calm animals easily accustomating to handling which makes them less stressful then the mice. This is why they are suitable models for behavioral studies including memory and learning behavior.

In addition, genetically modified rat can have different phenotype then the similarly genetically modified mouse and so making it more suitable study model. For example, the mouse model of Huntington´s disease shows almost no symptoms or only short-lasting phenotype. In contrast, the rat model for this disease exhibits adult-onset phenotype including a slowly progression of the disease typical for Huntington´s patients.

Rat strains

The most commonly used strains of the laboratory rat probably originate from the strains of Albino Norway rat *Rattus nirvegicus*. The majority of the strains are therefore Albino with white coat and pink eyes.

Rats can be classified as outbred, inbred, or F1 generation. The examples of outbred strains are Sprague Dawley (SD), Wistar, Long Evans (LE). The examples of inbred rats are Fisher 344, spontaneously hypertensive rat (SHR), and buffallo rats. There are also many mutant lines available.

Transgenesis in rats

Despite the advantages of rats as an experimental model, the mouse was always preferred in the field of genetics. The preference was strengthened after derivation of mouse embryonic stem cells (mES cells) in 1981. The mES cells have enabled the precise genetic modification in mouse genome *in vitro* and facilitated the development genome engineering technologies in the mouse. However, equipollent rat ES cell lines have not been available until relatively recently.

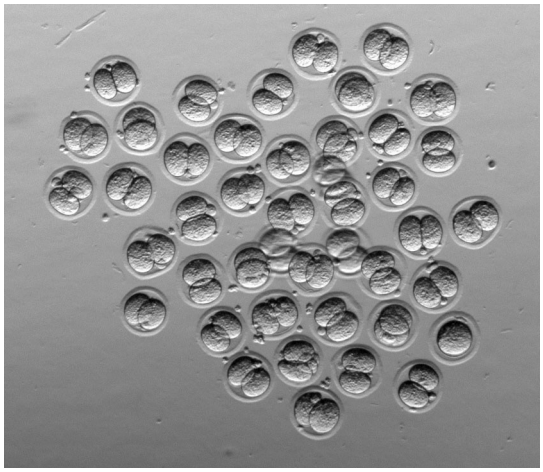


Figure 1. Rat 2-cell stage embryos

Manipulation of rat embryo is basically the same to the mice. Pronuclear microinjection has been used to generate transgenic rats with randomly integrated transgene. The randomly generated knock-out rat models have been produced with chemically induced N-ethyl-N-nitrosourea (ENU) mutagenesis and transposon-based gene-trap systems. However, the relatively fragile rat oocyte/zygote, higher sensitivity of rat embryos to *in vitro* conditions, and the absence of better knowledge of rat reproductive physiology delayed the production of transgenic rats and its application in research.

Male body weight (strain dependent)	300-520g
Female body weight (strain dependent)	250-300g
Body temperature	35.9-37.5C
Estrous cycle length	4-5 days
Gestation period	21-23 days
Litter size	6-12
Chromosome number	42

Table 1. Useful rat facts

Recently, several techniques have been developed to simplify the transgenesis in the rats – rat ES cells, rat spermatogonial stem cells, and the usage of programmable nucleases (ZFNs, TALENs, CRISPR/Cas9). Real rat ES cells were first derived with the establishment of inhibitor media (2i/3i) used for derivation of ES cells from the non-permissive strains. Such derived rat ES cells are germ line transmitting and when used for transfection they are capable to generate transgenic rat. The derivation and culture of spermatogonial cells together with the development of the technique for the transfection and screening of those cells have improved the efficiency of this approach. The transfected spermatogonial cells are injected into testes of young male followed by breeding and production of transgenic progeny. Similar as in the mouse, transgenic rats have been successfully

generated using programmable nucleases. Over all, it is the rat in which was the application of the ZFN published as the first. Programmable nucleases enable not only the indel/deletion, but also the integration of desired transgene.

