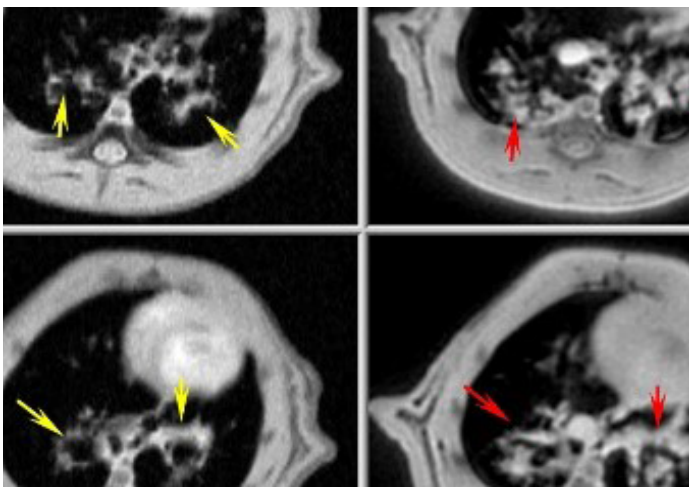
# Non-invasive Preclinical Lung Imaging: A Pharmaceutical Perspective




## Abstract

The incidence of respiratory disease is increasing throughout the world, and the demand for safe, effective drugs to tackle conditions such as asthma, chronic obstructive pulmonary disease (COPD), pneumonia and lung cancer is rising. But the journey of a drug candidate from discovery through to production is a long and costly one, with a low approval rate. Approximately nine out of every ten drug candidates fail to win approval, a significant factor in the overall high cost of drug development.[1] While the reasons for failure are numerous, the pharmaceutical industry benefits from finding ways to more effectively evaluate drugs in preclinical studies that have a higher chance of clinical success in humans. Improved characterization of diseases and pathologies assists researchers to better identify drug targets and the therapeutic candidates that interact with them. Over the past two decades, preclinical lung imaging methods such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT), and Positron Emission Tomography (PET) have developed considerably, allowing examination of the lungs in small rodents at anatomical, functional, and even molecular or target levels at high spatial and temporal resolution. This paper reviews these developments and provides an overview of the current non-invasive lung imaging technology capable of driving preclinical studies.


## Introduction

Growing demand for safe, effective medicines for pulmonary disorders and diseases has sparked more research into therapies that address the treatment of pathological lung conditions — including asthma, chronic obstructive pulmonary disease (COPD), pneumonia, and lung cancer. Yet drug discovery and development are arduous and costly endeavors, motivating the pharmaceutical industry to continually find new ways to evaluate drug candidates more effectively. As such, pharmaceutical researchers can improve chances for successful drug development with an earlier and deeper understanding of drug characteristics that may impact final approval.

Improving the characterization of compounds and their effects in early and relatively non-costly phases is one way to increase the chance of success in later phases of drug development. Research on pathophysiology, and its early diagnosis and characterization, can also contribute to a better understanding of disease mechanisms and therefore increase the probability of finding an appropriate treatment.

Over the past two decades, researchers have found value in using non-invasive bioanalytical technologies, such as imaging, for preclinical pulmonary disease drug discovery and development. Imaging techniques can be used to non-invasively investigate, *in vivo*, animal biology and metabolism, disease models, and pharmacokinetics and pharmacodynamics of drugs. These imaging techniques include Magnetic Resonance Imaging (MRI), Computed Tomography (CT), and Positron Emission Tomography (PET).

These tools, and combinations of them, have become invaluable in preclinical pharmaceutical research to examine lung tissue at high spatial and temporal resolution in small rodents at the anatomical, functional, and molecular or target levels. In addition to focusing on specific lung diseases, imaging enables the diagnosis and quantification of characteristics related to pathological conditions of the lung, which include inflammation, mucus secretion and clearance, emphysema, ventilation, perfusion, fibrosis, airway remodeling, and pulmonary arterial hypertension.

