10th Kraków Workshop on Novel Applications of Imaging and Spectroscopy in Medicine, Biology and Material Sciences

September 23-25, 2019
Kraków, Poland

Organised by:
H. Niewodniczański Institute of Nuclear Physics
Polish Academy of Sciences

Venue: Institute of Nuclear Physics PAN,
Main Building, Auditorium
Radzikowskiego 152, Kraków, Poland

Kindly supported by:
# Agenda

**Monday, September 23, IFJ PAN, Auditorium**

## Registration

11:00 - 18:00  
Registration desk, Auditorium Entrance, 1st Floor

## Welcome

11:30 - 11:45  
**Welcome Remarks**  
Prof. dr hab. Marek Jeżabek, Director IFJ PAN, Poland

### 1. **Chair: prof. Alex MacKay**

11:45 - 12:30  
Thoralf Niendorf, Berlin, Germany  
*Progress in Fluorine (19F) Magnetic Resonance: Technical Developments and Application in Disease Models*

12:30 - 13:00  
Greg Stanisz, Toronto, Canada  
*Early Evaluation of Cancer Therapy Using CEST & MT*

13:00 - 14:30  
Lunch, IFJ cantine

### 2. **Chair: prof. Greg Stanisz**

14:30 - 15:00  
Alex MacKay, Vancouver, Canada  
*Investigating the entire NMR signal from brain*

15:00 - 15:30  
Piotr Kozłowski, Vancouver, Canada  
*Quantitative MRI in clinically relevant models of spinal cord injury*

15:30 - 16:00  
Niels Schwaderlapp, Freiburg, Germany  
*Optogenetic-fMRI in experimental temporal lobe epilepsy*

16:00 - 16:15  
Anna Orzyłowska, Lublin, Poland  
*7T 1H MRS for detection of immediate cerebral response to probiotic treatment in rat model of depressive disorder*

16:15 - 16:30  
Stefan Gaździński, Warsaw, Poland  
*Structural Gray Matter Abnormalities in Morbidly Obese Patients with Type 2 Diabetes and their Recovery with Intragastrical Balloon Treatment*

16:30 - 17:00  
Coffee Break, IFJ cantine

### 3. **Chair:**

17:00 - 17:30  
Guilhem Collier, Sheffield, UK  
*MRI and MRS of dissolved hyperpolarized xenon-129*

17:30 - 18:00  
Tadeusz Pałasz, Kraków, Poland  
*MRI of lungs with hyperpolarized noble gases (3He, 129Xe)*

18:00 – 18:30  
Maria Sokół, Gliwice, Poland  
*NMR-based metabolomics in the evaluation of low-dose radiation cardiotoxicity*

18:30 – 18:45  
Krzysztof Jasiński, Kraków, Poland  
*The neurochemical profile of anorexic rats*
Tuesday, September 24, IFJ PAN, Auditorium

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<td>8:30 - 9:00</td>
<td>Chair: PhD Claudia Oerther</td>
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<td></td>
<td>Jim Delikatny, Philadelphia, USA Translating Optical Molecular Imaging</td>
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<tr>
<td>9:00 - 9:30</td>
<td>David Viertl, Lausanne, Switzerland MicroPET/CT-based internal radiation dosimetry of a $^{152}$Tb-labeled antibody in tumor-bearing mice</td>
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<td>9:30 - 9:50</td>
<td>Frantisek Spoutil, Prague, Czech Republic MicroCT for high throughput mouse phenotyping</td>
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<td>9:50 - 10:10</td>
<td>Jens Waldeck, Ettlingen, Germany Non-destructive in vivo and ex vivo insights into biological nanostructures via X-ray based microscopy and CT</td>
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<td>10:10 - 10:30</td>
<td>Arno Nauerth, Ettlingen, Germany Self-Gated Cardiac PET/MR</td>
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<td>10:30 - 11:00</td>
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<td>11:00 - 11:30</td>
<td>Chair: Dr Jens Waldeck</td>
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<td>11:00 - 11:30</td>
<td>Dorde Komljenovic, Heidelberg, Germany Ultra-high field MR imaging in preclinical oncology</td>
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<td>11:30 - 11:50</td>
<td>Claudia Oerther, Ettlingen, Germany Preclinical ultra-highfield MRI</td>
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<td>11:50 - 12:10</td>
<td>Astrid Wietelmann, Bad Nauheim, Germany Cardiac MRI of model species smaller than mice</td>
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<td>12:10 - 12:30</td>
<td>Michał Fiedorowicz, Warsaw, Poland Multimodal imaging for preclinical evaluation of tumors induced by renal cell carcinoma stem-like cells</td>
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<td>12:30 - 12:50</td>
<td>Oliver Štrbák, Martin, Slovakia Comparative MRI of Magnetoferritin as a Pathological Model System of Native Ferritin</td>
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<td>12:50 - 13:10</td>
<td>Igor Serša, Ljubljana, Slovenia Magnetic Resonance Microscopy of Blood Clots</td>
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<td>14:30 - 15:00</td>
<td>Chair: prof. Piotr Kozłowski</td>
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<td>14:30 - 15:00</td>
<td>Harald Möller, Leipzig, Germany PROspective Baseline Enhancement (PROBE): An Efficient and Versatile Editing Scheme for CEST</td>
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<td>15:00 - 15:30</td>
<td>Franciszek Hennek, Zurich, Switzerland Fast MRI with extreme field gradients</td>
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<td>15:30 - 16:00</td>
<td>Grzegorz Kwiatkowski, Zurich, Switzerland Accelerated MRI for robust quantification of cardiac perfusion in small animals</td>
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<td>16:00 - 16:30</td>
<td>Martin Uecker, Göttingen, Germany Model-Based Reconstruction Methods for Accelerated Quantitative MRI</td>
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<td>Conference Dinner, Old Town, “Kawaleria” Restaurant, Gołębia 4, Poster Prizes</td>
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<td>Khanlian Chung, Heidelberg, Germany</td>
<td>Adaptive cone beam scan trajectories for interventional applications</td>
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<td>9:00 - 9:30</td>
<td>Artur Krzyżak, Rafał Obuchowicz, Kraków, Poland</td>
<td>Practical aspects of applying the generalized Stejskal-Tanner equation to MRI</td>
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<td>Barbara Blasiak, Krakow, Poland</td>
<td>Molecular Imaging of Cancer using Targeted Contrast Agents</td>
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<td>9:50 - 10:10</td>
<td>Natalia Łopuszyńska, Kraków, Poland</td>
<td>Visualization of the distribution of 19F nuclei in Nafion loaded theranostic nanocapsules with the 3D Ultra-Short Echo Time pulse sequence at 9.4T</td>
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<td>Przemysław Dorożyński, Warsaw, Poland</td>
<td>Inhalable theranostic system for local tuberculosis treatment containing isoniazid loaded metal organic framework Fe-MIL-101-NH2</td>
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<td>Piotr Kulinowski, Kraków, Poland</td>
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<td>11:30 - 11:50</td>
<td>Anna Nikodem, Wrocław, Poland</td>
<td>Studies using microCT conducted to analyze degenerative changes of the hip and knee joints</td>
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<td>11:50 - 12:10</td>
<td>Magdalena Tomanik, Wrocław, Poland</td>
<td>Analysis of subchondral bone porosity for biomimetic scaffold design</td>
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<td>12:10 - 12:30</td>
<td>Iwona Habina, Kraków, Poland</td>
<td>Low Field NMR Relaxometry of poly(sodium acrylate)/sodium silicate hydrogels</td>
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<td>12:30 - 13:00</td>
<td>Władysław Węglarz, Kraków, Poland</td>
<td>Not only mice and rats – high field MRI of rocks</td>
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Abstracts of Oral Presentations
Molecular Imaging of Cancer using Targeted Contrast Agents

Barbara Blasiak¹,², Frank C. J. M. van Veggel³, Armita Dash³, John Matyas⁴, Dragana Ponjevic⁵, Abhinandan Banerjee⁵, Simon Trudel⁶, Garnette R. Sutherland², Boguslaw Tomanek¹,²,⁵

¹Polish Academy of Sciences, Institute of Nuclear Physics, Krakow, Poland; ²Department of Clinical Neurosciences, University of Calgary; ³Department of Chemistry, University of Victoria, British Columbia; ⁴Faculty of Veterinary Medicine, University of Calgary; ⁵University of Alberta, Department of Oncology, Edmonton, Canada; ⁶Department of Chemistry, University of Calgary, Calgary AB, Canada

Magnetic Resonance Imaging (MRI) has been used for early cancer detection, treatment monitoring and image guided surgery. MRI has excellent spatial resolution and soft tissue contrast but low specificity. Standard contrast enhanced MRI based on tumors vasculature, including Gd-based T₁ contrast agents, do not provide sufficiently high specificity for tumor diagnosis and thus contrast agents providing T₂ contrast have been applied to provide information on tumor specificity.

To improve the tumor contrast we have developed core/shell NaDyF₄/NaGdF₄ nanoparticles changing both T₁ and T₂ relaxation times of surrounding water molecules and conjugated them with tumor specific antibodies and proteins. The relaxation times (T₁ and T₂) of the nanoparticles with various score/shell sizes and concentrations were measured at 9.4T and 3T to find the optimum T₁/T₂ ratio for MRI. T₁- and T₂-weighted images using core/shell nanoparticles of the animal models of brain, breast and prostate cancer were collected and combined to provide enhanced contrast and edges. The contrast agents consisting of the core/shell nanoparticles with the optimal core and shell sizes are being developed to provide improved tumor contrast when the T₁ and T₂-weighted MR pulse sequences are applied. The results may improve the efficacy of the new contrast agents, thus potential suitability for the early detection of cancerous tissues.

Adaptive Cone-Beam Scan Trajectories for Interventional Applications

Khanlian Chung, Alena-Kathrin Schnurr, Tom Russ, Dominik Bauer, Gordian Kabelitz, Barbara Waldkirch, Lothar Schad, Frank Zöllner

Heidelberg University, Computer Assisted Clinical Medicine, Project Group “Image-guided Interventions”, Mannheim, Germany

Motivation

Image-guidance is essential for many minimal-invasive interventions. Physicians rely on a variety of imaging modalities such as MRI or X-ray. To cancel the individual drawbacks of every modality, new interventional techniques aim for using multimodal data sets. The combined data sets are more comprehensive so that interventions can be conducted with a greater insight. In this context, we present two approaches for:

Exploiting multimodal data sets during interventions

Bypassing training data issues with machine learning methods to enhance multimodal data sets

Exploiting multimodal data sets during interventions

Registration algorithms can be used to access external (multimodal) data sets. At our project group, we are working on adaptive cone-beam scan trajectories. The imaging geometry is hereby calibrated by iteratively comparing an external data set with the current acquired projections. Consequently, the live acquired images are aligned with the external data set which can be directly used for image reconstruction. This allows the use of prior knowledge and to reduce dose exposure and reconstruction enhancements.

Bypassing training data issues with machine learning methods to enhance multimodal data sets

Recently, more machine learning methods like deep learning are used for medical image processing. The training of networks rely on good and extensive training data. This is especially in medicine a huge challenge. Firstly, data protection regulations make it difficult to publish or distribute medical data. Secondly, the few publicly available data sets lack number or expert annotations. One approach to bypass this inaccessibility is the use of synthetic data for the training procedure. In particular, synthetic data can overcome the issues of limited data set size and inaccurate annotations. We have developed a network to generate pseudo-realistic CT-images based on a perfectly annotated body model (XCAT-phantom). The synthetic data then can be used to train new networks for image processing tasks.
**MRI and MRS of dissolved hyperpolarized xenon-129**

Guilhem J. Collier

POLARIS, Academic Unit of Radiology, University of Sheffield, UK

**Introduction:** Hyperpolarized (HP) $^{129}$Xe allows functional imaging of ventilation and gas exchange in the lung. It is soluble in biological tissues and exhibits two distinct resonance peaks corresponding to $^{129}$Xe dissolved in lung tissue parenchyma/plasma (TP, ~197ppm from $^{129}$Xe gas) and red blood cells (RBC, ~217ppm). Spectroscopic measurement of $^{129}$Xe dissolved phase uptake such as the RBC to TP peaks ratio has been shown to be sensitive to lung disease [1]; in particular, interstitial lung diseases (ILD) where increased septal thickness results in slower and reduced gas transfer. Imaging dissolved-phase $^{129}$Xe has been difficult as it represents only 1-2% of the total magnetization and due to short relaxation times $T_2$ (~2ms). Short $T_E$ sequences can overcome this limitation, enabling simultaneous imaging of $^{129}$Xe in its gas and dissolved phase compartments [2, 3]. In this work, spectroscopic measurement of dissolved xenon was performed in healthy volunteers and patients with ILD and RBC/TP ratio was derived. Additionally, a novel Dixon/IDEAL imaging technique, combined with a 3D radial trajectory, was developed to obtain maps of gas, RBC and TP compartments and imaging results were compared with global spectroscopic measurement.

![Image](image_url)

**Figure:** Ratio maps of RBC/TP (top) and TP/GAS (bottom) in a healthy volunteer (left) and a subject with ILD (right) showing reduced gas transfer and increase dissolved xenon uptake in tissue plasma compartment.


**Translating Optical Molecular Imaging**

Jim Delikatny, Sofya Osharovich, Alejandro Arroyo, Andrea Guzman, Michael Hart and Anatoliy Popov

Department of Radiology, University of Pennsylvania, Philadelphia, PA, 19104, USA

Near-infrared (NIR) fluorescence optical imaging is a powerful and transformative technology that has enabled the routine assessment of biological function *in vivo* in small animals. Translation of optical imaging has proven to be difficult due to absorption and scattering of NIR light by biological tissue, limiting detection to phenomena within 1 cm of the surface. One emerging application of NIR imaging is in the surgical operating theatre, where systemically injected or topically applied NIR contrast agents are used for tumor detection, resulting in more complete tumor excision, delineation of tumor margins, and detection of micrometastases. Our group has been developing and implementing NIR fluorescent probes for assessment of tumor metabolism. We have synthesized a series of fluorescent contrast agents that can report directly on enzyme activity or expression, or on local tumor microenvironment. These compounds were created via conjugates to molecules of biological interest or through attachment to a backbone structure that allows activation through cleavage or rearrangement. One major focus has been development of Cerenkov probes for detection of tumor microenvironment pH and redox status. A second focus of the lab has been on fluorescent probes that report on lipid metabolism, including enzymes in the phosphatidylcholine cycle upregulated during tumor progression, such as choline kinase and phospholipases A2 and C. Our choline kinase sensors are being translated in a clinical trial to aid in the surgical tumor removal and detection of tumor margins in client-owned canines who present to the veterinary clinic with primary lung cancer.
Inhalable theranostic system for local tuberculosis treatment containing isoniazid loaded metal organic framework Fe-MIL-101-NH2

Gabriela Wyszogrodzka 1, Przemysław Dorozżyński 2, Weronika Strzempek 2, Piotr Kulinowski 4, Edyta Pesta 5, Władysław P. Węgiel 4, Barbara Gil 6, Elżbieta Menaszek 1 and Stefano Giovagnoli 7

1 Jagiellonian University Medical College, Faculty of Pharmacy, Department of Pharmacobiology, Medyńska 9, 30-068 Kraków, Poland, 2 Medical University of Warsaw, Department of Drug Technology and Pharmaceutical Biotechnology, Banacha 1, 02-097 Warszawa, Poland, 3 Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Kraków, Poland, 4 Pedagogical University of Cracow, Institute of Technology, Podchorąży 2, 30-084 Kraków, Poland, 5 Department of Pharmaceutical Analysis, Research Network Lukasiewicz - Pharmaceutical Research Institute, Rydygiera 8, 01-793 Warszawa, Poland, 6 Institute of Nuclear Physics, Polish Academy of Sciences, Department of Magnetic Resonance Imaging, Radzikowskiego 152, 31-342 Kraków, Poland, 7 Department of Pharmaceutical Sciences, via del Liceo 1, University of Perugia, Perugia, 06122, Italy

Pulmonary anti-tuberculosis drug delivery is the logical strategy for maintaining local therapeutically effective concentrations while avoiding massive systemic exposure and consequently reducing side effects. The theranostic approach to local tuberculosis treatment allows drug delivery and imaging of the lungs for a better control and personalization of antibiotic therapy.