All above mentioned techniques are opening new possibilities in the field of rat transgenesis. Especially genome editing with the programmable nuclease will expand the generating of transgenic rats available for scientific research. The high efficiency of those techniques means that introduction of certain types of mutation into the rat genome is no longer the limiting factor.

Figure 2. Foster mother after embryo transfer

Service available in TAM

The Transgenic Unit in Czech Centre for Phenogenomics focused on transgenic rat generation using CRISPRs. We have generated so far 3 transgenic lines containing indel or deletion. All the lines were generated via pronuclear injection on Sprague Dawley background (Rj Han: SD). The service for transgenic rat generation is now fully available to all potential customers. Above that, we are now working on the establishment of the delivering the CRISPR/Cas9 into the zygote by electroporation (ELPO). The advantage of electroporation is in higher survival rate and better development of the zygotes after ELPO in comparison to pronuclear injection. The preliminary data looks very promising, and we believe that in near future the electroporation will be more efficient for generation of transgenic rats than the pronuclear injection.

Besides generation of transgenic rats, we have successfully frozen and thawed 2-cell stage embryos. Slow rate freezing of 2-cell stage embryos is available as an archiving service. The first project archived in our facility was done in cooperation with the Institute of Experimental Medicine. Transgenic Unit also re-derived 3 transgenic lines from the Institute of Physiology (SHR genetic background) and some other lines are now waiting for re-derivation.

In contrast to the mouse, the techniques as sperm freezing and *in vitro fertilization* (IVF) are not well established in the rat in general due to high fragility and sensitivity of rat spermatozoa. Transgenic Unit therefore does not offer service in rat sperm freezing and IVF.

All the newly generated rats are produced in specific pathogen free (SPF) conditions. The current capacity of the SPF facility for rat breeding is 1792 cages, which is the highest in Czech Republic. We are also the only facility producing SPF rats and only one of few in Europe. The lines coming from the other core facilities must be transferred into the SPF condition via embryo transfer. This is the only way how we can guarantee stable clean condition in our breeding facility. For short term breeding of rats coming from “less healthy controlled” facilities, we have a quarantine breeding room.

Transgenic Unit in Czech Centre for Phenogenomics is now offering all the above mentioned services. You are welcome to ask any questions. For more details and requests contact, please: tgunit@img.cas.cz

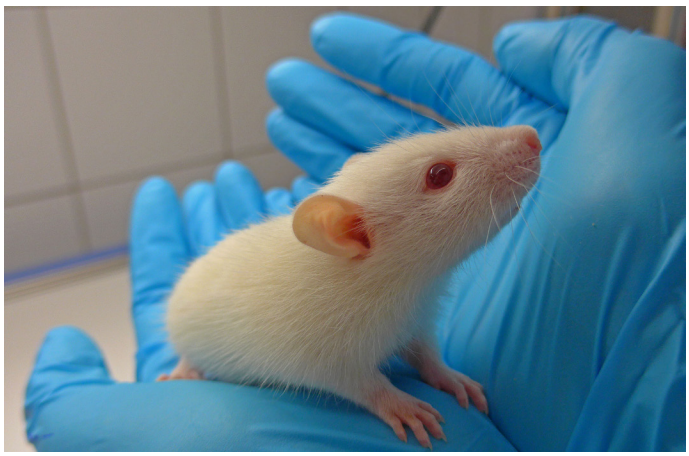


Figure 3. Sprague Dawley rat (pup)

Service	Genetic background	Details
Transgenic rat generation via pronuclear injection (electroporation)	Sprague Dawley	Including CRISPR/Cas9 design & production (optional), pronuclear injection or electroporation, embryo transfers
Genotyping of the transgenic rats		Including primer design, DNA isolation, and genotyping with PCR
Re-derivation/re-animation	as needed	Including assistance in embryo isolation/embryo thawing and embryo transfers
Archiving/cryopreservation	as needed	Including embryo isolation, cryopreservation, and testing of successful archiving (i.e. embryo thawing and embryo transfers), storage in LN2
Breeding of transgenic rats	as needed	Including breeding space, pair breeding & pups weaning, assistance in ear tagging