Dr. Nicolau Beckmann and his research team at the Novartis Institutes for BioMedical Research in Basel, Switzerland, have spent the past two decades studying how the use of imaging techniques in preclinical studies can more effectively evaluate drugs and enable a higher chance of clinical success in humans. In addition to respiratory disorders, such as asthma and COPD, there is a range of other chronic conditions that can affect the lungs, such as cardiovascular disease and cancer. Their work has demonstrated how improving understanding of the molecular events that characterize lung disease and pathology enables researchers to better identify drug targets and the therapeutic candidates that interact with them. Dr. Beckmann explains:

*"The lung is a very intricate and interesting organ. If there is pathology in one part of the lung, the rest of the organ can compensate. The lung will continue to function even if a part of it is damaged, because it is so vital. As such, standard measurements like spirometry to assess lung health aren't often indicative of disease. Imaging can detect early disease in these situations when conventional approaches may only show advanced disease states. It's a very important argument in favor of using imaging techniques in pharmaceutical research, particularly for the lung."*

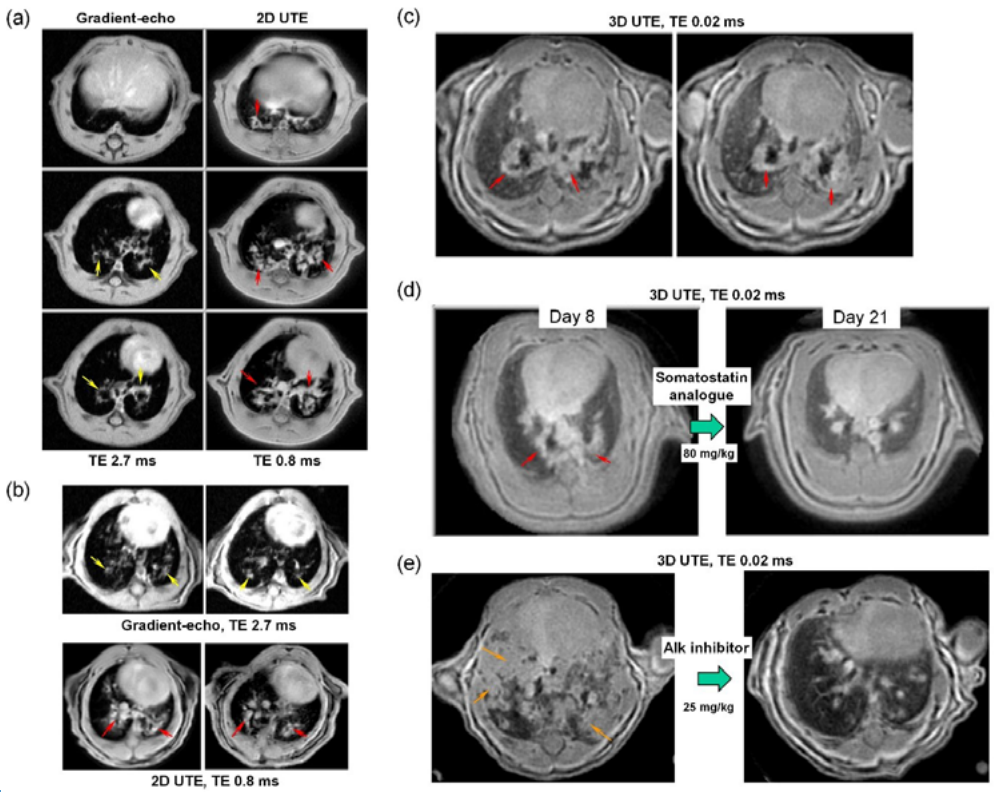



Figure 1 – Development of ultrashort echo time (UTE) for imaging the lung in small rodents increased significantly the sensitivity for detecting pathology in a shorter acquisition time. A bleomycin (BLM) model of pulmonary fibrosis illustrates this well. (a) Two-dimensional multislice gradient-echo and UTE acquisitions performed at 4.7 T on the same rat, at day 15 after BLM (4 mg/kg) administration. Image slices have the same geometrical parameters and positions for both acquisitions. It is clear that UTE allowed a more accurate detection of pathology reflecting tissue remodeling and fibrosis as evidenced post-mortem by histology. (b) The same is valid for gradient echo and UTE images at 4.7 T following administration of BLM (0.6 mg/kg) in mice. Of note, while the gradient-echo acquisitions lasted 22 min, UTE images were acquired in only 4 min in both rats and mice. Due to suseptibility effects, measuring the lung at a higher magnetic field is more demanding. Images c-e show representative images extracted from 3D UTE data sets acquired at 7 T with a TE of 0.02 ms. Acquisition times were 10 min for both species. (c) Images from a mouse at day 14 after BLM (0.6 mg/kg) administration. In addition to tissue remodeling/fibrosis along main airways (red arrows), the technique clearly allowed detection of parenchymal signal. (d) Rat images acquired on the same rat at days 8 and 21 post-BLM (3 mg/kg) administration. A somatostatin analogue was administered on day 9. The tissue remodeling/fibrosis detected along main airways on day 8 resolved on day 21. This shows that the compound had therapeutic effects on established fibrosis in rats. (e) UTE is obviously very useful for the detection of other disease conditions as well, as illustrated here with a mouse lung cancer model. The compound, an Alk inhibitor, basically erased the prominent tumors (orange arrows). All images shown here were acquired from isoflurane-anesthetized, spontaneously breathing animals, without the use of any gating.