For this reason, Metal-Organic Framework (MOF) Fe-MIL-101-NH2 nanoparticles were loaded with isoniazid. MOFs are porous materials whose structure is built by inorganic single ion or ion cluster nodes joined together by organic linkers. To optimize their functionality towards inhalation drug delivery a 2-factorial design of spray-drying with poly(lactide-co-glycolide) (PLGA) and leucine was employed. Essential functionality of the inhalation drug delivery system is determined powder aerodynamic properties and these properties were assessed using Twin Stage Impinger based on ‘emitted dose’ and ‘fine particle fraction’ parameters. In vitro drug release experiments were performed by the dialysis bag method and quantified by HPLC. Cell viability and amount of isoniazid accumulated in macrophages were assessed on murine macrophages RAW 246.9. Magnetic resonance imaging (MRI) contrast capabilities were tested on porous lung tissue phantom with three-dimensional ultrashort echo time imaging sequence (UTE). The phantom consisted of set of sponges separated by an impermeable barrier wetted with water MOF suspensions of MOF concentrations ranging from 0.03 to 1 mg/ml. Additionally the very preliminary study was conducted on lower pulmonary tract extracted from sacrificed rats.

The final product showed good aerodynamic properties. Optimized microparticles revealed modified drug release, with about 34 % and 67 % of drug release after 1 and 24 h, respectively. It also allowed easier uptake by macrophages in relation to raw isoniazid-MOF. Classical magnetic resonance image intensity contrast could not be obtained due to highly inhomogeneous phantom/lower respiratory tract structure. However, normalized image intensity histogram analysis allowed capturing differences (contrast) between pure water and
various concentrations of MOF in suspension e.g. in terms of histogram amplitude and intensity range (upper intercept).

As a result of the study, starting from raw MOF nanoparticles, a fully functional inhalable theranostic system (microparticles) with a potential application in personalized tuberculosis pulmonary therapy was developed.

**Multimodal imaging for preclinical evaluation of tumors induced by renal cell carcinoma stem-like cells**

Michał Fiedorowicz¹, Mohhamed I. Khan², Damian Strzememki¹, Jarosław Orzel¹,³, Marlena Welniak-Kaminska¹, Agnieszka Sobiborowicz⁴,⁵, Michał Wieteska¹,³, Zbigniew Rogulski Z.⁶, Łukasz Cheda¹, Weronika Wargocka⁶, Krzysztof Kilian¹, Cezary Szczylik², Anna M. Czarnecka¹,²,⁵ ¹Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland. ²Department of Oncology with Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland. ³Faculty of Electronics and Information Technology, Warsaw University of Technology, Warsaw, Poland. ⁴First Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland. ⁵Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Warsaw, Poland. ⁶Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland. ⁷Heavy Ion Laboratory, Faculty of Physics, University of Warsaw, Warsaw, Poland

Renal cell carcinoma (RCC) and in particular its most common type clear cell RCC (ccRCC) is associated with generally poor prognosis since it is largely resistant to chemo- and radiotherapy. Development of tumor, disease progression, aggressiveness, metastasis and drug resistance seems to be associated with presence of a subpopulation of cancer cells called cancer stem cells or tumor initiating cells (CSCs/TICs). However, tumorigenic potential of CSCs/TICs isolated from established RCC cell lines is unknown and multimodal imaging would enable in-depth characterization of tumors induced by these small subpopulations of cancer cells. The aim of our study was to characterize tumors induced by potential stem-like cells isolated from established human RCC cell line. Subpopulations of CD105+, CD105−, CD44+ and CD44− as well as CD44+/CD105− CD44+/CD105+ and CD44−/CD105− cells were isolated by FACS from Caki-1 RCC cell line. Sorted cells were injected subcutaneously into NOD SCID mice and tumor growth was monitored with MRI/MRS and PET/CT. Tumor growth was observed after implantation of CD105+, CD105−, CD44+ and CD44− as well as CD44+/CD105− CD44+/CD105+ and CD44−/CD105+ cells were isolated by FACS from Caki-1 RCC cell line. Sorted cells were injected subcutaneously into NOD SCID mice and tumor growth was monitored with MRI/MRS and PET/CT. Tumor growth was observed after implantation of CD105+, CD105−, CD44+ and CD44− as well as CD44+/CD105− CD44+/CD105+ and CD44−/CD105− but not CD105− or CD44+/CD105−. Implantation of CD44+/CD105− cells induced tumors that were characterized by longer T1 and distinct metabolic pattern than other tumors. All the tumors were characterized by low uptake of [18F]FDG. We have demonstrated that multimodal imaging approach allow characterization of tumors induced by stem-like RCC cells and indicate that co-expression of multiple markers is be crucial to define cancer stem cell signature.

The study was supported by Polish National Science Centre grant (DEC-2014/13/B/NZ1/04010). Project was carried out with the use of CePT infrastructure financed by the European Union – the European Regional Development Fund in the Operational Programme “Innovative Economy” for 2007–2013.

**Structural Gray Matter Abnormalities in Morbidly Obese Patients with Type 2 Diabetes and their Recovery with Intragastric Balloon Treatment**

Stefan P. Gazdzinski, Andrzej Gazdziński, Aleksandra Mojkowska, Agata Gażdzińska, Maria Gorycka, and Ryszard Pachó

Military Institute of Aviation Medicine, Warsaw, Poland

**Background:** Obesity is associated with structural brain abnormalities in both otherwise healthy individuals and in morbidly obese patients. In particular, in multiple studies type 2 diabetes (T2D) was associated with gray matter volume reductions. However, no studies evaluated changes in brain structure induced by weight loss and/or T2D remission. Insertion of intra-gastric balloon (IGB) in the stomach of the patient for six months is a bariatric treatment leading to massive weight loss (up to 40 kg) over six months and often to remission of T2D. We hypothesized lower cortical thickness and less gray matter volume before IGB insertion than in controls. We also evaluated, if these measures change with weigh loss induced by IGB treatment.

**Methods:** The study was performed among 23 morbidly obese patients (average weight:
The control group (CON) consisted of nine healthy volunteers with BMI in the normal range. The data were processed with FreeSurfer 5.3. Mean cortical thickness was compared between groups and the changes in the above parameters during treatment were evaluated. White matter abnormalities were scored on FLAIR images (T1/TE/TR=2470.21/150.28/3000ms, 0.4688x0.4688x4mm³) by an experienced radiologist using Fazekas scale.

**Results:** The patients had, in general, a very small number of white matter lesions. The frequencies of no lesion/punctate foci/ early confluent lesions were not different between all groups $\chi^2(4)=4.59$, p=0.30. The data processed with Freesurfer required extensive manual corrections due to large amounts of subcutaneous fat around the brains of the morbidly obese patients. Compared to CON, OD had 3.7% and 4.1% lower cortical thickness in the left and right hemispheres, respectively (p<0.02). OB were not different from CON (p>0.33). Over the treatment, in the OD, the mean cortical thickness increased by 2.5% in the left hemisphere (p=0.02) and 2.0% in the right hemisphere (p=0.04).

**Discussion:** The results suggest that morbid obesity together with type 2 diabetes leads to brain atrophy at middle age, consistent with other studies. Surprisingly, we did not see any abnormalities in the patients without type 2 diabetes, suggesting that co-morbid type 2 diabetes, not just being obese, is associated with brain atrophy in middle age. This conclusion is supported by increases in gray matter thickness in the OD group during the treatment, likely due to remission of symptoms of type 2 diabetes.

This study was supported by the Polish National Science Centre: grant 2013/09/B/NZ7/03763.

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**Low Field NMR Relaxometry of poly(sodium acrylate)/sodium silicate hydrogels**

I. Habina-Skrzyzniarz, A. T. Krzyżak, J. Mastalska-Popławska, A. Stempkowska

1AGH University of Science and Technology, Faculty of Geology, Geophysics and Environmental Protection, Krakow, Poland; 2AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Krakow, Poland; 3AGH University of Science and Technology, Faculty of Mining and Geoengineering, Krakow, Poland

Of the many materials used to build MRI phantoms, hydrogels occupy a significant place [1]. In many of them, the addition of paramagnetic ions allows to obtain appropriate relaxing properties. It is worth noting, however, that these characteristics depend also on gel concentration and porous structure of the samples [2].

In the presented work samples of poly(sodium acrylate)/sodium silicate hydrogels [3] were examined using low field NMR systems: Rock Core Analyzer (RCA) operating at magnetic field of 0.05 T and Mobile Universal Surface Explorer (MoUSE) at 0.5 T and with magnetic field gradient of 24 T/m. Hydrogel samples were a mixture of water glass R-145 and poly(sodium acrylate) (pANa, 20 wt%), prepared in a weight ratio of 1:10 to 7:1. T1 and T2 relaxation times were recorded using Inversion Recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) sequences, respectively. The obtained data were developed using the inverse Laplace transform (ILT) and thus the relaxation time distributions were obtained from which the location of the main component: T2,max and the geometric mean: T2,gm were determined. Two-dimensional IR-T1-T2-CPMG measurements were also recorded for the selected samples. In our study the dependence of T1,2,max and T1,2,gm and 1/T1,2,max and 1/T1,2,gm values was analyzed in relation to the concentration of sodium ions [Na⁺]. Diffusion weighted T2 experiments, which were performed on the NMR MoUSE system with a set of different echo times: 35.5, 60 and 100 μs, revealed additional intricate characteristics of the examined materials. For the relationship of T1,2([Na⁺]) a shortening of the relaxation time was observed. High agreement was obtained between for T1- and T2 values, in most cases even overlapping on the obtained charts. The character of relaxivities changes, 1/T1,2([Na⁺]), for hydrogel samples was analyzed and compared with pure acrylates and salt solutions, revealing more complex nature of the mixtures samples, for which this relation was far from the linear and the character of the data was best represented by an exponential or polynomial function of the 3rd degree.
Fast MRI with Extreme Gradients
Franciszek Hennel, Bertram Wilm, Manuela Roesler, Benjamin Dietrich, Markus Weiger and Klaas P. Pruessmann

Institute for Biomedical Engineering, ETH Zurich and University of Zurich, Switzerland

Introduction
Echo-Planar Imaging (EPI) and other ultra-fast MRI methods require high and fast-switching gradients to produce high-resolution images without distortions and blur. We report first results obtained with EPI using a recently developed insert coil generating 100 or 200 mT/m maximum gradient dependent on amplifier configuration. The key elements of this design are the limited spatial range of the gradient field, which allows its full usage for human head and limbs without peripheral nerve stimulation, and a low inductance allowing the slew rate of 1200 T/m/s with the 100 mT/m configuration. It appears that using EPI with this unprecedented gradient strength requires special means to deal with the “Nyquist ghost” caused by high-order eddy currents. The original solution presented here is based on dynamic field monitoring. All experiments were carried out on a 3T Achieva scanner (Philips Healthcare, The Netherlands) with the gradient insert mentioned above connected to the system’s standard amplifiers. A custom-made transmit-receive 8-channel array was used. The reconstruction involved a regularized least-squares inversion of the high-order encoding matrix including gradient- and sensitivity encoding, B0 map, as well as the eddy-current-related phase terms measured with the field camera at multiple positions. Concomitant field contributions were also included based on transverse field maps derived from the coil design. The high-order reconstruction procedure, highly time-consuming in its full form, was significantly accelerated by Fourier-transforming each echo and making 1-dimensional algebraic inversions in the hybrid space, as recently proposed for de-ghosting and unwarping of multiband EPI.

Echo-planar images of the human head acquired at 3T with the gradient insert show a very low level of distortion, even at high resolution (1mm in-plane) without the need of high parallel acceleration factors. This is demonstrated by a comparison of the data acquired for the same volunteer with the high performance gradient insert (left) and with the standard whole body gradients of the same system (30mT/m, right), both fully sampled (no acceleration).

References
### The neurochemical profile of anorexic rats

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<thead>
<tr>
<th>1Krzysztof Jasiński, 2Kamil Skowron, 3Magdalena Kurnik-Łucka, 4Władysław P. Węglarz, 5Krzysztof Gil</th>
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<td>1Institute of Nuclear Physics PAN, Kraków, Poland, 2Department of Pathophysiology, Jagiellonian University Medical College, Krakow, Poland</td>
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Eating disorders are defined as a group of mental illnesses marked by severe disturbances in eating behaviors, distress, and excessive preoccupation about weight and body shape. **Anorexia nervosa** (AN) is an eating disorder characterized by the desire to lose bodyweight or to maintain body weight at a lower level than normal. The treatment and recovery is often a long and complex process requiring a robust multidisciplinary team approach, however, it is hampered by a high relapse rate. Only about half of the patients recover, one third partially recover but continues to have symptoms and 20% develop anorexia nervosa as a chronic disorder. AN has a considerable mortality rate, the highest of any psychiatric disorder and is increasingly recognized as an important cause of morbidity and mortality in young people. Although AN is clinically well-characterized, the pathogenesis underlying the disease is still not well established. Moreover, no specific pharmacological treatment is available.

We used an animal model of AN induced by restricted feeding and voluntary physical activity: Activity-Based Anorexia (ABA) to study neurochemical changes in rat hypothalamus during pathology progression. Female Wistar rats were divided into three groups: control, ABA and ABA-KISS injected daily with Kisspeptin-10. During experiment body weight, food intake and running wheel activity were monitored. Localized MRS was performed when animals lost approximately 25% of initial body weight. MRS measurements were performed on a 9.4T Bruker BioSpec 94/20 MR scanner with a transmit volume coil and a rat head receive RF surface coil. Based on anatomical $T_2$ weighted multi-slice images, 1$^H$-MRS 3x3x3mm voxel was positioned in the hypothalamus. For acquisition respiratory-gated PRESS sequence with 14 ms echo time and 1600 averages was used. MRS data acquisition took 50 to 70 minutes depending on animal breath rate. Obtained data were fit with LCModel. Administration of Kisspeptine changed the pattern of weight loss between ABA and ABA-KISS groups. Moreover there was significantly lower body weight loss in ABA-KISS group comparing to ABA group. A statistically significant difference between the control group and the ABA group ($p <0.05$) is observed at the concentration of phosphocreatine (PCr), GABA and glutathione (GSH). The concentrations of both PCr and GSH increased in the ABA group, while the GABA level dropped drastically.

Although there were no statistically significant differences in metabolite concentrations between groups ABA and ABA-KISS, it seems that kisspeptin reverses the effects of anorexia for some metabolites.

ABA rat model combining restricted feeding and physical activity is useful for the study of pathophysiological alterations and pharmacological treatment response.

### Ultra-high field MR imaging in preclinical oncology

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<thead>
<tr>
<th>Dorde Komljenovic and Mark E. Ladd</th>
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<td>Division of Medical Physics in Radiology, German Cancer Research Center, Heidelberg, Germany</td>
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The current RECIST classification (Response Evaluation Criteria In Solid Tumors) used to assess tumor burden by recurrent imaging/measurement of defined target and non-target tumor lesions aims to provide objective assessment of treatment response in a variety of solid tumors and is primarily based on tumor size. Rapid development of diagnostic imaging methods, however, has resulted in the identification of various functional parameters that may provide additional valuable information regarding the course of the tumor disease. Such information may enable the assessment of therapy response even before macroscopically observable tumor changes occur.

Several of these functional parameters can be captured using magnetic resonance imaging, one of the most commonly used imaging techniques in clinical oncology in the western world, where human imagers currently mainly operate at moderate magnetic field strengths of 1.5 T and 3 T. In experimental oncology settings, small-animal imaging is a widely used approach. Nowadays, the vast majority of small animals used for imaging applications in preclinical oncology are mice and rats due to availability, low cost, possibility for genetic engineering, and suitability to serve as models for a variety of tumor entities relevant in human pathology. Such well-defined models may simplify the transfer of knowledge from experimental to clinical conditions. To acquire similar anatomical structures as in humans, resolutions need to be
significantly increased when small-animal imaging is conducted. Current standard magnetic field strengths in small-animal MRI are 7 T and 9.4 T. In parallel with the increase in the strength of the main magnet in preclinical scanners, substantial efforts are taken to improve coil set-ups, including cryo-cooled coils, to further enhance sensitivity. To address specific applications demanding superior sensitivity, some preclinical scanners now operate at field strengths ranging from 11.7 T to 21 T.