Table 2. Overview of rat service available in CCP

Resource	Web link	Details
Rat genome database	http://www.rgd.mcw.edu	Genetic, genomic, phenotype, and disease data, strains and gene info, sequences, etc.
National Bioresource for the Rat (NBPR), Japan	http://www.anim.med.kyoto-u.ac.jp/nbr/repository.aspx	Repository of >700 rat strains including reporters, Cre and disease lines, cryopreserved embryos and sperms
Rat Resource and Research Centre (RRRC), USA	http://www.rrrc.us	Repository of >350 rat strains including reporters, Cre and disease lines, cryopreserved embryos, sperms and ES cells

Table 3. Useful links for further reading

References:

Meek, S., et al. From engineering to editing the rat genome. Mamm Genome **28**:, 302-314 (2017)

Suckow, M.A., et al. The Laboratory Rat. 2nd edition, Elsevier, AP (2006)

Anegon, I. Rat Genomics, Methods in Mol. Biol. 597, Springer Protocols, (2010)

Pease, S. and Saunders, T.L. Advanced Protocols for Animal Transgenesis, Springer Protocols (2011)

SLIDE SCANNING TECHNOLOGY

Jan Kučera

Histopathology Unit

In recent years the world goes digital in almost every field of life. This burst of digitalisation era is given by rapidly increasing computing strength of present and future computers. Currently, the concept of digitalisation is entering the field of morphological branches of medical diagnosis and science such as histopathology. Nowadays techniques enable the transformation of the physical glass slide into a binary computer language of zeros and ones within "a click of a button".

Production of digital slides brings five main advantages for researchers, clinicians, and educators: Archiving, Sharing, Consulting, Educating, Analysing. Once the solid glass slide is digitalized there is just limited need for its onward physical existence. This greatly reduces the storage space required to archive physical samples. The digitalised image can be much easier transferred and shared with colleagues and co-operators across the work facility, state or even world for consultation and comparison of opinions. Such slides can be easily used for educational purposes and lastly it can be subjected to repeated deep analysis of tissue morphological components thus transferring descriptive and "sensational" semiquantitative assessment into clearly comparative language of numbers.