## Non-invasive preclinical lung imaging

Pneumology is a branch of internal medicine involving the prevention, diagnosis, and treatment of disorders affecting the respiratory system. Over the past two decades, technological advances in imaging technology have led to a better understanding of lung disorders, as well as to a better identification of key features for confirming diagnosis of lung disease such as pneumonia, pulmonary embolism or pulmonary hypertension, early detection of lung tumors, and staging bronchial carcinoma.[2] These improvements have generated more interest in the use of imaging techniques for the development of new therapies for respiratory diseases. Dr. Beckmann describes these advancements:

*"These imaging techniques have developed over the last 20 years, and I'm always impressed by the breadth of applications that they allow us to pursue. For example, the acquisition speed of MRI has increased as the electronics and computer power have improved. Technology has also made important leaps for data reconstruction and analysis, and better instrument stability has improved the reproducibility of our experiments. More specifically, access to ultra-short echo time acquisition (UTE) for MRI experiments was an important development that enabled us to analyze tissues that are difficult with more traditional techniques (fig. 1). As a result, today we're able to do things that weren't possible two decades ago."*

As technology has advanced, pharmaceutical R&D has shifted towards targeted therapies for treating patients. In drug discovery and development, a biomarker-driven approach to developing targeted therapies and patient selection strategies can improve early decision-making on compound safety and efficacy by providing early research that validates a therapeutic concept, endorses a candidate molecule, and facilitates dose selection.

Advances in developing imaging biomarkers for respiratory diseases have benefited drug development research. What differentiates imaging biomarkers from others — for example, blood serum and urine analytes that have been used for decades in medicine and drug development, or more recent proteomics biomarkers — is the fact that imaging readouts tend to be much more closely related to the disease phenotype, thus facilitating direct associations between therapy and effect.

After a lead compound has been validated and optimized, it is essential to test it in a relevant animal model of disease to obtain information concerning drug efficacy, absorption, distribution, metabolism, and elimination. This phase is where imaging holds significant benefits for the pharmaceutical industry. Dr. Beckmann explains:

*"Pharmaceutical companies rely on understanding the biology underlying the disease process for developing compounds or therapies. For the lungs specifically, that requires inducing a disease status like lung inflammation. Our research here at Novartis found that imaging techniques made significant improvements to our preclinical work. For example, MRI can detect inflammation in the lung very clearly. So, we elicit the disease status, quantify it with MRI, and then administer the compound to assess its efficacy also using MRI. Standard techniques, such as histology, would not detect the dynamics of the compound, but MRI can. That enabled us to get a compound into clinical trials, and eventually to market, much faster. Now we can test a larger number of compounds, and we've expanded our studies of lung diseases by using imaging techniques."*

Today, cost-efficient and high-throughput longitudinal study of animal models can be achieved using imaging techniques such as MRI, micro-CT, and PET.

## MRI

The principal strengths of MRI are its non-invasiveness, high spatial resolution, and excellent soft tissue contrasting capabilities. The signal is governed by a number of parameters, and this wealth of information renders it a valuable tool for diagnosis, tissue characterization and *in vivo* morphometry, and for deriving functional information.