Possible applications of MRI in preclinical oncology are constantly expanding. MRI offers superior spatial and temporal resolution and does not require radioactivity or gene transfer into cells. Further, it is generally not limited by tissue depth and considers both endogenous tissue signal as well as exogenously applied contrast agents. As neo-angiogenesis is one of the hallmarks of cancer, characterization of tumor vasculature using contrast-enhanced MRI techniques continues to be extensively studied. Additionally, state-of-the-art preclinical MRI scanners are increasingly available as hybrid modalities (e.g. combining the complementarity of nuclear medicine methods) to merge morphological and functional read-outs and, therefore, provide a significant gain in the acquired information. These developments have led to intense research into multifunctional probes applicable in preclinical imaging platforms, often engineered to contain a therapeutic component and therefore fulfilling the prerequisites for a theranostic approach.

### Quantitative MRI in clinically relevant models of spinal cord injury

Andrew Yung, Stephen Mattucci, Jie Liu, Caron Fournier, Wolfram Tetzlaff, Thomas Oxland, Piotr Kozlowski

**UBC MRI Research Centre, ICORD, Vancouver, BC, Canada**

**Introduction**

The type of initial mechanical injury to the cord has been shown to influence the degree of neurological dysfunction throughout the primary and secondary phases of spinal cord injury (SCI), and is therefore important to identify and track in order to guide treatment. Imaging techniques that are sensitive to damage in the white matter microstructure are attractive in characterizing the injury mechanism. Here, we investigate the ability of diffusion tensor imaging (DTI) to distinguish between three experimental rat models of spinal cord injury mechanisms – contusion, dislocation, and distraction, at various time points after injury.

**Materials and methods**

Sprague-Dawley rats (male, 300g) were injured at the C5/C6 level with the UBC multi-mechanism SCI apparatus¹. Three clinically relevant injury models were applied: contusion (direct compressive impact from an actuator tip), dislocation (dorso-ventral shearing of the spinal cord), and distraction (stretching of cord along the rostro-caudal axis). Cords were perfusion-fixed and extracted at 3 hours, 24 hours and 7 days post-injury (N=9-12 for a particular time point and injury model). DTI-EPI was acquired with a Bruker 7T preclinical scanner at 11 axial slices centered around the lesion epicentre at 50 μm in-plane resolution and 1mm slice thickness (TE/TR =38.61/2750 ms, 8 shots, 6 directions, b=1000 s/mm², NA=18, 128x128, FOV=6.4mm, 11 slices). White Matter Damage (WMD) and Damage Occurrence Ratio (DRO) maps, generated from DTI metrics, were used to compare spatial patterns of WMD between injury models and times post-injury².

**Results and Discussion**

Damage classification was most robust using thresholds in the longitudinal diffusivity, which supports previous studies that show that longitudinal diffusivity is the most robust DTI metric in depicting damage in SCI. Furthermore, the spatial damage patterns from all subjects in the same group were consolidated into a "damage occurrence ratio map", which illustrates an average damage shape that characterizes the injury mechanism.

Our analysis has yielded a dataset which highlights the differences in injury pattern due to the initial mode of mechanical injury. For example, contusion produced an initial injury that emanated radially outward from the central canal, with subsequent damage along the caudal corticospinal tract and rostral gracile fasciculus; dislocation injuries showed a high level of involvement in the lateral and ventral white matter which became less apparent by 7 days post-injury, and distraction injuries were found to be less focal and more distributed rostro-caudally.

This work represents a first step in adopting the use of the primary injury mechanism as a
clinical prognostic factor in SCI, which may help to inform the trialing of existing neuroprotective treatment candidates, the development of new therapies as well as personalize the management of SCI for the individual patient.

Acknowledgment
This work was supported by the Wings for Life Spinal Cord Research Foundation.

References:
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Practical aspects of applying the Generalized Stejskal-Tanner equation to MRI

Artur T. Krzyżak, Rafał Obuchowicz

AGH University of Science and Technology, Faculty of Geology, Geophysics and Environmental Protection, Krakow, Poland

Introduction. The issue of imaging coefficients and diffusion tensor in MRI experiments using the spatial distribution of matrix-b was proposed by Bammer in 2002 [1]. This approach is based on the calculation of the so-called coil tensor, applying data from the manufacturer about the heterogeneity of the distribution of gradients generated by gradient coils. Another approach from 2008 is based on using the diffusion tensor pattern to calculate the spatial distribution of the matrix-b [2]. In this case, we take into account all possible components that generate heterogeneity of the magnetic field gradients, without the need for any knowledge of the MR sequence parameters. In 2015 [3] an example of application for the pre-clinical 9.4T system was published and at the same time officially named BSD-DTI (B-matrix Spatial Diffusion in MRI). Over the past few years, several papers have also been published that used the spatial distribution of the matrix-b [5-8]. It should be noted that all above experimental works contradicted the assumptions of the Stejskal-Tanner equation usually used to calculate the diffusion tensor. Its basic assumption is the stability of the magnetic field gradient distribution in space. This contradiction was resolved last year by deriving the equation Generalized Stejskal-Tanner equation $\ln \left( \frac{A(2\tau)}{A(0)} \right) = -b \cdot D$, valid for any distribution of magnetic field gradients [4].

Materials and methods. The BSD-DTI method was used on clinical systems 1.5 and 3T. Calculations of diffusion tensor were carried out for the brain, in addition to heart and leg muscles, in volunteer and patient studies.

Results and conclusions. In the case of testing the diffusion tensor distribution for isotropic phantoms, we observe from 2-3 to even several times the distribution improvement compared to the classical approach [8]. For images after fiber-tracking, in particular for the brain, there is also clearly noticeable improvement in the visualization of nerve fibers.

Conclusions. The GS-T equation allows for the most accurate description of diffusion coefficients in MR imaging. The use of the BSD-DTI technique to obtain spatial b-matrix distribution allows to achieve much better visualization effects of fiber-tracts compared to the standard approach.

References
# Acknowledgments
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## Hydration of compressed sodium alginate pharmaceutical matrices - spatiotemporal analysis

Ewelina Juszczyk 1, Piotr Kulinowski 2*, Encarna García-Montoya 3, Dorota Majda 4, Władysław P. Węglarz 5, Przemysław Dorożyński 6

1 Celon Pharma SA, Ogrodowa 2A, 05-092 Łomianki / Kiełpin, Poland, 2 Pedagogical University of Cracow, Institute of Technology, Podchorążych 2, 30-084 Kraków, Poland, 3 University of Barcelona, Faculty of Pharmacy, Pharmacy and Pharmaceutical Technology Department, Joan XXIII 27-32, CP 08028 Barcelona, Spain, 4 Jagiellonian University, Faculty of Chemistry, Department of Chemical Technology, Gronostajowa 2, 30-387 Kraków, Poland, 5 Institute of Nuclear Physics, Polish Academy of Sciences, Department of Magnetic Resonance Imaging, Radzikowskiego 152, 31-342 Kraków, Poland, 6 Medical University of Warsaw, Department of Drug Technology and Pharmaceutical Biotechnology, Banacha 1, 02-097 Warszawa, Poland

The aim of the study was to analyze the main aspects of processes occurring in polymeric matrix tablets during hydration:

- concentration and distribution of water in the matrix,
- molecular mobility of water,
- interaction of water with polymeric material.

For the purpose of the study the matrix tablets containing pure sodium alginate (Protanal LF 240 D, FMC Biopolymers, USA) or mixtures of sodium alginate with salicylic acid or sodium salicylate (1:1) were prepared. The hydration was carried out in distilled water and hydrochloric acid solution (0.1m/L).

The implementation of the assumed goals required the development of a methodology allowing spatiotemporal characterization of selected physicochemical parameters of the matrix tablets during hydration.

The matrix of tablets were hydrated unilaterally in specially designed holder. In particular time points (1, 2, 3, 4 hours) the hydrated tablets were cut into 1 mm slices. The samples taken form each slice were tested using Karl-Fischer titration method (Metrohm, USA) and differential scanning calorimetry (DSC) (Mettler-Toledo, Switzerland). The MRI studies (Bruker Biospin 9.4T, Germany) were carried out using multi-spin-echo (MSME) and ultrashort echo time (UTE) imaging sequences.

Water distribution within the matrix tablets with sodium alginate was spatially and temporally determined. The distribution of water and its penetration into matrix tablets depended on the medium in which hydration was carried out. The presence and physico-chemical properties of drug substance influenced the water transport within the matrix tablets: sodium salicylate favored water penetration into the matrix, salicylic acid did not affected the degree of tablet's hydration comparing with pure sodium alginate matrix. DSC studies showed that water penetration into the polymer matrix was associated with the interaction between polymer and water molecules, the molecular properties of the system depended on the nature of the interaction between sodium alginate and the solvent.

Regarding MRI results, highly hydrated matrix layers were characterized by long (~200 ms) T2 decay constants, while for deeper slices (less hydrated) inside the sodium alginate matrix tablets, T2 was shorter than 3.5 ms and it was not possible to measure it properly with MSME sequence regardless of hydration time. In the tablets containing mixture of salicylic acid and sodium alginate similar observations were made. In the case of tablets with sodium salicylate the water signal was acquired from the entire volume of the tablet starting from 2 hours of hydration.

## Accelerated MRI for robust quantification of cardiac perfusion in small animals

Grzegorz Kwiatkowski, Sebastian Kozerke

Institute for Biomedical Engineering, University and ETH Zurich, Zurich, Switzerland

Assessment of myocardial perfusion is considered to be of significant value for the evaluation of patient outcome. Parallel to progress in perfusion imaging in humans, translation of efficient and reproducible protocols for quantitative estimation of myocardial perfusion in animal models has become highly desirable, especially in the context of a multiparametric characterization of myocardial tissue. Currently, myocardial perfusion in rodents is typically estimated using arterial spin labelling (ASL), which suffers from long acquisition times (15–60 min) and as such is prone to variations in both heart and respiratory rate. On the other
hand, dynamic contrast-enhanced (DCE) perfusion MRI has been used widely in clinical practice relying on dynamical MR imaging immediately after administration of a bolus of a suitable contrast agent, with a typical acquisition time of less than 2 min. However, the subsequent translation of DCE to studies in rodents has been challenging due to high demands on the imaging sequence. Fast heart rates (400–550 bpm) and the fast breathing pace (60–120 bpm) require high temporal resolution, typically of less than 200 ms. This communication will focus on several aspects of quantitative cardiac perfusion estimation in small animals (mice and rats). The requirements of the acquisition sequence will be discussed with respect to the practical aspects of measurements in small animals at high magnetic fields, including data reconstruction and perfusion quantification. In particular, the application of different acceleration strategies will be discussed. Examples of cardiac contrast enhanced first pass perfusion in mice and rats acquired with an accelerated, dual-contrast gradient echo sequence will be shown.

**Figure 1.** A) A schematic diagram of the dual-contrast gradient echo sequence. A single dynamics is segmented over two consecutive heart beats. B) The ky-t undersampling pattern used for data acquisition (white – acquired, black – skipped). C) The time series of cardiac short axis images recorded during first pass of the gadolinium contrast agent.


### Visualization of the distribution of 19F nuclei in Nafion-loaded theranostic nanocapsules with the 3D Ultra-Short Echo Time pulse sequence at 9.4T

Natalia Łopuszyńska1, Krzysztof Jasiński1, Krzysztof Szczepanowicz2, Piotr Warszyński2, Władysław P. Węglarz1

1 Department of Magnetic Resonance Imaging, Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland, 2 Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Kraków, Poland

The term ‘theranostic’ relates to an object that combines two modalities: therapy and diagnostic imaging. Utilization of such materials instead of two separate substances for each application introduces a possibility of overcoming differences in biodistribution and selectivity of administered therapeutic and imaging agents [1]. One of the available imaging modalities is a heteronuclear magnetic resonance imaging, which allows to obtain a spatial distribution of X-nucleus imposed on a conventional 1H image.

As one of possible theranostic systems, nanocapsules with molecules containing 19F atoms as MRI markers were investigated. It is desirable for the 19F MRI detectable tracer to be characterized by the high fluorine content, simple 19FNMR spectrum, short T1 and long T2. However, mainly due to advances in MRI instrumentation, and a various pulse sequences development, the range of fluorine compounds that can be used as markers can be expanded. Here we present the application of the 3D UTE sequence at 9.4 T for imaging of a compound with a complex 19FNMR spectrum and very short (in range of single milliseconds) T2.
As an example of a molecule with high fluorine content that can be effectively embedded in nanocapsules shell, the Nafion polymer was chosen. The $^{19}$F MR spectroscopy as well as $^1$H and $^{19}$F 3DUTE imaging were performed at the 9.4 T Bruker Biospec 94/20 research MRI scanner with in-house built small transmit-receive RF ribbon solenoid coil, which can be tuned either to $^1$H or $^{19}$F resonant frequency. Paravision 5.1 and Topspin 2.0 software were used for MR imaging and spectroscopy as well as relaxation times measurements. First, the MR spectroscopy (with a 17 μs 90° pulse and SW: 59.52 kHz) and T1 and T2 measurements were performed in order to choose the peak with the highest NMR signal. For T1 measurements in TopSpin 2.0, pseudo-2D version of a standard Inversion Recovery experiment was used while T2 values for individual resonances were estimated by calculating the reciprocal of their full width at half maximum (FWHM) values.

The 3DUTE sequence was first applied to Nafion solutions of a different concentration. Imaging parameters were as follow: TR: 8 ms, TE: 0.16 ms, RF pulse BW: 4.27 kHz, FOV: 4.0x4.0x4.0cm, FA: 6.4°. For $^1$H images: MTX: 128x128x128 and NA: 1, resulting in total acquisition time $\sim$6m 51s, while for $^{19}$F images: MTX: 32x32x32, NA: 64 and acquisition time: $\sim$27m 6s. Subsequently, the imaging of a phantom containing theranostic carriers was performed with imaging parameters as before, but with two different numbers of averages for $^{19}$F images: NA: 256 or 500, resulting in acq time: $\sim$1h 48min 24s and $\sim$3h 31m 44s respectively.

We obtained a full MR spectrum of Nafion with five resonances. These signals were assigned to chemical groups containing equivalent fluorine nuclei. T1 and T2 relaxation times of individual groups were measured and an estimation of the number of $^{19}$F nuclei for each resonance was made. For all imaging experiments, a peak at +42 ppm was chosen due relatively high MR signal and being well separated from the other resonances. We managed to acquire $^{19}$F 3DUTE images of all Nafion dilutions and of the sample containing theranostic nanocapsules. For the lowest analyzed concentration, imaging within a reasonable acquisition time (< 30 minutes) produced an image with SNR = 5, which was sufficient to reliably determine the spatial distribution of $^{19}$F nuclei in a sample. The $^{19}$F 3DUTE imaging of theranostic nanocapsules with 256 averages gave SNR = 12, and increasing number of averages to 500 (acq. Time: $\sim$3h 32 min) caused improvement in SNR to 20. Therefore we showed that by adjusting imaging parameters of appropriate sequence even a compound with less preferable characteristics can be used as a MRI-detectable tracer, which creates an opportunity of designing an efficient process of the production of theranostatic nanocarriers for drug delivery.


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Natalia Łopuszyńska acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16

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**Investigating the entire NMR signal from brain**

*Alex MacKay*

Dept of Radiology / Dept of Physics & Astronomy, University of British Columbia, Vancouver, Canada

The human brain is a highly complex organ and the proton NMR signal from brain has contributions from spins in widely different micro-environments. In this presentation, we investigate the $T_1$ and $T_2$ behaviour of spins in the various micro-environments in brain.

From the NMR point of view one can separate the NMR signal from brain into four components: non-aqueous protons in myelin, non-aqueous protons not in myelin, water trapped between myelin bilayers and water in the intra- and extra-cellular spaces [1]. Practically all non-aqueous components in brain exhibit dipolar broadened lineshapes, their signal decays to zero in less than 100 μs and their signal is inaccessible to conventional MRI. Practically all of the water in brain has $T_2$ times greater than 10 ms and is the sole direct contributor to conventional MRI. However the MR signal from water, and consequently the MRI contrast, is substantially influenced by non-aqueous protons. Furthermore, this influence of the non-aqueous protons on MR contrast depends upon the MR sequence. Recent work has demonstrated that measuring brain $T_1$ using different MR sequences results in different $T_1$ time estimates [2,3].
It is well known that $T_2$ relaxation in brain is multi-component with separate contributions from myelin water and water in the intra- and extra-cellular spaces [4]. Investigators have also found that $T_1$ relaxation is also multi-component; additional components have been ascribed to myelin water and to cross-relaxation with non-aqueous protons [5].