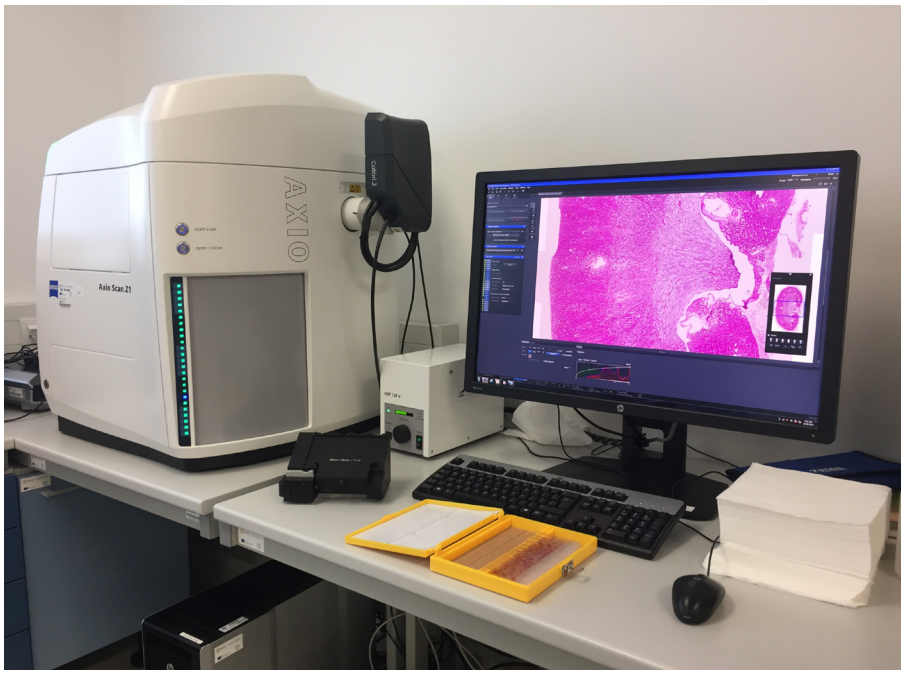


Figure 1: CCPs Axio Scan.Z1 installation

The ZEISS Axio Scan.Z1 is a fast and reliable slide scanner for both bright-field and fluorescence imaging of standard histological slides. It is able to digitalise the specimen in a reliable and reproducible way, producing a high quality virtual slide, which is especially useful for fast bleaching fluorescent staining. This scanner is currently available at CCP for customers and partners (Fig.1).

Our system's technical specifications are following:

With a modular tray concept we can use both standard (26 x 76 mm) specimen slide, and "double width" (52 x 76 mm) sized slides for scientists with special demands who need to scan large specimen which would not fit onto standard slide dimensions. The machine magazine can be then loaded with 25 trays of four slides or two slides giving 100 specimens or 50 "double width" sized specimens to be scanned. The individual slides are not moved by the slide loader which decreases the possibility of the slide breaking and sample destruction.

After loading of specimen into the machine the samples are previewed and preview scans of both identification and specimen area of the slide are produced. For unique labelling of specimen, 1D and 2D barcodes can be used. The system can also use optical character recognition. If predefined, the system is then able to assign scanning options/profiles and file names according to the obtained identification information which is especially useful when creating a broad virtual slide library whether for educational, medical or research use.

Magnification	Type	Numerical aperture	Maximal Theoretical Resolution (for 520nm light) [µm/pixel]
5x	Fluorite	0.25	
10x	Plan-Apochromat	0.45	0.44
20x	Plan-Apochromat	0.80	0.22
40x	Plan-Apochromat	0.95	0.11

Table 1: Objective specifications

With a tissue detection wizard, the system is able of automatic sample/section region detection on the previewed slides. Acquisition then takes place on the pre-defined region of the specimen. This feature makes scanning more effective with reduction of both time needed for specimen scanning and amount of data produced. In case of faintly stained sections where the automatic tissue detection does not detect the specimen properly, manual selection/adjustment of a region of interest is possible.

For thumbnail generation, separate camera with reflected light illumination is used. Specimen area in bright-field is previewed with separate camera using transmitted light illumination. For fluorescence a 5x objective with transmitted light illumination (RAC) or reflected light illumination (fluorescence) is used.

Light Sources

For brightfield applications AxioScan.Z1 is equipped with a transmitted light VIS-LED 400-700 nm light source with maximum at 460 nm. For fluorescence acquisition the system is equipped with both metalhalide and fast LED Colibri.2 light sources. MetalHalide HXP 120 V mercury short-arc bulb illuminator is a separate compact unit with inclusive built-in power supply, lamp module and infrared filter. It is equipped with motorised brightness switch and integrated motorised shutter, that works stepper motor controlled, fast and vibration free.

Colibri.2 is a fast LED fluorescence light source able to turn on and off in milliseconds minimising the time of sample exposure to the damaging excitation light. This feature, in addition to fluorophore excitation with a monochromatic light source, reduces phototoxic effects to the samples. Colibri.2 is equipped with 4 LED (365, 470, 560, 630 nm) fluorescence light sources.

Fluorescence filter sets for DAPI (Filter set 49, shift free), CFP (Filter set 47, shift free) , eGFP (Filter set 38 HE, shift free), Cy3 (Filter set 43, shift free), mPlum (Filter set 64 HE, shift free), Cy5 (Filter set 50, shift free), and one tri-band filter (Filter set 62 HE, BFP + GFP + HcRed, shift free) for fast fully automatic exchange of excitation wavelength during LED fluorescence acquisition are installed. With the possibility of switching the excitation wavelengths in milliseconds and using tri-band and quad-band filter sets, no mechanical components will move in the optical beam path, thus increasing image sharpness and again reducing the phototoxic effect on sample. All filters are localised in 10-position ACR wheel for filter cubes and filter number and characteristics can be thus further enlarged or exchanged for actual needs.