MRI is a particularly attractive imaging option for the evaluation of animal models since it provides good spatial resolution without the need for harmful radiation. It also has excellent contrast to distinguish between normal and pathological tissue. In addition to providing anatomical information, MRI can perform functional evaluation through quantification of flow, tissue diffusion, and perfusion or visualization of changes in blood oxygenation. Both structural and functional MRI are especially valuable for non-invasive animal studies. Dr. Beckmann explains:

*"MRI is a very flexible technique that allows us to analyze from the brain down to the limbs and to address a multitude of diseases. Because of its non-invasive nature, imaging technology like MRI enables us to repeat measurements on the same subject without harm. This provides the basis for the use of MRI in preclinical drug development. Another fundamental aspect is the translational character of MRI, because you can use it on both humans and small animals."*

A review by Beckmann and colleagues provides an evaluation of MRI applied to various models of airway diseases.[3]

## Micro-CT

CT is considered the gold standard for clinical lung imaging, and micro-CT provides X-ray imaging in 3D by the same method used in hospital CT scans, but on a small scale with massively increased resolution. Micro-CT systems providing high-resolution images and rapid data acquisition are a cost-effective means for detecting tissue structures, skeletal abnormalities, and tumors in small animals.

Micro-CT can be used to supplement data from other molecular imaging techniques by providing images of the very fine scale internal structure of objects non-destructively. It can generate 3D images of a sample's morphology and internal microstructure with resolution down to the micron level. Strategies to reduce radiation burden while keeping the spatial resolution have rendered the technique useful for repetitive, longitudinal measurements in the same subject.

## PET

PET detects radiation emitted from tracer substances injected into the body and labeled with positron emitting isotopes. These isotopes are bound to a targeted tracer. For example, the most prominent tracer $^{18}$FDG combines $^{18}$F-labeled fluorine with a type of glucose to target, in particular, cancer cells. PET detects gamma rays generated at the target site when a positron emitted from tracer is captured and annihilated by an electron in the tissue.

Being one of the most sensitive imaging approaches with picomolar amounts of radiolabel being readily detected and quantified *in vivo*, PET is the method of choice for pharmacokinetic studies of biologically active compounds, for instance drugs or drug candidates.

## Multimodality

These imaging techniques are rather complementary than in competition, as there is no "all-in-one" imaging modality providing optimal sensitivity, specificity, and temporo-spatial resolution. Anatomical modalities (e.g., CT, MRI) mainly reveal the structure of the tissues and organs, while the functional PET visualizes the physiology and function of the tissue.

For example, MRI or CT with their high spatial resolution provide a good anatomical reference for molecular data obtained with high sensitivity PET. This might be achieved by post-processing of data obtained in different imaging sessions or by simultaneous multimodality small-animal imaging such as PET/MRI or PET/CT.

## Translation of preclinical findings

Preclinical and clinical studies in the drug development pipeline should be mutually supportive. This link between the preclinical and clinical stages is reinforced by non-invasive bioanalytical technologies, such as imaging. The development of imaging strategies that meet the requirements for use in a clinical setting may facilitate the translation from animal models to human subjects because they minimize changes in experimental paradigms while the model organism is changed. For instance, small-animal imaging may help to improve the characterization of clinical readouts and, conversely, imaging in humans may support the refinement of animal models. Comparisons of MRI images in preclinical and clinical lung research have found a striking similarity, indicating the translational potential of MRI.[4] As a result, Dr. Beckmann believes using non-invasive imaging in preclinical investigations could help to further improve the protocols of clinical MRI. He explains:

*"Our preclinical work proceeds to clinical trials only when we are absolutely sure we have a viable and safe drug candidate. When conducting clinical trials, you have an ethical responsibility to make sure you are not unintentionally causing harm. So, if you use the same technique and you get similar results in the clinical trials as you did in the preclinical studies, the confidence that the compound is doing what you want increases. Imaging is one of the most important techniques in this sense. It helps us get the maximum information possible to be sure we've made a good decision to advance a compound to the next phase."*

## Impact of non-invasive imaging on the 3Rs

Biologically relevant small animal models have become important tools for the study of the fundamental aspects of human system function and dysfunction. In preclinical research, which encompasses all endeavors before testing compounds in humans, a significant portion of the activities is performed in small rodents, including mice. The main reasons for using mice are the possibility of using genetically altered animals and the availability of several disease models in this species. Additionally, smaller amounts of compound are needed for *in vivo* drug testing in mice than in higher species.