This presentation reports on an ex-vivo study on bovine brain using a spectrometer capable of measuring entire proton NMR signal and the aforementioned four pool model. The results demonstrate how the signal from water, i.e. the MRI signal, is influenced by the magnetization of the non-aqueous signal and explain how the results of $T_1$ measurements depend upon the measurement technique.


**PROspective Baseline Enhancement (PROBE): An Efficient and Versatile Editing Scheme for CEST**

**Harald E. Möller, Tobias Lenich, André Pampel**

Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

In chemical exchange saturation transfer (CEST) experiments in vivo, the presence of multiple unspecific contributions—for example, from macromolecules through magnetization transfer (MT)—results in a complex background that confounds the detection of the metabolite peaks of interest. Direct water saturation (DWS) will further distort the baseline. To account for such contributions, we have developed a method dubbed PROspective Baseline Enhancement. It compensates for intermingled background effects in $Z$-spectra and achieves sensitivity enhancement of peaks related to CEST or Nuclear Overhauser Effects (NOE). The pulse sequence-specific background compensation is achieved through variation of the pulsed saturation power, $\omega_{1,max}$, as a function of the chemical shift, $\delta$. Following a “scout acquisition” of a standard $Z$-spectrum as a separate experiment, the background is modeled through an appropriate spin system. Subsequently, an optimization procedure yields $\omega_{1,max}(\delta)$ to obtain a flat baseline. Contributions from metabolites not considered in the optimization procedure (in particular, those from the compounds of interest) are enhanced as distinct perturbations to the baseline. Artifacts due to local variations of the static field, $B_0$, and the radiofrequency field, $B_1^+$, can be straightforwardly corrected based on separately acquired field maps and entries from a dictionary.

As proof of concept, measurements of lactate in the presence of cross-linked bovine serum albumin (BSA) were performed at 7 T. Further explorative experiments included human subjects at 3 T. Nuisance contributions from DWS, macromolecular MT, and exchanging background protons were successfully removed from the $Z$-spectrum in phantoms and in brain tissue. The lactate methyl, methine, and hydroxyl peaks were readily observable in vitro. The peak areas correlated linearly with known concentrations.

Reference:

**Self-Gated Cardiac PET/MRI**

**Arno Nauerth**

Bruker BioSpin MRI GmbH, Ettlingen, Germany

IntraGate, a Self-Gated cardiac MRI technique, derives the cardiac and respiratory cycles
from so-called navigator scans which are acquired concomitantly to the MRI imaging sequence. Cardiac cine movies are retrospectively calculated according to the cycles derived therefrom. Thus, no ECG electrodes are required, which significantly simplifies the animal setup. IntraGate offers many other unique qualities: (i) the sequence is running in a steady state (ii) the full cardiac cycle is reconstructed, and (iii) it allows applications on moving objects where conventional gating & triggering simply fail.

This technique has been extended to multi-modal system like PET-MR scanners where both modalities run in parallel.

In this talk we introduce Self-Gated cardiac imaging on MR scanners equipped with a PET insert where the cardiac and respiratory signal is derived from the MR scanner and where this information is used to retrospectively reconstruct synchronous cardiac cines of the simultaneously acquired PET-and MR experiment. It is also shown how this technique can be applied to multiple animals.

### Progress in Fluorine (\(^{19}\)F) Magnetic Resonance: Technical Developments and Application in Disease Models

**Thoralf Niendorf**

Berlin Ultrahigh Field Facility (B.U.F.F.), Max-Delbrueck Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

The development of ultrahigh field magnetic resonance (UHF-MR) is moving forward at an amazing speed that is breaking through technical barriers almost as fast as they appear. UHF-MR has a staggering number of potential uses in neuroscience, neurology, neuroradiology and other related fields of preclinical research and clinical science.

Fluorine-19 (\(^{19}\)F) Magnetic Resonance (MR) methods have found their application in a wide range of biomedical research areas. One branch of research is tracking of cells, including inflammatory cells, in vivo with the help of fluorine \(^{19}\)F nanoparticles (NPs). Due to the absence of organic \(^{19}\)F in living organisms, the acquired \(^{19}\)F MR images are free from background signal, such that \(^{19}\)F/\(^{1}\)H MRI is able to localize labelled cells in vivo with complete signal selectivity and specificity. The possibility of quantifying inflammatory cells by \(^{19}\)F MR spectroscopy is another advantage, allowing a quantitative assessment of inflammation and of anti-inflammatory strategies. However, the usefulness of \(^{19}\)F MR to a wide range of biomedical imaging applications is hampered by the low availability of \(^{19}\)F spins in the living organism. This is compounded by the fact that the signal sensitivity of current state-of-the-art MR equipment remains limited, making \(^{19}\)F MR measurements of fluorine compounds present at low concentrations an extremely challenging task. To this end this presentation documents advances and progress of \(^{19}\)F UHF-MR with the goal to engage the interest of clinical adopters, basic scientists, engineers, and translational researchers from many areas. To meet this goal the traits, challenges and opportunities of in vivo \(^{19}\)F UHF-MRI will be surveyed. The considerations run from technical advances to early applications in preclinical research and clinical science. Examples for strategies that enhance signal-to-noise efficiency, e.g. more sensitive hardware including cryogenically radiofrequency probes and more time efficient pulse sequences are demonstrated. Digital signal processing, particularly compressed sensing (CS), is another new avenue to boost detection performance for probing \(^{19}\)F in vivo.

A concluding section ventures a glance beyond the horizon including explorations into Extreme Field MR (EF-MR) which \(^{19}\)F MR at 21 Tesla, which is an important leap of the imagination because it aims to fill a crucial “resolution gap” in our understanding of biology. It is the speakers hope that this presentation will convey the seeds of this vision and inspire the audience to become pioneers in these amazingly promising areas of biomedical research.

### Studies using microCT conducted to analyze degenerative changes of the hip and knee joints

**Anna Nikodem**
Preclinical ultra-high field MRI

Claudia Oerther

Bruker BioSpin MRI GmbH, Ettlingen, Germany

Currently, the majority of clinical MRI systems operate at moderate field strengths of 1.5 Tesla and 3 Tesla. For small animal imaging, resolutions need to be significantly increased in order to visualize similar structures as in humans. Since the sensitivity increases with the field strength, field strengths of 7 Tesla and 9.4 Tesla are therefore standard in the preclinical field. Beyond this, preclinical UHF systems ranging from 11.7 Tesla to 21 Tesla address specific applications, which demand the highest sensitivity. Even the most demanding applications become feasible when ultra high field strengths are combined with optimal coil setups, such as receive-only arrays, the sensitivity of which increases super-linearly with magnetic field strength, or MRI CryoProbes, which provide an even additional sensitivity boost.

The advantages of UHF MRI go beyond the sensitivity gain itself. UHF MRI facilitates a range of imaging methods and applications. Increased chemical shift, increased Blood Oxygenation Level Dependent (BOLD) contrast, altered relaxation times, and increased susceptibility effects make it predestined for several MRI methods such as MR Spectroscopy (MRS), BOLD Functional MRI (fMRI), Chemical Exchange Saturation Transfer (CEST), Susceptibility weighted Imaging (SWI), and Quantitative Susceptibility Mapping (QSM). Taken together UHF MRI can open up completely new avenues in the understanding of biological processes.

7T 1H MRS for detection of immediate cerebral response to probiotic treatment in rat model of depressive disorder

Anna Orzylowska1, Artur Łazorczyk2, Paulina Kozioł2, Agata Chudzik3, Katarzyna Kochalska4, Tymoteusz Słowiński, Anna Pankowska2, Radosław Rola3, and Greg J Stanisz1,4,5

1Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Lublin, Poland, 2Department of Electroradiology Medical University of Lublin, Lublin, Poland, 3Center of Experimental Medicine, Medical University of Lublin, Lublin, Poland, 4Physical Sciences, Sunnybrook Research Institute, Toronto, ON, Canada, 5Medical Biophysics, University of Toronto, Toronto, ON, Canada

Introduction: 1H MRS is known to be an efficient tool for studying cerebral metabolic changes caused by depression in humans1, as well as in animal models2. It has been a reliable method to detect glutamate-glutamine/GABA cycle equilibrium restoration in depressed animals after therapy with long-term feeding with Lactobacillus rhamnosus, JB-1 bacteria, as the effect of gut-brain axis communication3. Here we intended to detect very subtle process of brain metabolic response after the single intake of JB-1 in chronic unpredictable mild stress (CUMS) rat model4, with the use of short-TE 1H MRS at 7T.

Material & Methods: 14 male Wistar rats were subjected to CUMS protocol for five weeks, and then half of them were fed with Lactobacillus rhamnosus, JB-1 (LR-JB1™) in single dose of 2x10⁹ CFU, and second half with PBS as Placebo. Single voxel MRS was performed from right hippocampus before CUMS, after CUMS and 2 hours after JB-1/Placebo gavaging (7T PharmaScan 70/16 US scanner, Bruker Biospin, GmbH, Germany; voxel size: 2.0 x 2.0 x 5.5 mm³; PRESS sequence: TR/TE = 2500/16 ms, BW = 3 kHz, data points = 4096, NA = 1024, VAPOR scheme for water suppression). jMRUI software was employed for the MRS data analysis5, using basis-set of 17 metabolites signals simulated with NMRScopeB plugin. The unsuppressed water MRS signal was used for an absolute quantitation of metabolites concentrations.

Results: After CUMS all the animals presented depressive-like behaviour as compared to the baseline. Prolonged stress resulted in significant decrease in GABA, glutamate, glutamine, glutathione, and creatine. The JB-1 feeding restored GABA level to the baseline (from 1.8 ± 0.4 to 2.2 ± 0.3, vs. 2.3 ± 0.4 mM at baseline), while glutamine was further reduced. No changes in any metabolites were observed after placebo diet.

Conclusion: The use of short TE MRS in high field allowed for separation of closely-overlapped signals of glutamate, glutamine, glutathione and GABA, and thus successfully detect subtle changes in GABA concentration. The results clearly reflected gut-brain communication as immediate brain response to enrichment of intestinal microbiota with JB-1
strain. The onset of therapeutic effect occurred already after first dose of JB-1, before behavioural changes were noticed.

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References

MRI of lungs with hyperpolarized noble gases (3He, 129Xe)

Tadeusz Pałasz1 and Noble Gases Optical Polarization Group: Tomasz Dohnalik1, Bartosz Gowacz1, Łutosława Mikowska1, Zbigniew Olejniczak2, Mateusz Suchanek3

1Marian Smoluchowski Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-348 Kraków, Poland. 2Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31-342 Kraków, Poland. 3Department of Physics, University of Agriculture, Mickiewicza 21, 31-120 Kraków, Poland

The World Health Organization (WHO) reports that chronic obstructive pulmonary disease (COPD) in 2002 was the fifth leading cause of death; and currently, according to WHO estimates, 65 million people have moderate or chronic COPD [1]. Magnetic resonance imaging (MRI) is an innovative diagnostic method for lung diseases, including COPD. However, a small concentration of water vapor in the lungs excludes imaging based on hydrogen nuclei. Suitable contrasts are noble gases 3He and 129Xe [2], characterized by nuclear spin 1/2. During the medical examination, immediately before taking the MR image, the patient inhales a portion of the inert gas mixed with nitrogen. To obtain a useful MR image of a volume filled with 3He or 129Xe, its nuclear polarization must be increased. This is achieved by optical pumping and collisions with metastability exchange (MEOP) or spin exchange (SEOP). The nuclear polarization of noble gases produced by these methods is much higher (up to five orders of magnitude) than the thermal polarization in the magnetic field of the MR scanner [3]. MEOP and SEOP methods will be discussed, as well as polarizers built by Noble Gases Optical Polarization Group, including the innovative MEOP polarizer [4] operating in the strong magnetic field of the MR scanner and under increased pressure of 3He [5]. MR images of lungs filled with polarized 3He, made using a special ventilator for hyperpolarised gases will also be presented (studies were conducted in cooperation with the John Paul II Hospital in Krakow and TransCom International).

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References
Optogenetic fMRI in experimental temporal lobe epilepsy

Niels Schwaderlapp¹, Enya Paschen², Jürgen Hennig¹, Carola Haas², Pierre LeVan¹

¹ Department of Radiology, Medical Physics, University Medical Center Freiburg, Germany
² Department of Neurosurgery, Experimental Epilepsy Research Group, University Medical Center Freiburg, Germany

Spontaneous epileptic seizures, the hallmark of epilepsy, can be studied by EEG with high temporal, but only at low spatial resolution. Due to the limited time of an MRI scan, only interictal events can be studied by fMRI. However, fMRI would be extremely useful to describe the propagation of epileptic activity at high spatial resolution and to identify possible treatment targets. We resolve this issue by preclinical research in the mouse model of temporal lobe epilepsy, which replicates several features of the human disease. Optogenetic stimulation allows the precise control of neuronal activity. In our study, ChR2 was injected into the entorhinal cortex and ChR2-expressing cells of the perforant path in the septal pole of the hippocampus were stimulated. This is also the location where spontaneous seizures would arise, the induced epileptic seizures thus mimic spontaneous activity. Preclinical fMRI was used to describe the seizure onset and spread across the brain. Compared to healthy control mice, the seizures in epileptic mice were stronger and spread to more brain areas. We also optimized the sensitivity of resting state fMRI, which was acquired immediately before seizure induction. We found a clear relation between resting state connectivity and seizure spread. Furthermore, different seizure patterns between healthy and epileptic mice could be related to differences in the resting state connectivity. This systematic research of epileptic activity by fMRI is only possible in preclinical research but can guide future clinical research and diagnosis.

Magnetic Resonance Microscopy of Blood Clots

Igor Serša

Jožef Stefan Institute, Ljubljana, Slovenia

Blood clots can form in arteries where they block blood flow and result in acute events such as stroke or heart attack or can form in veins, dislodge latter and cause blood flow obstruction usually in lungs by the event known as pulmonary embolism. All these conditions are life threatening and an immediate intervention to reestablish normal blood flow is needed. This can be done mechanically by the percutaneous transluminal angioplasty (PTA) procedure or chemically by thrombolysis usually using the thrombolytic agent tissue plasmin activator (tPA). For both procedures success of the treatment depends on blood clot characteristics such as length, composition, age ... Many of these can be precisely determined by magnetic resonance. Therefore, scanning of blood clots by MRI prior to the intervention could ease the treatment planning and ultimately increase its success. Unfortunately, current MRI technology does not enable such scanning in vivo mainly because of the lack of sensitivity and resolution. However, this can be already done ex vivo by magnetic resonance microscopy (MRM) where sensitivity and resolution is sufficient. MRM can help in understanding the process of thrombolysis and the role of its parameters such as degree of clot occlusion, blood flow velocity through the clot’s reperfusion channel, clot retraction, type of thrombolytic agent used ... on the rate of thrombolysis. Such study was performed on model blood clots made of human blood that were inserted in the artificial perfusion system [1]. Its results demonstrate that faster blood flow through the reperfusion channel promotes thrombolysis. Based on the results a mathematical model for the rate of thrombolysis was made [2]. The model assumes equality between the work done by shearing forces of flowing blood on the surface of the clot and the work needed for removal of the portion of the clot. Furthermore, MRM was found efficient in analysis of blood clot composition. It was shown that T1-weight imaging is efficient in discrimination between red blood cell (RBC)-rich regions and platelet-rich regions [3], which was confirmed also in a study on pulmonary emboli ex vivo [4]. Multiparametric MR imaging using ADC and T2 mapping enables more sensitive characterization of blood clots. Thus, ADC and T2 mapping was employed to study differences in ADC and T2 distributions during the course of thrombolysis of retracted and non-retracted whole-blood clots [5]. These two maps can be used also to obtain two-dimensional ADC-T2 histograms. It was shown that the histograms are efficient for discrimination among different clot types, specifically among venous thrombi, acute and chronic pulmonary emboli and sedimented clots [6]. Another type of MRM applications was focused more to blood flow related phenomena, such as organization of up to hundreds of millibars at 4.7 Tesla. The European Physical Journal D, 2013, 67, 9.
1. platelets into layers dictated by the blood flow during the clot formation [7] or studies of blood clot perfusion with a thrombolytic agent [8]. While MR imaging of blood clots in vivo on clinical MR scanners is still challenging, MRM of artificial or extracted blood clots ex vivo already proved exceptional potentials of the method for blood clot characterization and its prognostic potentials for the treatment outcome.