The system is equipped with calibrated high-quality plan-apochromatic objectives with 5x-40x magnification. List with further characteristics of objectives are presented in Table 1.

Geometric calibration and colour calibration guarantees that virtual slides will be reproduced precisely at any time with comparability both between systems and over time. This can be especially useful for researchers evaluating samples collected during extended time periods, for example during time scheduled sample collection in pre-clinical studies or when comparing rare cases collected over years. Comparability between machines assures the possibility to compare similar

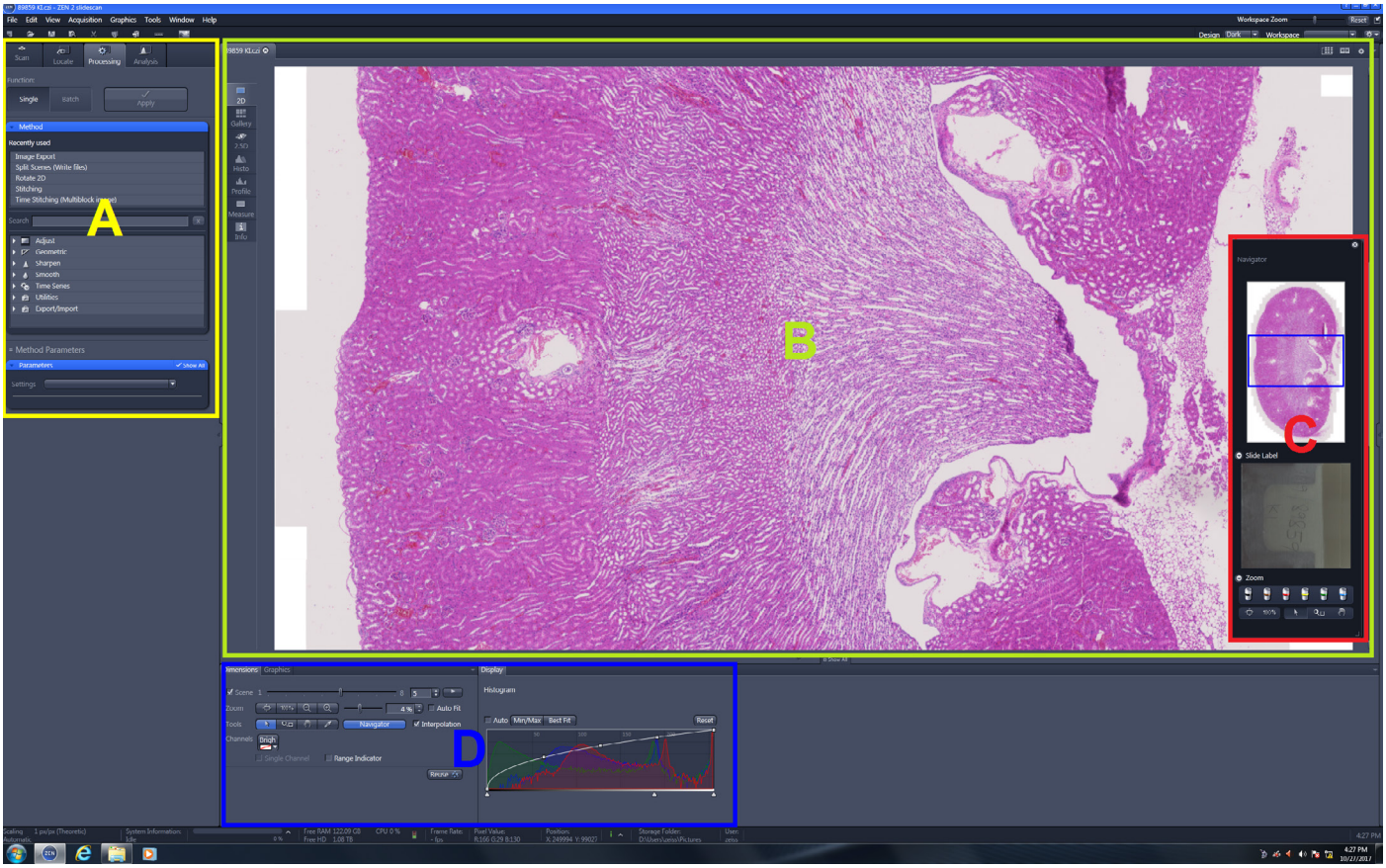


Figure 2: ZEN software interface

Scan/Machine/Processing/Analysis settings panel, B) Acquired image insight, C) Scanned area navigator D) Insight panel display and annotation settings

samples scanned on same machine on the other side of the world.

For fast bright-field acquisition, a highly sensitive camera Hitachi HV-F202SCL with RGB non-interpolated chip of 1600 x 1200 effective pixel size 4,4 x 4,4 µm and digitalisation into 12 bit per pixel can be used. The camera is able to acquire up to 30 images per second with electronic shutter speed up to 1/100 000.

Fast monochromatic acquisition is provided with 4MPix active air cooled CMOS Hamamatsu Orca-flash 4.0 monochrome camera, with pixel size 6,5 x 6,5 µm and 16 bit digitalisation range and ability to capture up to 30 images per second. With the shortest possible exposure time of 10 µs it ensures maximum specimen protection from extended light exposure. Maximal exposure time can be set up to 10 sec for extremely weak signals. In our centre, we currently use an exposure time of 200 µs as our standard, although we are able to customize exposure times according to individual samples and chromogen or fluorophore needs.

Focusing of specimen during acquisition is automatically assured by the software in coarse and fine steps with a defined strategy of focus point map created at low and high magnification for accuracy. The number of points is variable and relates to sample flatness. Different algorithms are available for spatial distribution of focus points.