### NMR-based metabolomics in the evaluation of low-dose radiation cardiotoxicity

Michalina Gramatyka, Agnieszka Skorupa, Mateusz Ciszek, Łukasz Boguszewicz, Maria Sokół

Department of Medical Physics, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland.

Metabolomics is a technique designed to measure and identify metabolites present in the studied system. The composition of the metabolome can change as a result of external factors, therefore the analysis of the metabolic profile and changes occurring in it enables early detection of the abnormal state of the organism. One of harmful factors affecting the metabolic profile is ionizing radiation, possessing, among others, cardiotoxic properties. While the cardiotoxicity of high doses of radiation is indisputable, the toxicity of low doses remains unclear. Changes induced by ionizing radiation in the heart often appear many years after exposure, however, damage at the cellular level, leading to the development of cardiovascular diseases, occurs much earlier.

The aim of our study was to evaluate whether and how the low, single dose of ionizing radiation will affect the metabolic profile of heart cells and muscle. In first model system human cardiomyocytes were exposed to 2 Gy ionizing radiation and their survival was assessed by clonogenic assay and TUNEL test. We used HR MAS NMR technique to measure changes in metabolic profile of living cells. In second model system mouse hearts were irradiated with 2Gy and subjected to histological (Masson's Trichrom and TUNEL) and metabolomic (NMR spectrometry) analyses.

Although the cell-based in vitro tests and histologic tests did not reveal any radiation-induced changes, disturbances in the metabolic profile were observed in both studied systems: in the cardiomyocytes and in the murine hearts. In cardiomyocytes, 48 hours after exposure, we observed changes in metabolites associated with oxidative stress, disturbances of energetic pathways and damage of membrane structures. In the hearts, 48 hours after exposure, we observed changes related to disorders in beta-oxidation, ATP production, oxidative stress and cell membrane stability. 20 weeks after the heart irradiation, the levels of most metabolites returned to control values, however, some disturbances were still observed, indicating the impairment of antioxidant defense mechanism and energy production through beta-oxidation. As radiation-related effects were not detected at the level of tissue histology or in vitro cellular tests, the metabolomics-based tests using NMR spectroscopy seems to be more suitable for the evaluation of cardiac tissue response to radiation.

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biggest challenge in translation of genetic information after sequencing of human genome.

To achieve this, the mouse model is used for large scale gene function identification under umbrella of International Mouse Phenotyping Consorcium (IMPC.org). The aim of this effort is to characterize every gene in mouse genome by systematic phenotyping of the knockout mouse strains. During the phenotyping process, plethora of physiological data is gathered from every single gene knockout model. The large scale imaging is one of the important sources of phenotype data, either following embryonic development or adult morphology with context of whole body composition analysis, the X-ray computed microtomography (microCT) has become a gold standard within the last years. The three-dimensional (3D) context, availability of data for additional analysis (e.g. volumetry, bone density, or body composition), and in-vivo approaches in case of adults are the main advantages when compared to classic histology and bone morphology. On the other hand, the amount of data is enormous and thus making the data storage, analysis and sharing the bottle-neck of the microCT method. To overcome this obstacle, we incorporate bioinformatics solutions for high through put image segmentation and data analysis processes as well automatization of identification of developmental defects in embryos and application of such algorithms also for automatised morphological analysis of adult skeletons. The maximalization of usage and sharing of 3D imaging data within the scientific comunity is critical point for full understandig of genetic network at the level of a whole organism which could help us to understand origin of rare diseases, developmental malformations but also the process of mammalian evolution.

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Early Evaluation of Cancer Therapy Using Saturation Transfer MRI
(CEST is Obsolete, MT is In?)

Greg J Stanisz

Department of Medical Biophysics, University of Toronto, Sunnybrook Research Institute, Toronto, Canada

Chemical exchange saturation transfer (CEST) is a promising MRI technique that probes tumour microenvironment through detection of mobile proteins and peptides. CEST MRI is analogous to MRS in that multiple proton groups at different resonance frequencies can be studied. While the most important feature of MRS is its molecular specificity, it is generally hampered by very low sensitivity because of the millimolar concentration of the directly detected proton pools. CEST provides molecular specificity with MRI sensitivity, which explains the great interest in this rapidly expanding field.

Magnetization Transfer (MT) is a contrast mechanism sensitive to the concentration of macromolecular protons (mainly associated with cell membranes) and their exchange with free water protons. Quantitative MT (qMT) enables measurement of the characteristics of the macromolecular pool, including bound proton fraction, relaxation times of the bound and free water pools, as well as the exchange rate between the two pools. These parameters are sensitive to treatment effect and reflect the treatment-induced changes in the tumour earlier than the clinically used imaging metric, which is based on changes in tumour size.

Over the past 5 years we have explored the feasibility of CEST/MT imaging and its application in determining response of glioblastoma and brain metastases to radiotherapy and/or chemotherapy. We have scanned more than 300 patients — at multiple time-points before, during and after their treatment — at both 1.5T and 3T Philips MRI scanners and have perfected the imaging protocol and also patient friendliness aspects of the protocol. Our early studies in animal models of cancer have demonstrated that saturation transfer MRI allows for non-invasive evaluation of tumour regions with high metabolic activity. Encouraged by these results we applied CEST in patients with brain metastases (BM) undergoing Stereotactic Radiosurgery (SRS) and showed that:

a. Changes in tumour metabolism measured by CEST allow for separation of responders from non-responders one-week post treatment in BM. Quantitative MRI (qMRI) can also predict how much shrinkage of the original tumour mass may occur after one month.

b. Certain features of CEST can predict tumour response before treatment is administered.

c. CEST has the potential to assess treatment-induced side-effects of radiation and is capable of distinguishing between tumour progression and radiation induced necrosis in BM.

In high grade gliomas HGG (glioblastoma) we demonstrated that saturation transfer MRI can...
identify patients that will progress soon after the treatment as early as two weeks into their 6-week chemo-radiation treatment. Even before beginning treatment several saturation transfer metrics could assess tumor aggressiveness and predict response. Magnetization transfer was also capable of showing the extent of the disease (i.e. damaged normal tissue due to glioma infiltration) better than clinically MRI (post-Gd T1w and T2w FLAIR).

At the same time, in normal tissue, saturation transfer can probe damage to the normal appearing white matter (NAWM) that is invisible to clinical MRI. We observed altered metabolism in NAWM of glioblastoma patients due to presence of tumor (even before any chemo-radiation). Treatment effects on normal tissue were also visible on saturation transfer MRI and we observed increased metabolism and damaged NAWM due to chemo-radiation in 55% of patients (such damage was not detectable on post-Gd T1w and T2w FLAIR). These patients are at higher risk to suffer from cognitive dysfunction and deteriorated quality of life over the course of their disease. This is in agreement with the literature findings that approximately half of HGG patients will experience cognitive and quality of life issues. Normal brain tissue has rich macromolecular content resulting in high MT signal. We have shown significant reduction in MT in tumors and its surrounding edema (visible in T2w FLAIR). The degree of change in MT signal in tumors (which is due to replacement of normal tissue with tumor) and edema (which is due to inflammatory response) are different which enable distinguishing these two tissue types. More importantly, the contrast between combined tumour and edema and normal brain tissue is very well pronounced and can be segmented with high accuracy. Considering in the combined tumour and edema region is used in prescribing radiotherapy plan, this saturation transfer contrast could be used to replace Gd-injection. In a pilot study of 20 glioblastoma patients we observed sufficient contrast for contouring planning target volume (PTV) using MT-weighted MRI. In another study in brain metastases, combining MT, CEST and T2w FLAIR allowed for segmenting tumor and edema boundaries (enabling GTC and PTV contouring) with high accuracy. These feasibility studies are of significant importance for active daily monitoring of treatment plan using MR-Linac to avoid excess Gd injection and adaptive treatment planning to take tumor size change during treatment into account. Avoiding repeated Gd injection to monitor brain cancer patients is also of significant interest and is feasible using saturation transfer MRI. These preliminary findings make saturation transfer and ideal candidate to provide a comprehensive characterization of the disease, not only for assessing tumor progression, but also for monitoring normal brain tissue and the treatment side-effects that play a crucial role in the management of gliomas.

Fingerprinting for MR spectroscopy
Zenon Starčuk jr., Jana Starčuková, Iveta Pavlova
Institute of Scientific Instruments of the CAS, Královopolská 147, 61200 Brno, Czech Republic

Fingerprinting [1] has been found useful for obtaining quantitative MR images from acquisitions considerably shortened by the combination of two principles: (1) fingerprint creation by irregular sampling of the image k-space and varying the pulse sequence parameters in a pattern that is sparse in comparison with the full sampling as dictated by the Nyquist conditions and model fitting accuracy (such as TE, TR, flip angle etc. for T2, T1, spin density, B1+ estimation), followed by not necessarily perfect image reconstruction, and (2) fingerprint identification by matching the pixel intensities of the artifact-beset reconstructed images to patterns precalculated and stored in a dictionary. Application to MR spectroscopy (MRS) has been so far limited to specific applications [2, 3] utilizing metabolite singlets only, but the success of fingerprinting in MRI motivates the exploration of possible more general utilization also in spectroscopy.

This contribution identifies the basic assumptions on which the MRI fingerprinting is based and finds the modes of their potential reuse in spectroscopy. Some of the MRI assumptions (such as the very low number of local sample parameters and distinctive response of non-equilibrium magnetization to pulse sequence parameters) are not generally available in MRS. In MRS (and MRSI) the number of parameters describing a voxel is in principle much larger (per metabolite at least: relative concentration, T2, T1, per voxel at least line broadening, frequency shift, global phase), and thus the applicability of principle (2) is limited; unless trivial, spectrum decomposition into metabolite components is better accomplished by conventional model fitting. Besides relaxometric and diffusometric designs suitable for singlet
resonances, an approach based on non-balanced SSFP applicable for coupled spin systems in MRS and MRSI is discussed and illustrated with simulation and prototype experimental results.


Comparative MRI of Magnetoferritin as a Pathological Model System of Native Ferritin

Oliver Štrbák1, Lucia Balejčíková2,3, Martina Miháliková1, Michal Bittšanský1, Peter Kopčanský2, Petra Hnilicová1, and Dušan Dobrota4

1Division of Neurosciences at Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala Hora 4, 036 01 Martin, Slovakia. 2Institute of Hydrology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia. 3Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia. 4Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala Hora 4, 036 01 Martin, Slovakia.

Various pathological processes including neurodegenerative disorders are associated with the accumulation of iron. It is believed that a precursor of iron accumulation is ferritin. Physiological ferritin is due to low relaxivity only weakly detected by magnetic resonance imaging (MRI) techniques. On the other hand, pathological ferritin is associated with disrupted iron homeostasis and structural changes in the mineral core that lead to the hypointensive artefacts in MRI. On the basis of recent findings regarding the pathological ferritin structure, we prepared the magnetoferritin particles as a suitable pathological ferritin model system. Our main goal is to establish an MRI methodology with the possibility of distinguishing native ferritin from magnetoferritin, as a model system of iron accumulation in pathological processes. MRI measurements were performed at 7 T BRUKER system using longitudinal ($T_1$) and transversal ($T_2$) relaxation time mapping protocols. Relative contrast, relaxation time, relaxation rate and relaxivity of native ferritin and magnetoferritin with different loading factor were analysed and compared. The results clearly show a significant difference between native ferritin and magnetoferritin in $T_2$-weighted protocols. The difference in $T_1$-weighted protocol is also obvious but not as considerable. We also show that transversal and longitudinal relaxivity ratio provides a powerful tool in discrimination of native ferritin (physiological ferritin) from magnetoferritin (pathological ferritin). These findings could contribute to the exploitation of iron oxides accumulation in a noninvasive diagnostics of pathological processes related to disrupted iron homeostasis (e.g. neurodegenerative disorders, neuroinflammation, cirrhosis, etc.).

Analysis of subchondral bone porosity for biomimetic scaffold design

Magdalena Tomanik
Department of Biomedical Engineering, Mechatronics and Theory of Mechanisms, Wroclaw University of Science and Technology

With the increase in the median age of our population bone disorder is a significant issue. Commonly damaged bone can be restored by bone grafts but the advanced in biomaterial technology allows now to substitute traditional grafts by synthetic ones. Initially, biomaterials were selected based on their biomechanical properties then engineered to be bioactive or bioreabsorbable to enhance tissue growth. Nowadays designed scaffolds are intended to induce bone formation and vascularization by a selection of the geometry with a particular focus on the porosity and biodegradable materials which can be enriched with different growth factors, drugs, genes or stem cell for faster tissue regeneration [1]. Multi-layered scaffolds have been taken under consideration for the articular surface replacement, by mimicking the specific biochemical, physical and mechanical conditions there are believed to better fit then unify constructions [2-4].

Critical role in bone formation, both in vitro and in vivo, is played by the porosity, pore size and its distribution in the scaffold. Migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularization, will depend on pore size and its wall roughness which also was found to play an important role in cell adhesion. Besides, a porous surface improves mechanical interlocking between the implant biomaterial and the surrounding natural bone, providing greater mechanical stability at this critical interface. However, it should be noted that with the increase of the porosity above some level may
cause a decrease in mechanical properties and affects the scaffold strength.

The aim of the study was to determine the porosity gradient and the quantitative changes in subchondral bone of distal femur epiphysis. Microarchitecture properties of the bone specimens were investigated with the use of microCT. The two-dimensional analysis of the bone porosity has shown the variability of the parameter relative to the distance from the articular surface. Moreover, obtained characteristics allowed to separate the subchondral plate for the cancellous bone region. The carried out three-dimensional analysis allowed the calculation of the geometrical and structural parameters of the bone tissue and have shown how the bone structure differs. Based on specific ranges of porosity and trabeculae thickness of native tissue, the biomimetic scaffold can be designed.

References

Model-Based Reconstruction Methods for Accelerated Quantitative MRI

Martin Uecker, Nick Scholand, Zhengguo Tan, Xiaqing Wang
Institute for Interventional and Diagnostic Radiology, University Medical Center Göttingen and German Centre for Cardiovascular Research (DZHK)

Quantitative mapping of relaxation parameters and other physical quantities in MRI is usually very time consuming. The reason is that traditional methods need to acquire multiple images with different contrast and then perform a pixel-wise fit to a physical signal model to identify the underlying physical parameters. Acquisition of all images required for a well-posed fit takes a long time because different contrasts need to be prepared (often multiple times). Moreover, a complete k-space has to be read out for each individual contrast to be able to reconstruct an image-domain representation of the information using the Fourier transform. To avoid this problem, novel iterative methods estimate the parameter maps directly from highly undersampled k-space data – completely avoiding the reconstruction of intermediate images. Because there is no requirement for two k-space positions to have the same contrast, steady-state signals do not need to be prepared and transient signals can be used for data acquisition. This opens the way for extremely efficient quantitative mapping. Model-based reconstruction techniques can be classified in methods using non-linear models, linear subspace models, and dictionary-based methods. In particular, we show examples from single-shot $T_1$ mapping using a combination of model-based reconstruction, parallel imaging, and compressed sensing, real-time imaging with model-based water-fat separation and field mapping, and new results which extend non-linear model-based reconstruction methods from simpler exponential models to the full Bloch equations.