For thicker sections, it is possible to use Z-stacking and connected Extended Depth of Field calculation mode, which produces a merged image made from several Z-stacked single images acquired in different focus planes.

ZEISS Axio Scan.Z1 scanning automat is controlled by ZEN software from Zeiss (Fig.2). ZEN is a familiar software designed for high throughput workflow of capturing virtual slides and can be further extended with image analysis modules for accurate data processing and assessment. ZEN native format for storing multidimensional image data (XYZ and λ) is CZI, and it can be viewed and annotated with freely downloadable ZEN lite software. Annotations are then stored in separate image layer. It is also possible to export your data into wide variety of popular image formats (such as TIFF, JPEG, OME TIFF), nevertheless, CZI format is open for processing and analysis in third-party software (see list at www.zeiss.com)

APPLICATIONS OF THE SCANNER

- bright-field and fluorescence scanning
- 10x, 20x, 40x magnification
- DAPI, CFP, eGFP, Cy3, mPlum, Cy5 and tri-band BFP + GFP + HcRed Fluorescence filters
- Fast bright-field camera
- sensitive monochromatic camera
- automatic tissue detection and autofocusing
- CZI format third-party SW compatible

To finish the comprehensive list of Axio Scan.Z1 technical data there is one example of high resolution image with which I as the author and operator of this machine would like to kindly ask you to consider your needs for scanning magnification: At the attached Figure 3 you can see comparison of same area scanned at 10x, 20x and 40x. Both 10x and 20x have good image quality and resolution, but offer also significant scanning time savings in comparison with 40x. 20x is 5 times faster than 40x with only cca 15% less resolution, and 10x is 5 times faster than 20x with only cca 40% less resolution. Data size of the relevant image file doubles with 10x to 20x transience and almost quadruple with 10x to 40x transience.