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MicroPET/CT-based internal radiation dosimetry of a 152Tb-labeled antibody in tumor-bearing mice

Francesco Cicone¹, Silvano Gnesini², Thibaut Denoël¹, Thierry Stora³, Nicholas P. van der Meulen⁴,⁵, Cristina Müller⁴, Christiaan Vermeulen⁴, Martina Benešová⁵, Ulli Köster⁶, Karl Johnston⁷, Ernesto Amato⁷, Lucrezia Auditore⁷, George Coukos⁸, Michael Stabin⁵, Niklaus Schaefer¹, David Viertl¹, and John O. Prior¹

¹Department of Nuclear Medicine and Molecular Imaging, Lausanne University Hospital, Lausanne, Switzerland. ²Institute of Radiation Physics, Lausanne University Hospital, Lausanne, Switzerland. ³ISOLDE/CERN, Geneva, Switzerland. ⁴Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, Switzerland. ⁵Laboratory of Radiochemistry, Paul Scherrer Institute, Villigen, Switzerland. ⁶Institut Laue-Langevin, Grenoble, France. ⁷Section of Radiological Sciences, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy. ⁸Department of Oncology and Ludwig Center for Cancer Research, Lausanne, Switzerland. ⁹NV5/Dade Moeller, Richland, WA USA

Biodistribution studies in mice by ex vivo organ harvesting is currently the gold standard pre-clinical technique for dose to organs estimation. However, imaging is becoming increasingly popular as it allows longitudinal study from single animals, and a direct correlation with deterministic radiation effects. We assessed the feasibility of microPET-based dosimetry of an antibody fragment labeled with the positron emitter ¹⁵²Tb. Image-based absorbed dose estimates were compared with those obtained from the extrapolation to ¹⁵²Tb of a classical biodistribution experiment using the same antibody fragment labeled with ¹ⁱ¹In. ¹⁵²Tb was produced by proton-induced spallation in a tantalum target, followed by mass separation and cation exchange chromatography. The endosialin-targeting scFv78-Fc fusion protein was conjugated with the chelator p-SCN-Bn-CHX-A⁵-DTPA, followed by labeling with either ¹⁵²Tb or ¹¹¹In. Micro-PET images of four immunodeficient female mice bearing RD-ES tumor xenografts were acquired 4, 24, and 48 h after the i.v. injection of ¹⁵²Tb-CHX-DTPA-scFv78-Fc. After count/activity camera calibration, time-integrated activity coefficients (TIACs) were obtained for the following compartments: heart, lungs, liver, kidneys, intestines, tumor, and whole body, manually segmented on CT. For comparison, radiation dose estimates of ¹⁵²Tb-CHX-DTPA-scFv78-Fc were extrapolated from mice dissected 4, 24, 48, and 96 h after the injection of ¹¹¹In-CHX-DTPA-scFv78-Fc (3–5 mice per group). Imaging-derived and biodistribution-derived organ TIACs were used as input in the 25 g mouse model of OLINDA/EXM², 2.0, after appropriate mass rescaling. Tumor absorbed doses were obtained using the OLINDA2 sphere model. Finally, the relative percent difference (RD%) between absorbed doses obtained from imaging and biodistribution were calculated. RD% between microPET-based dosimetry and biodistribution-based dose extrapolations were +12, -14, and +17% for the liver, the kidneys, and the tumors, respectively. Compared to biodistribution, the imaging method significantly overestimates the absorbed doses to the heart and the lungs (+89 and +117% dose difference, respectively). MicroPET-based dosimetry of ¹⁵²Tb is feasible, and the comparison with organ harvesting resulted in acceptable dose discrepancies for body districts that can be segmented on CT. These encouraging results warrant additional validation using radiolabeled biomolecules with a different biodistribution pattern.

Non-destructive in vivo and ex vivo insights into biological nano-structures via X-ray based microscopy and CT

Jens Waldeck

Bruker BioSpin MRI GmbH, Rudolf-Plank-Str. 23, 76275 Ettlingen, Germany

Both Computed Tomographic (CT) as well as X-ray based microscopy offer non-destructive imaging capabilities. While micro-CT allows for the follow-up of e.g. diseases in animal models in vivo longitudinally, X-ray based microscopy is often limited to ex vivo applications due to its harsh radiation doses, however, allow for nano-structure detection and analysis. Radiation doses in animals and tissues due to the usage of X-rays effect biology and thus
may hamper scientific conclusions. What is true for (ancient) DNA during the evaluation of sub-fossil bones\(^1\), stays relevant when using in vivo imaging approaches\(^2,3\). In the past, high resolution micro-CT (μCT) often exceeded the recommended radiation dose (Gray) per day (<1 Gy/d), while ex vivo μCT applications often applied several hundreds or even thousands Gray to the scanned specimens leading to DNA degradations as well. However, the usage of specialized cameras\(^4\), specially designed low does X-ray filters\(^5\) and highly precise capturing methods\(^6\) in combination with optimized scanning protocols\(^6\) support the usage of μCT and X-ray based microscopy in vivo and ex vivo without limitations to the Signal-to-Noise Ratio (SNR) but at a reduces dose, and thus subsequently no limitations in the image quality and image analysis capabilities. In addition, results will be obtained in a biological relevant environment without having the need to sacrifice animals at every given and needed timepoint. Following at least the paradigm of the 3Rs by reducing the amount of used animals by a factor of 60-95% should not be hampered by the fact that these animals are exposed to radiation doses\(^3\). Thus, "refining" the dose is not only in line with the 3Rs, but also a needed consequence to obtain biological relevant and unshakable scientific results. Thus, obtaining micro-or nano-structure information's non-destructively might sometimes be a challenge. However, applying the right parameters and settings and keeping an eye on the overall goal allow for uncompromised results.

Literature


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<td><strong>Astrid Wietelmann</strong>, Clemens Müller, Arno Nauerth, Didier Stainier, Anabela Bensimon-Brito</td>
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- **Astrid Wietelmann**, Kerckhoff-Klinik, Bad Nauheim, Germany
- **Clemens Müller**, Bruker Biospin MRI GmbH, Ettlingen, Germany
- **Arno Nauerth**, Max-Planck-Institute of Heart and Lung Research, Department III Developmental Genetics, Bad Nauheim, Germany
- **Didier Stainier**, Max-Planck-Institute for Heart and Lung Research, MRI & μ-CT Service Group, Bad Nauheim, Germany
- **Anabela Bensimon-Brito**, Max-Planck-Institute for Heart and Lung Research, MRI & μ-CT Service Group, Bad Nauheim, Germany

**MRI** is a well-established modality for in-vivo imaging of the cardiovascular system in rats and mice and became the gold standard method for the quantitative evaluation of cardiac function at our institute. The main advantage of this technique is the live monitoring of cardiac function in our animal models. Smaller, non-mammalian organisms like newt and zebrafish are valuable alternative model systems mainly due to their unique regenerative capacity. However, live imaging, especially of adult zebrafish hearts, is particularly challenging due to the small size of the specimens. By optimizing cine retrospective reconstructions using the evaluation of MR signals by a selfgated sequence, we were able to successfully acquire images of the adult zebrafish beating hearts.
Not only Mice and Rats - High Field MRI of Rocks

Władysław P. Węglarz

Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland

Nuclear magnetic resonance (NMR) is a powerful tool for the laboratory-scale petrophysical characterization of oil bearing rocks. Until recently, considerable attention has been paid to low magnetic field technologies, as they assure the minimization of the influence of magnetic-susceptibility-induced internal gradients, compromising the observed NMR signal. However, measurements in low magnetic field have significant flaw due to the inherently poor signal-to-noise ratio (SNR), which limits the application of advanced imaging techniques. Recently, MR imaging of rocks in high magnetic field becomes more popular. Application of the so called zero echo time (ZTE) pulse sequence improves possibility for time effective 3D imaging of samples with short $T_2$. Collection of the set of data points on FID during TR period significantly accelerates measurements for 3D imaging, comparing to other commonly used Single Point Imaging (SPI). ZTE is purely frequency-encoded methods, and acquiring of the signal starts immediately after excitation using, e.g., radial centre-out trajectories, without the need for preceding encoding gradients. An excellent overview of the ZTE imaging method can be found in Weiger et. al.1

In this work application of 3D ZTE imaging for assessment of local hydration level in highly heterogeneous dolomite, as well as tight sandstone rocks are presented2,3. Correlation of the obtained results of the water content and spatial distribution in rock material, with the low field MR relaxometry as well as with gravimetric data provides interesting insight into interpretation of the relaxation data in terms of assessment of sample’s porosity. Correlation with high resolution micro-CT images of the same rock samples allow for direct assessment of the role of sample’s composition and structure in the dynamics of water hydration into the porous rock’s structure.

Acknowledgments

This work was supported by the research projects Blue Gas (BG2/ShaleCarp/14) and Applied Research Program (PBS2/A2/16/2013 (NMR-Rocks)), financed by the National Centre for Research and Development, Poland.

Fig. 1 Scheme of ZTE pulse sequence.

Fig. 2 Example of 2D cross-sections through 3D ZTE images of different dolomite plug cores.

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Elevation of 1H-MR glutamate signal after maximal aerobic capacity exercise in the human brain

Oxidation processes in tea observed by NMR relaxation measurements
Abstracts of Posters
Feasibility of single-voxel MR spectroscopy in the cervical spinal cord of rats

Michal Bittšanský¹, Soňa Balentová², Petra Hnilicová¹, Dagmar Kalenská³, Oliver Štrbák¹, Dušan Dobrota³

¹Biomedical Center Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia.
²Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia.
³Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Introduction
We investigated the feasibility of MR spectroscopy in vivo in the area of cervical spine of rodents, which is technically challenging.

Materials and methods
MR spectra were acquired using single-voxel PRESS in 20 healthy rats in the area of the vertebrae C2-C4 using Bruker Biospec 7T experimental scanner and 20mm surface coil for signal reception. Spectra were processed in LCModel and quantified with the reference to the water signal.

Results
Bruker-supplied high-order shimming using B0 field mapping and sufficient signal to noise ratio enabled quantification of several metabolites in the cervical spine. Signals of NAA, tCre, tCho, mI and a few other metabolites were quantified in the animals at age 3 and 6 months.

Conclusion
Our experiments have shown successful quantifiable measurements of metabolites in the area of the cervical spine of rats.

This work was supported by the Science Grant Agency - projects VEGA 1/0243/18 and the Grant of the Ministry of Health 2018/11-UKMT-7.

Transition metal oxide nanoparticles as versatile platforms for contrast enhancement in high field magnetic resonance imaging

Abhinandan Banerjee⁵, Barbara Blasiak⁶, Gabriel E. Bertolesi⁵, Chang-Chun Ling⁵, Boguslaw Tomanek⁶, and Simon Trudel⁵

⁵Department of Chemistry, University of Calgary, Calgary, AB, Canada, ⁶Department of Clinical Neurosciences, University of Calgary, Calgary & Institute of Nuclear Physics, Polish Academy of Sciences, Krakow, Poland

Bio-imaging protocols in healthcare lead to reduced costs, greater efficiency, more accurate diagnosis, and an overall improvement in the standard of care for the patient. Magnetic nanoparticles (NPs) with customizable surfaces are promising materials in this context, since upon administration to the patient, they can augment magnetic resonance imaging (MRI). When combined with the possibility of optical imaging of tissues, these NPs can lead to greater sensitivity, better prognosis, and improved guided surgical excision of sensitive pathologies.

In the Trudel group, we design, synthesize, and characterize transition metal oxide NPs with tunable magnetic properties and surface functionalities as model bionanoprobes for augmented imaging. In this poster presentation, we present our recent work on the application of metal oxide NPs in biomedical imaging. We show that maltol, an FDA-approved food flavoring, is a suitable capping agent for coating Fe₃O₄ NPs in order to synthesize efficient T₂ MRI contrast agents with demonstrated inertness to human cell lines [1]. We also show that bimetallic spinel nano-ferrites (MFe₂O₄) are better candidates for T₂ contrast enhancement compared to magnetite [2]. We demonstrate that MnO NPs capped with pyrrolidin-2-one function as potential bionanoprobe active for two orthogonal imaging modalities: T₁ MRI and fluorescence [3]. Highly anisotropic MnO NPs are also being studied by us ex vivo for possible T₁ contrast enhancement, as well as for better understanding of the influence of NP morphology on their r₁ relaxivity values [4]. Our contrast agents are particularly suited for high-field and ultrahigh-field MRI. We expect this study to lead to the development of new classes of biocompatible multifunctional nanoprobes for biomedical imaging.
Environmental conditions may substantially limit animal food availability, and often the imbalance between energy ingestion and energy requirements has to be covered through catabolism of body reserve. Inclement weather conditions like heavy precipitation, storms, or sandstorms may last for days and leave many birds without a possibility to feed. Although birds have a relatively high basal metabolic rate and small body size compared to mammals they may endure long periods of food deprivation during which birds may lose up to 50% of body/organ mass over few days. Enduring rates of aerobic metabolism generate reactive oxygen species (ROS) that, when not scavenged, may result in increased oxidative damage caused by imbalance between antioxidant defence and ROS production.

We will investigate if fasting conditions have a positive effect on removing oxidative damage from the tissues of Zebra finches. Therefore, we employ repeated quantification of organ size through magnetic resonance imaging (MRI) to estimate the change in organ volume in CONTROL and food deprived birds (FASTED). Our prediction is that we can explain variation in the oxidative damage of biomolecules or endogenous antioxidant capacity through the changes in organ mass. Use of an animal experimental MRI provides a new tool in research on phenotypic plasticity of organ size in birds, and in conjunction with our measurements of organ specific parameters for oxidative stress may contribute to our general understanding of how animals my combat oxidative stress, a main player in the ageing process.

### INTRODUCTION

MRS is used to assess the biochemical composition of tissue at a very sensitive level (~1 mM in high field systems). Often, the metabolic changes that are the target of the study are on detection limit and it is crucial to perform measurement in stable conditions. In the case of preclinical studies, protocols are often extensive, which extends the time of anesthesia and may affect this stability. The purpose of study was to investigate the influence of anesthesia duration on neurometabolic equilibrium in rat hippocampus using short-TE ¹H MRS at 7T.

### METHODS

Five healthy male Wistar rats were anesthetized under 3% isoflurane at start and 2.2% for maintenance during whole protocol. MRS spectra were acquired using 7T PharmaScan 70/16 US scanner (Bruker Biospin, GmbH, Germany). The volume of interest of 2 x 2 x 5.5 mm³ was placed in right hippocampus. PRESS sequence was used: TR/TE = 2500/16 ms, BW = 3 kHz, data points = 4096, NA = 1024, VAPOR scheme for water suppression. MRS was performed at three time points every 40 min, starting 40 min after anesthesia induction. The whole scanning time did not exceed 3 h 10 min. MRS data were...
RESULTS Based on the one-way for repeated measurements ANOVA analysis, concentrations of glutamate (Glu) and total choline (GPC+PCh) were significantly affected by anesthesia duration. The Glu concentration significantly (p = 0.02) decreased from 9.2 ± 0.3 at 40 min post anesthesia induction to 8.7 ± 0.4 at 1 hour 20 min to 8.5 ± 0.4 at the end of 3 hour scan. Respectively, GPC+PCh (p = 0.03) changed from 1.3 ± 0.07 to 1.2 ± 0.05 to 1.13 ± 0.05.

CONCLUSIONS The study shows that anesthesia time change concentrations of such brain metabolites as Glu and Cho group, as anesthesia affects cerebral blood flow and metabolism. Therefore, in research focused on these compounds the use of a suitably short protocol should be considered.

ACKNOWLEDGEMENTS Work was supported by National Science Centre, Poland (2015/17/B/NZ4/02986).

Semi-automatic application for tumor volumetry from preclinical MRI

Monika Drabik¹,², Michal Fiedorowicz¹, Ireneusz Grudziński³, Piotr Bogorodzki¹,²

¹Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
²Faculty of Electronics and Information Technology, Warsaw University of Technology, Warsaw, Poland.
³Department of Applied Toxicology, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland

Fast and accurate determination of the tumor volume is crucial for preclinical oncology studies and in particular for studies that evaluate drug effectiveness. Involvement of in vivo imaging techniques in preclinical oncology research allow more precise quantification of tumor volume than traditional methods based on two dimensional measurements with caliper. However, processing of the image data remains a problematic issue. Anatomical MRI usually consists of 24-48 slices that need to be carefully evaluated. It is labor-intensive and the results can depend on the level of experience of the examiner. In case of tumors that appear homogenous in tomographic images like CT or MRI and could be easily distinguished from surrounding tissues, application of automated/semi-automated volumetric evaluation (e.g. region growth algorithm) may be possible and increase the efficiency of data processing. The goal of this work was to develop application for automatic segmentation of tumor – to save time and provide more accurate volume measure. The application that we developed is based on region growing algorithm: one pixel within the tumor in 2-D slice is marked manually and nearest pixels with similar signal intensity are marked and added to the tumor volume. Next, morphological filters are used to smooth the edges of the tumor. Process is propagated within slices and finally volume of the tumor is calculated. Validation of the application accuracy has been performed by comparison of the calculated tumor volume with weight of the excised tumor.

This work was financially supported by GEMNS project granted in the European Union’s Seventh Framework Program under ERA-NET EuroNanoMed II (European Innovative Research and Technological Development Projects in Nanomedicine).Project was carried out with the use of CePT infrastructure financed by the European Union – the European Regional Development Fund in the Operational Programme “Innovative Economy” for 2007–2013.

The influence of pH and Na⁺ on chemical exchange processes in aqueous solutions of mexiletine

Jan Kobierski¹, Krzysztof Jasiński², Wojciech Jawień¹, Władysław P. Węglarz²

¹Department of Pharmaceutical Biophysics, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland
²Department of Magnetic Resonance Imaging, The Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland

Chemical Exchange Saturation Transfer (CEST ¹H MRI) is an innovative method of contrast
imaging that can be used to investigate kinetics of drug distribution. It becomes particularly important in determining the patient's dosage requirements. This technique can be used if the solute protons have a sufficiently fast exchange rate (residence time in millisecond range) and the saturation time is sufficiently long (second range).

The exchange rate depends on many factors, such as temperature, pH and concentration of paramagnetic ions. These parameters can vary widely in living organisms, which can prevent the application of CEST in vivo.

We present results of CEST ¹H MRI experiments conducted on a 9.4T horizontal scanner. Quantitative analysis of CEST effect for a wide range of pH and NaCl concentration at various mexiletine concentrations were determined to assess the applicability of the CEST technique in vivo.

References:

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<th>Developmental changes in metabolite concentration and T₂ relaxation in the Wistar rat brain assessed by ¹HMRS and CPMG sequences</th>
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**Katarzyna Kochalska¹2, Anna Pankowska¹2, Artur Lazorczyk¹2, Tymoteusz Slowik¹, Agnieszka Michalak¹, Olga Wronikowska³, Radoslaw Pietura², Barbara Budzyńska³**

¹ Department of Radiography, Medical University of Lublin, ² Center of Experimental Medicine, Medical University of Lublin, Poland, ³ Independent Laboratory of Behavioral Research, Medical University of Lublin

**Purpose:** Brain development is a continuous process combined with significant volume and biochemical changes. Studies results of rat brain maturation using ¹HMRS are subtle and slightly unconcordant. Among others, biochemical changes and their time of occurrence depend on the laboratory rat strain or sex. The main objective of this study is to establish metabolic alteration and intercurrent changes of T₂ relaxation associated with Wistar rat brain maturation, assessed by ¹HMRS and CPMG sequences.

**Methods:** The examined cases consisted of 5 healthy male Wistar rats (250g), that were examined on postnatal days 28 and 42. Single-voxel ¹HMRS experiments were performed on a 7T MRI scanner (Bruker Pharma Scan). Voxel size was 20 μL (2x2x5mm³) located in the right hippocampus, spectra acquired using the PRESS sequence (TE/TR=16 ms/3000ms). MR spectra were processed using LCModel™ software (Version 6.3-1). T₂ maps were measured by multi slice multi echo (MSME) method, based on CPMG sequences. MSME was applied 64 different echo times (TE), with a repetition time (TR) = 3000 ms, slice thickness = 1 mm, number of slices = 5, field of view (FOV) = 30 x 30 mm, and matrix size = 128 x 128 pixels, which serves the spatial resolution of 0.234 x 0.234. T₂ relaxation were calculated manually by Bruker post- processing macro FitinIsa, from ROI defined at right hippocampus.

**Results:** The results from two aging states, which were obtained by MSME sequences, revealed significant decrease of T₂ relaxation for day 42 compared to 28 postnatal day (Wilcoxon test; p =0.04). Due to observed alterations in brain water content, the analysis of ¹HMRS spectra using water as the internal reference was eliminated to avoid underestimated metabolites levels. Concentration of metabolites were assessment as ratio to signal the sum of creatine and phosphocretine (Cr+PCr). ¹HMRS data showed higher NAA/Cr+PCr ratio at day 42 compared to day 28, the results of NAA/Cr+PCr ratio were as follows: 1.16 ± 0.02 and 1.07 ± 0.02 (Wilcoxon test; p=0.04). Gln/Cr+PCr ratio for day 28 was 0.53 ± 0.02, whereas for 48 postnatal day, it was significantly lower at 0.46 ± 0.02 (Wilcoxon test; p=0.04).

**Conclusion:** Statistically significant T₂ relaxation changes can by explain by myelination and reorganization processes of brain during development and aging, consequently contribute lower extracellular space accessible for brain water. The major metabolic changes in rat brain, obviously appear within the first two postnatal month, corresponding

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with the period of active brain development, myelination and the rapid increase in energy metabolism. Statistically, the significant increase of NAA/Cr+PCr ratio during maturation, results from the active growth of regional neuronal cells and axons. Results regarding a decrease of Gln/Cr+PCr ratio are at variance with some papers due to the fact that the concentration changes of weakly represented metabolites, such as Gln or GABA, were not as evident. For an accurate estimation, contributions of the overlapping resonances should be always taking into account. The current data may provide a reference for MRI/MRS studies of brain disease models which should not start before the end of maturation process to exclude serious results confounds.

Acknowledgements: The research is supported by National Science Centre, Poland (2017/25/B/NZ7/02410).

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<td><strong>Artur T. Krzyżak</strong>¹, <strong>Weronika Mazur</strong>¹, <strong>Jacek Matyszkiewicz</strong>², <strong>Alicja Kochman</strong>²</td>
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¹Department of Fossil Fuels, Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, ²Department of Environmental Analysis, Geological Mapping and Economic Geology, Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology

**Background:** Cherts are highly siliceous sedimentary rocks that occur in the form of beds or nodules. The potential sources of silica in these rocks were discussed by many authors, but some of these concepts do not fit the origin of cherts from Kraków-Częstochowa Upland (KCU). Low Field NMR is widely used for studies on properties of rocks. Its sensitivity to very fast relaxing hydrogen species allowed the investigation of physically and chemically bound protons in different silicates. In the work we present the application of ¹H LF-NMR 1D and 2D relaxometry for studies of porosity in cherts from the KCU.

**Methods:** We measured three samples of nodular and one representative sample of bedded chert. Performed 1D-¹T₁, 1D-¹T₂ and 2D-¹T₁-¹T₂ experiments delivered a set of NMR parameters that were used as variables in the Principal Component Analysis.

**Results:** Results from 1D-¹T₂ experiments showed that the total porosity is in the range of 1.08-2.34%. After heating, all of the samples retained relatively high amount of water. Interestingly, 1D-¹T₂ distributions of all of the samples contain four distinct peaks located in the same regions within the range of T₂=0.3-100 ms. ¹T₁-¹T₂ maps delivered T₁/T₂ ratios reflecting the desorption energy. Moreover, geochemical analysis showed that studied samples contained ~98% of silica, which allowed us to compare the results with the analysis of pure silica materials [1].

**Discussion/Conclusions:** Clearly visible 1D-¹T₂ distributions of dry samples suggest the existence of inclusions, but the reproducible spectra forced us to speculate that they are filled with water and siliceous materials with different surface roughness. All of the information collected from the experiments helped us to identify several hydrogen populations in the samples: (1) chemically bound protons (silanols), (2) H₂O retained in the structure, (3) H₂O loosely held in open pores, (4) water strongly adsorbed on the surface of the pores. PCA showed the separation of nodular and bedded cherts. Estimated Pore Size Distribution showed that open porosity is dominated by mesopores in bedded chert, while by micropores in nodular cherts that do not contain mesoporosity. Considering that water/hydrogen content in silicates varies with environmental conditions, we believe that investigation of cherts porosity will help to identify their origin and characterize the differences between bedded and nodular cherts.

**Acknowledgments** This research was funded by the National Science Centre, Poland, on the basis of contract No. UMO-2017/27/B/ST10/00594 (J.M., A.K.) and by The National Centre of Research and Development (contract No. STRATEGMED2/265761/10/NCBR/2015) (W.M., A.T.K.). W.M. has been partly supported by the EU Project POWER.03.02.00-00-I004/16.

Comparison of Contrast Agents Uptake in Tumour Imaging

Tereza Michalcikova, Frantisek Spoutil, Jan Prochazka, Radislav Sedlacek

Institute of Molecular Genetics of the AS CR, v.v.i., Videnska 1083, 142 20 Prague 4, Czech Republic

Micro-CT imaging is a very useful non-invasive imaging technique for studying the structures and tissues of various organisms. Although this method has proven to be suitable for imaging mineralized tissues, imaging of soft tissues is more difficult due to the need for contrast media. Except for bones, fat and lungs, tissues have almost the same density. The potential of contrast agents has been revealed recently and several have since been discovered. Contrast agents are solutions containing high atomic number element that bind more or less specifically to certain cell types or structures of the contrasting sample. Lugol’s solution consists of dissolved iodine in potassium iodine and binds to glycogen-rich tissues, and PMA (phosphomolybdenic acid) and PTA (phosphotungstic acid) specifically bind to proteins, including collagen. PTA most commonly shows a specific affinity for collagen-rich structures of connective tissue and PMA for primarily collagen and probably phospholipid structures. The less widespread contrast agent is a silver. It is known for being very easily reduced out of its solution silver nitrate in reducing environment and it was previously described as an element capable of absorbing X-rays but it has not been used as a soft tissue contrast agent to date. The aim was to compare and identify the ideal contrast for micro-CT imaging (Fig. 1).

Reduced silver is a suitable element for imaging of soft tissue but additional optimization of the contrast protocol is necessary. Lugol’s solution and PTA contrasted the entire tumour tissue very well, allowing the study of internal structure and heterogeneity based on signal density. A tumour contrasted with PMA solution has the lowest signal strength under nearly the same contrast and scan conditions (tumour was contrasted with PMA twice as long as in the case of other contrasts). However PMA binds with greater specificity to the muscle tissue, rather than to the tumour tissue, which does not make it a suitable contrast for tissue itself, but in the case of studying tumour cells invasiveness into surrounding tissue, PMA appears to be a very suitable contrast.

Figure 1: Virtual images of tumours stained with Lugol’s solution, PMA (phosphomolybdenic acid), PTA (phosphotungstic acid) and silver nitrate. Pseudocolours refer to changes in signal density – see bar.

Production of hyperpolarized $^3$He and $^{129}$Xe for human lung MRI

Tomasz Dohnalik$^1$, Bartosz Głowacz$^1$, Lutosława Mikowska$^1$, Zbigniew Olejniczak$^{1,2}$, Tadeusz Pałasz$^1$ and Mateusz Suchanek$^3$

$^1$ Marian Smoluchowski Institute of Physics, Jagiellonian University, Kraków, Poland, $^2$ Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland, $^3$ Department of Physics, University of Agriculture, Kraków, Poland

The MRI of human lungs using hyperpolarized noble gases (HP-MRI) provides new capabilities for the diagnosis of lung diseases [1]. The nuclear polarization of the noble gases that is obtained in polarizers based on optical pumping exceeds by several orders of magnitude the thermal equilibrium value. Therefore, when inhaled by the patient, it compensates for the low gas density in the lungs and allows to obtain high resolution MR images. Contrary to traditional lung function tests, which provide only global information about lung condition, HP-MRI gives precise localization and size of ventilation defects. The technique has been successfully applied to assess the ventilation impairments in patients.
with asthma, Chronic Obstructive Pulmonary Disease and cystic fibrosis [1,2]. Moreover, changes in the lung microstructures caused by ageing, smoking, emphysema and other diseases have been determined quantitatively by monitoring apparent diffusion coefficient of inhaled HP gases [1,3].

Our group has constructed polarizers for $^3$He and $^{129}$Xe gases, which are most popular in the lung MRI. A mobile, compact $^3$He polarizer operates on the Metastability Exchange Optical Pumping (MEOP) principle [4,5], producing sufficient for a single patient portion of 30% polarized gas in 40 minutes. The administration of HP gas is controlled by a dedicated ventilator, which ensures the helium gas recovery [6]. A series of lung images of volunteers were obtained, using the 1.5 T medical scanner [6].

The $^{129}$Xe polarizer based on the Spin Exchange Optical Pumping (SEOP) principle [7] operates in the stop-flow mode, providing HP xenon gas of about 14% polarization [8]. The images of the phantom filled with HP $^{129}$Xe gas were obtained, and several improvements of the experimental setup are in progress, to implement this method for human lung imaging. Moreover, since xenon can dissolve in living tissues, the lung perfusion studies and brain imaging are possible [1]. For this purpose, the NMR spectroscopic studies of xenon dissolved in human blood are planned.


Using chemical exchange saturation transfer method for comparison of metabolite levels changes in the animal model of chronic unpredictable mild stress

Anna Pankowska¹, Artur Łazorczyk¹, Katarzyna Kochalska¹, Agata Chudzik², Tymoteusz Słowięk³, Marta Andres-Mach⁴, Wilfred W. Lam⁴, Radosław Pietura⁵, Radosław Rola¹, Greg Stanisz²³⁶, Anna Orzyłowska²

¹Department of Radiography, Medical University of Lublin, Poland, ²Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Poland, ³Center of Experimental Medicine, Medical University of Lublin, Poland, ⁴Isobolographic Analysis Laboratory, Institute of Rural Health, Lublin, Poland, ⁵Physical Sciences, Sunnybrook Research Institute, Toronto, ON, Canada, ⁶Department of Medical Biophysics, University of Toronto, ON, Canada

PURPOSE: Use of the chemical exchange saturation transfer (CEST) method to evaluate metabolic changes which occur in the brain in an animal model of chronic unpredictable mild stress (CUMS) disorder.

METHODS: Experiments were performed on 7 T MRI animal scanner. Z-spectra were acquired with two $\text{B}_1$ saturations of 0.5 and 0.75 µT and pulse duration of 4900 ms. Additional spectra sensitive to Magnetization Transfer and Direct Water Saturation Effect were performed with a $\text{B}_1$ peak amplitude of 3.0 µT, 5.0 µT and 0.1 µT respectively. Two anatomical regions (cortex and hippocampus) were selected for analysis in two groups of animals: healthy control (n=10) and depressed group (n=11) exposed to eight weeks of stress protocol.

RESULTS: Analysis of Z-spectrum parameters (MTR, CEST and rNOE contributions) in five selected peaks (3.5 ppm, 3.0 ppm, 2.0 ppm, -3.2 and -3.6 ppm) revealed significant differences between groups. The biggest changes in a range of ~3.45% were found in the cortex (with saturation $\text{B}_1$ of 0.75 µT) in rNOE contribution peak (~3.6 ppm, p=0.0005). Also in the hippocampus, there was a significant decrease of MTR parameter observed in all selected offset (mostly with saturation $\text{B}_1$ of 0.5 µT). The use of the two-pool magnetization transfer fitting model didn’t show any substantial changes in fitted parameters, only calculated parameter $\text{R}_1$ differed significantly between groups (p<0.05).

CONCLUSION: Applying CEST as a novel method of in vivo metabolic imagining proved to be successful in differentiation metabolic changes which appeared in rats hippocampus and cortex due to chronic stress exposure.
**ACKNOWLEDGMENTS**: Work was supported by the National Science Centre, Poland (2015/17/B/NZ4/02986).

**Figure 2** CEST and rNOE contributions in cortex measured with B\textsubscript{1} saturation amplitude of 0.75 $\mu$T in 3.5 ppm, 3.0 ppm, 2.0 ppm, -3.2 and -3.6 ppm peaks.