For further information about AxioScan.Z1 system workload and capacity our future customers and co-operators are kindly asked to contact responsible person at www.phenogenomics.cz.

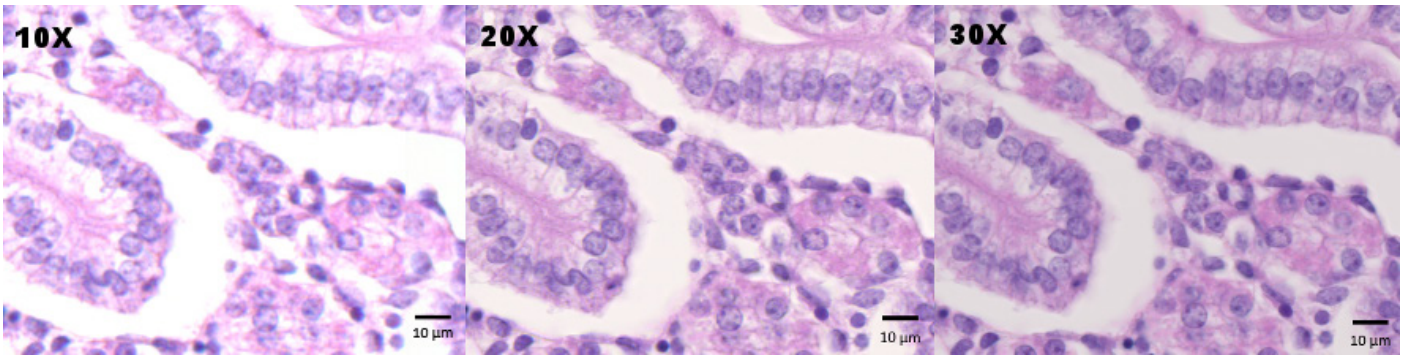
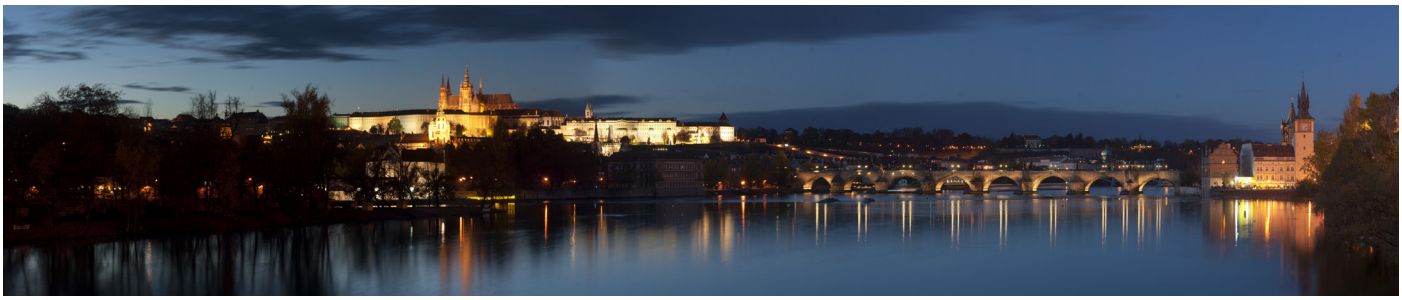


Figure 3: Comparison of image quality scanned at 10x, 20x and 40x magnification.

CAREERS



CCP comprises a young, multidisciplinary and international team. We believe in the personal and professional development of our staff and seek, where possible, to facilitate the attendance of relevant conferences and courses. We offer a competitive salary and various working contracts. Please click here for application details

Pathologist (rodent pathology)

To advance and further improve services of our histopathology lab we are seeking experienced pathologist who will be responsible for analyses and descriptions of mouse and rat tissue samples, especially:

– to provide expertise in the pathology of genetically-engineered mouse (GEM) and rat models

– to provide full pathology analysis including complete gross- and histopathological evaluation supplied with image-based report, digital images, and recommendations

– to perform phenotype investigation and characterization together with histology-lab managing scientist; this includes necropsy, macroimaging, tissue sample collection, supervision of histological processing, histopathological evaluation, digital photomicrographs, and consultations.

– to follow and implement GLP rules and manage work of lab technicians

– to drive his/her own research projects and actively participate within the other projects of the Centre.

Successful applicant should have DVM or MD (or equivalent advanced degree in relevant field) and relevant research and/or hands-on experience. Capability to work in English speaking environment is a must, previous experience with SOPs for GLP is an asset.

Senior Laboratory Technician / Transgenic Core Specialist (Embryonic Stem Cells / Transgenesis)

The Transgenic and Archiving Module of the CCP is seeking a motivated senior technician to join our international team.

Your key responsibilities will be work dedicated to embryonic stem cell (ESC) laboratory. This includes ESCs culture, back-up freezing, and their use and optimization for genome targeting by classical techniques and programmable nucleases (CRISPR/Cas) and validation of generated ESC clones. Partly you will support the team in rodent handling and operation (embryo transfer into foster mothers). In addition, assistance in general lab management, administrative tasks, and reviewing/supervising the daily subgroup work program will also belong to your tasks.

You should possess a master's degree in biology, molecular biology, biotechnology, or related subject and must have experience in mouse ESCs culture, general laboratory work, and organization. Experience in rodent handling as well as knowledge in rodent genetics and transgenic technologies would be advantageous. Candidates must be fluent in English, have good interpersonal communication skills, and be able to work independently as well as part of a team. If you are an organized and responsible person, have a keen perception and like to establish new techniques in the field of rodent transgenesis, you are welcome to apply for this job.

For more information or to apply for any of these positions, contact Mr Libor Danek (ccp@phenogenomics.cz). All applications should be made in English, include a letter of interest and a structured CV.

Upcoming Events

Keystone Symposia: Bioenergetics and Metabolic Disease

21st - 25th January 2018

http://www.keystonesymposia.org/18J4

New innovative technology in Rodent temperature telemetry workshop

23rd - 24th January 2018 | Vestec, Czech Republic

https://www.infrafrontier.eu/

Healthy Ageing: From Molecules to Organisms

31st January – 2nd February 2018 | Hinxton, Cambridge, UK

https://coursesandconferences.wellcomegenomecampus.org/events/item.aspx?e=658&dm_i=2SUU,KGV9,UWTV4,2551R,1

NEUBIAS Conference

27th January 2018 - 2nd February 2018 | Szeged, Hungary

http://eubias.org/NEUBIAS/

3rd Programmable nucleases (CRISPR/Cas9) Transgenesis Course

16th - 20th April 2018 | Vestec, Czech Republic

http://www.phenogenomics.cz/

Mouse vs Human Comparative Morphology (Module I)

Essentials for accurate interpretation of Precision Medicine models

18th-23th June 2018 | Barcelona, Spain

Mouse vs Human Comparative Morphology (Module II)

Essentials for accurate interpretation of Precision Medicine models

3rd - 6th September 2018 | Vestec, Czech Republic

Journal Club

Lefrançais E. *et al.* The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature* **544**, 105-109 (2017).

Dickinson ME. *et al*. High-throughput discovery of novel developmental phenotypes. *Nature* **537**, 508-514 (2016).

Stowers JR. *et al*. Virtual reality for freely moving animals. *Nature Methods* **14,** 995-1002 (2017).

Reardon S. Sex matters in experiments on party drug — in mice. *Nature* (2017).

Gaudelli NM. *et al.* Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 (2017).

Liao HK. *et al.* In Vivo Target Gene Activation via CRISPR/Cas9-Mediated Trans-epigenetic Modulation. *Cell* **71**(7), 1495-1507 (2017).

Czech Centre for Phenogenomics

Delivering and Characterizing Research Models

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