### Effect of lipid removal from the rat brain sample with the CLARITY procedure on the CEST spectrum in 7T MRI

Tymoteusz Slowik\textsuperscript{1}, Agata Chudzik\textsuperscript{2}, Anna Pankowska\textsuperscript{1,3}, Wilfred W Lam\textsuperscript{4}, Anna Orzylowska\textsuperscript{2}, Radoslaw Rola\textsuperscript{2}, Greg J Stanisz\textsuperscript{2,4,5}

\textsuperscript{1}Center of Experimental Medicine, Medical University of Lublin, Lublin, Poland, \textsuperscript{2}Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Lublin, Poland, \textsuperscript{3}Department of Electroradiology, Medical University of Lublin, Lublin, Poland, \textsuperscript{4}Physical Sciences, Sunnybrook Research Institute, Toronto, ON, Canada, \textsuperscript{5}Medical Biophysics, University of Toronto, Toronto, ON, Canada

**Introduction**: Chemical Exchange Saturation Transfer (CEST) and rNOE (relayed Nuclear Overhauser Effect) MRI is sensitive to labile proteins and metabolites and magnetization transfer (MT) to semi-solid macromolecules. This study aimed to assess the differences in Z-spectra (plots of water signal attenuation as a function of radiofrequency saturation frequency offset) in the rat brain hippocampus in vivo and after the post-mortem lipid removal procedure CLARITY (Clear, Lipid-exchanged, Acrylamide-hybridized Rigid, Imaging/immunostaining compatible, Tissue hYdrogel).

**Methods**: MRI (7T PharmaScan 70/16 US, Bruker, Germany) was performed twice: in vivo and post-mortem (after CLARITY). Single axial slices covering the hippocampus were acquired using an MT-prepared EPI sequence (TE: 37 ms, TR: 5 s, NA: 3, FOV: 30 × 30 mm\textsuperscript{2}, slice thickness: 1 mm), using two block saturations pulses of 0.5 and 0.75 $\mu$T peak amplitude B\textsubscript{1}, duration of 4900 ms and frequency offsets from -6 to + 6 ppm (139 offsets in vivo and 424 offsets post-mortem). Z-spectra were calculated from the segmented hippocampal area, B\textsubscript{0}-corrected and averaged over the ROI. A 5 mm slice was fixed with a hydrogel solution containing acrylamide, paraformaldehyde, bis-acrylamide and thermal initiator VA-044. For clearing, a sodium dodecyl sulphate and boric acid solution was used.

**Results**: The lipid removal substantially decreased the CEST signal within the whole range of spectrum received with both saturation B\textsubscript{1} amplitudes (Fig. 1). After CLARITY the Z-spectra were flattened compared to in vivo, with remaining distinct peaks at +3.5, +2.8, +2 and -3.5 ppm. With a saturation B\textsubscript{1} of 0.5 $\mu$T, the average signal decrease was 12 ± 3% and with a B\textsubscript{1} of 0.75 $\mu$T, it was 21 ± 4%.

**Conclusion**: This study showed that the macromolecular MT contribution into in vivo Z-spectra originates mostly from lipids, since the CLARITY technique removed the MT component from the spectrum. It is in agreement with biological data, from which lipids are known to be the biggest group of macromolecules (around 11%) of the brain tissue.

**Support**: National Science Centre, Poland (2015/17/B/NZ4/02986).
The main aim of our approach is to gain a comprehensive view of mobility of small molecules in confinement as reported by $^2$H NMR. Applicability of deuteron NMR methods in studies of molecular mobility was tested on a series of small molecules: $D_2$, $CD_4$, $D_2O$, $ND_3$, $CD_3OD$ and $(CD_3)_2CO$ confined in nanocages of NaX and NaY zeolites. A range of loadings provides another dimension in studies of molecular mobility. Observed features reflect evolution on decreasing temperature of molecular dynamics from gaseous state over liquid-like rotational phase to immobilized molecules. The temperature defined as $T_S$ separates two ranges with basically different mobility. Molecules become immobilized below the temperature $T_S$, which appears to be an important parameter related to the strength of interactions with zeolite framework. High mobility, both translational and rotational, is responsible for features observed in spectra and relaxation at temperatures above $T_S$.

A transition from translational to rotational mobility on decreasing temperature was a common observation above $T_S$, with transition temperature $T_{TR}$ as a significant parameter. The transition temperature $T_{TR}$ is related to the strength of mutual interactions in guest/host systems. The spin system is assumed to consist of two subsystems with different mobility: rotational $R$ and translational $R$ relaxation rates. We observe in the experiment single relaxation rate $R = wR' + (1 - w)R''$, where $w$ depends on relative abundance of molecules in both subsystems [3]. The reduced value of the quadrupole coupling constant, reduced with respect to the value expected for rotational mobility, underlines that decision. The weighting factor, and activation energies for respective mobilities characterize individual cases. High activation energies were obtained for translational mobility, however on a much simpler way, compared to other methods (QENS, PFG NMR).

The case of each considered molecule appears to be a distinctive one. Mutual interactions and interactions with adsorption centres on the cage walls, play direct and indirect role in features observed for NMR observables. These involved, for example, formation of water clusters [3, 4, 5], location at Levis sites for ammonia [6] and acetone [7], hydrogen bonding hindering rotation of ammonia [6], and methanol trimers [8], at low and high loading, respectively.

Fig. 1 Mean Z-spectra from the hippocampus in vivo (blue) and after the CLARITY procedure (yellow), obtained with two $B_1$ saturation amplitudes of a) 0.5 and b) 0.75 µT.
Elevation of 1H-MR glutamate signal after maximal aerobic capacity exercise in the human brain

Martlena Welnia-Kamińska1 Maciej Świątkiewicz1 Michał Fiedorowicz1, Jarosław Orzel1,2, Michał Madeyski1, Bartosz Kossowski1,2,4, Józef Langfort1, Paweł Grieb1, Piotr Bogorodzki1,2,4,5, Ewelina Zawadzka-Bartczak1, Jerzy Walecki1,4,5, Stefan Gaździński1,3

1 Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland 2 Faculty of Electronics and Information Technology, Warsaw University of Technology, Warsaw, Poland. 3 Military Institute of Aviation Medicine, Warsaw, Poland. 4 Nalecz Institute of Biocybernetics and Biomedical Engineering Polish Academy of Sciences, Warsaw, Poland. 5 Laboratory of Brain Imaging, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland. 6 Diagnostic Radiology Department, Central Clinical Hospital of the Ministry of the Interior in Warsaw, Warsaw, Poland. 7 Centre of Postgraduate Medical Education, Warsaw, Poland

During exercise many brain areas are significantly activated, which should certainly intensify glutamate (Glu) turnover. Glu that is the main excitatory neurotransmitter in the central nervous system, is efficiently recycled between synapses and presynaptic terminals through glutamate-glutamine cycle that uses 60–80% of energy in the resting brain. Our previous results [Świątkiewicz et al. 2017. Front Physiol 31:8:19. doi:10.3389/fphys.2017.00019. eCollection 2017] showed an elevation of Glu and glutamine (Gln) MRS signals after exhaustive exercise in the rat model. On the other hand, present data on effects of exercise on brain metabolites seem to be inconclusive. This study was aimed to verify the hypothesis that intensification of glutamate (Glu) turnover. Glu that is the main excitatory neurotransmitter in the central nervous system, is efficiently recycled between synapses and presynaptic terminals through glutamate-glutamine cycle that uses 60–80% of energy in the resting brain. Our previous results [Świątkiewicz et al. 2017. Front Physiol 31:8:19. doi:10.3389/fphys.2017.00019. eCollection 2017] showed an elevation of Glu and glutamine (Gln) MRS signals after exhaustive exercise in the rat model. On the other hand, present data on effects of exercise on brain metabolites seem to be inconclusive. This study was aimed to verify the hypothesis that intensification of glutamate-glutamine cycle can also be observed in humans subjected to exhaustive exercise. The subjects (males, 18±1 years old, n=14 per group) underwent a treadmill test assessing maximal aerobic capacity, the control group did not perform the test. MR examination was performed with 3.0T GE Discovery MR750w scanner and 1H PROBE-PRESS sequence (TR=1500ms, TE=35ms and TE = 288ms, VOI = 3x3x6cm placed in occipito-parietal cortex). MR spectra were obtained <10 min after the treadmill test and then after one hour. Data were processed with LC Model — both metabolite to total creatine ratios and signals scaled to the water peak were analyzed. Significantly higher signal of Glx, Glu (both metabolite to tCr ratio and water-scaled) and Gln (scaled to tCr but not water-scaled) was observed in the group subjected to treadmill test immediately after the exercise than in the control group. One hour after the exercise the Glx, Glu and Gln signals were similar as in control group. Our results support the view that Glu/Gln cycle in the brain is activated during physical exercise.

Oxidation processes in tea observed by NMR relaxation measurements

Dorota Wierzuchowska4, Magdalena Witk5, Agnieszka Zawiślak5 and Barbara Blicharska3

4 Institute of Physics, Pedagogical University, Podchorążych 2, 30-084 Kraków, Poland. 5 Department of Refrigeration and Food Concentrates, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-149 Kraków, Poland. 3 Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-348 Kraków, Poland
It is well known that tea infusions contain substances with antioxidant properties. Samples of various commercially available tea were used to test the ability of NMR relaxation measurements to study the oxidation processes. The oxidation was initiated by adding 3% hydrogen peroxide to the tea infusion sample, and spin-lattice relaxation time $T_1$ was measured immediately afterwards. After a short exponential decay of $T_1$, its recovery was observed. Similar behavior of $T_1$ time courses was observed in aqueous solutions of vitamin C [1]. We therefore concluded that the recovery of $T_1$ was due to the presence of antioxidants in tea.

Analysis of relaxation times measurements obtained from tea samples may be useful for studying the kinetics of antioxidant activity [1, 2].

Attendees List
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<tr>
<td>Izabela Baranowska</td>
<td>Medical University of Lublin</td>
<td>Lublin, Poland</td>
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<td>Artur Birczyński</td>
<td>Institute of Nuclear Physics PAN</td>
<td>Kraków, Poland</td>
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<td>Barbara Blicharska</td>
<td>Institute of Physics</td>
<td>Kraków, Poland</td>
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<tr>
<td>Barbara Błasiak</td>
<td>Institute of Nuclear Physics PAN</td>
<td>Krakow, Poland</td>
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<tr>
<td>Piotr Bogorodzki</td>
<td>Mossakowski Medical Research Centre, PAS</td>
<td>Warsaw, Poland</td>
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<td>Amadeusz Bryla</td>
<td>Jagiellonian University</td>
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<td>Agata Chudzik</td>
<td>Medical University of Lublin</td>
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<td>Khanlian Chung</td>
<td>Heidelberg University</td>
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<td>University of Sheffield</td>
<td>Sheffield, UK</td>
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<td>Comef</td>
<td>Katowice, Poland</td>
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<td>Jim Delikatny</td>
<td>University of Pennsylvania</td>
<td>Philadelphia, USA</td>
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<td>Przemysław Dorożyński</td>
<td>Warsaw Medical University</td>
<td>Warsaw, Poland</td>
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<td>Monika Drabik</td>
<td>Mossakowski Medical Research Centre, PAS</td>
<td>Warsaw, Poland</td>
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<tr>
<td>Michal Fiedorowicz</td>
<td>Mossakowski Medical Research Centre, PAS</td>
<td>Warsaw, Poland</td>
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<tr>
<td>Henryk Figiel</td>
<td>AGH University of Science and Technology</td>
<td>Kraków, Poland</td>
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<td>Stefan Gaździński</td>
<td>Military Institute of Aviation Medicine</td>
<td>Warsaw, Poland</td>
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<tr>
<td>Michalina Gramatyka</td>
<td>Maria Sklodowska-Curie Memorial Center and Institute of Oncology Gliwice Branch</td>
<td>Gliwice, Poland</td>
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<tr>
<td>Iwona Habina-Skrzyniarz</td>
<td>AGH University of Science and Technology</td>
<td>Kraków, Poland</td>
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<td>Franciszek Hennel</td>
<td>University of Zurich and ETH Zurich</td>
<td>Zurich, Switzerland</td>
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<td>Krzysztof Jasiński</td>
<td>Institute of Nuclear Physics PAN</td>
<td>Kraków, Poland</td>
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<tr>
<td>Jan Kobierski</td>
<td>Jagiellonian University Medical College</td>
<td>Kraków, Poland</td>
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<tr>
<td>Katarzyna Kochalska</td>
<td>Medical University of Lublin</td>
<td>Lublin, Poland</td>
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Dorde Komljenovic  
*German Cancer Research Center*  
Heidelberg, Germany

Piotr Kozłowski  
*UBC MRI Research Centre*  
Vancouver, Canada

Artur Krzyżak  
*AGH University of Science and Technology*  
Kraków, Poland

Piotr Kulinowski  
*Pedagogical University of Krakow*  
Kraków, Poland

Grzegorz Kwiatkowski  
*ETH Zurich*  
Zurich, Switzerland

Zdzisław Lalowicz  
*Institute of Nuclear Physics PAN*  
Kraków, Poland

Radosław Leszczyński  
*RHL-Service*  
Poznań, Poland

Natalia Łopuszyńska  
*Institute of Nuclear Physics PAN*  
Kraków, Poland

Alex MacKay  
*University of British Columbia*  
Vancouver, Canada

Weronika Mazur  
*AGH University of Science and Technology*  
Kraków, Poland

Tereza Michalčíková  
*Institute of Molecular Genetics of the ASCR, v. v. i.*  
Prague, Czech Republic

Lutosława Mikowska  
*Jagiellonian University*  
Kraków, Poland

Harald Möller  
*Max Planck Institute for Human Cognitive and Brain Sciences*  
Leipzig, Germany

Arno Nauerth  
*Bruker BioSpin MRI*  
Ettingen, Germany

Thoralf Niendorf  
*Berlin Ultrahigh Field Facility (B.U.F.F.), Max-Delbrueck Center for Molecular Medicine in the Helmholtz Association*  
Berlin, Germany

Anna Nikodem  
*Politechnika Wroclawska*  
Wroclaw, Poland

Maria Noga  
*Institute of Nuclear Physics PAN*  
Kraków, Poland

Claudia Oerther  
*Bruker BioSpin MRI GmbH*  
Ettingen, Germany

Zbigniew Olejniczak  
*Institute of Nuclear Physics PAN*  
Krakow, Poland

Anna Orzyłowska  
*Medical University of Lublin*  
Lublin, Poland

Tadeusz Pałasz  
*Jagiellonian University*  
Kraków, Poland

Wojciech Rutkowski  
*Institute of Nuclear Physics PAN*  
Kraków, Poland
Niels Schwaderlapp  
*University Medical Center Freiburg*  
Freiburg, Germany

Igor Serša  
*Jožef Stefan Institute*  
Ljubljana, Slovenia

Tymoteusz Słowik  
*Medical University of Lublin*  
Lublin, Poland

František Špoutil  
*Institute of Molecular Genetics of the ASCR, v. v. i.*  
Prague, Czech Republic

Greg Stanisz  
*University of Toronto, Sunnybrook Research Institute*  
Toronto, Canada

Zenon Starcuk Jr.  
*Institute of Scientific Instruments, CAS*  
Brno, Czech Republic

Oliver Strbak  
*Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava*  
Martin, Slovakia

Agnieszka Szymocha  
*Uniwersytet Rolniczy*  
Kraków, Poland

Magdalena Tomanik  
*Politechnika Wrocławska*  
Wrocław, Poland

Martin Uecker  
*University Medical Center Göttingen*  
Goettingen, Germany

Miroslav Vecheta  
*ACCELA s.r.o.*  
Prague, Czech Republic

David Vierl  
*Lausanne University Hospital*  
Lausanne, Switzerland

Jens Waldeck  
*Bruker BioSpin*  
Ettlingen, Germany

Marlena Welniak-Kamińska  
*Mossakowski Medical Research Centre, PAS*  
Warsaw, Poland

Władysław Węglarz  
*Institute of Nuclear Physics PAN*  
Kraków, Poland

Dorota Wierzuchowska  
*Pedagogical University*  
Kraków, Poland

Astrid Wietelmann  
*Max-Planck-Institute for Heart and Lung Research*  
Bad Nauheim, Germany
Maps
Adresses:

**The H.Niewodniczański Institute of Nuclear Physics PAS**
Radzikowskiego 152; 31-342 Krakow

**Q Hotel**
Radzikowskiego 142; 31-342 Krakow

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