Imaging technology can support the Replacement, Reduction and Refinement of animal studies, otherwise known as the 3Rs.[5] For example, traditional methods of investigating lung disease in animal models, such as bronchoalveolar lavage (BAL) analysis and histology, often rely on invasive handling, restraint or even euthanasia of the animal, followed by sampling and measurement of body fluids and tissues. However, non-invasive imaging techniques that provide images of body systems are becoming increasingly available to use as alternatives, even for small animals such as rodents.

With the use of imaging, also toxicological effects of compounds on structure or function of organs can be studied *in vivo* without tissue dissection, sectioning, and staining. As a result, these *in vivo* assessments can eliminate the need to sacrifice the animal. Additionally, non-invasive imaging may considerably simplify the assessment of compound efficacy in small rodents, as the animal can be used as its own control, limiting any intra-subject variability and greatly improving the statistical power of results.

MRI is a particularly attractive imaging option for the evaluation of animal models since it provides high spatial resolution combined with excellent contrast to distinguish between normal and pathological tissue. In addition to providing anatomical information, MRI can perform functional evaluation, both of which are especially valuable for non-invasive animal studies. Dr. Beckmann explains:

*"In animal models, you are not only measuring whether the compounds are doing what you expect and what you intend, but also to determine that the compound is not harmful. This often means performing studies of long duration, between weeks to several months. Imaging becomes of particular interest in this context because of the ability to perform repeated measurements. The disease process and the effects of a compound on it can be followed longitudinally over time on the same subject, thus providing invaluable information on the effectiveness of a therapy while reducing the number of animals in the experiments. Depending on the protocol, reductions by up to 90% in the animal usage are feasible."*

### Bruker Preclinical Imaging Modalities and Solutions

Bruker offers advanced preclinical imaging solutions for a broad spectrum of application fields, such as oncology, neurology, cardiology, inflammation, infectious diseases, cancer research, functional and anatomical neuroimaging, orthopedics, cardiac imaging, and stroke models. The range of techniques includes MRI, nuclear molecular imaging PET, micro CT, and Magnetic Particle Imaging (MPI).

### MRI

Bruker's small animal MRI systems for preclinical MRI research deliver images of living organisms with high spatial and temporal resolution.

**BioSpec:** The ultimate in preclinical MR imaging is made possible with Bruker's BioSpec instruments. These instruments, which are available with field strengths up to 18 Tesla, enable groundbreaking research whether it be addressing fundamental questions or treatment of diseases. Combined with MRI CryoProbes, they deliver highest spatial resolution *in-vivo*.

**BioSpec 3T:** The BioSpec 3T is a compact, easy to site, instrument with a small footprint. Its superior Maxwell magnet design eliminates the need for liquid helium and completely overcomes reliability limitations of previous cryogen-free magnets that quench within minutes after a cooling disruption. It is designed for the study of mice and rats.

### Micro-CT

Bruker microtomography is available in a range of easy-to-use desktop instruments, which generate 3D images of sample morphology and internal microstructure with resolution down to the micron level.

**SkyScan 1278:** The Bruker SkyScan 1278 micro-CT system for *in vivo* imaging addresses the needs of scientists working in the areas of physiological response to disease and regenerative medicine. As part of the development of the SkyScan 1278, Bruker has created a new micro-CT spatial beam shaper, which reduces the absorbed dose of radiation by up to five times while maintaining high quality image output.

### Multimodal PET

Multimodal imaging is facilitated by the combination of technologies in a single, easy-to-use instrument, such as PET/MR and the PET/CT Si78.

**PET Insert:** The PET insert for simultaneous PET/MR spatially and temporally correlates the observation of metabolic, physiological and functional processes using two powerful complementary molecular imaging techniques. It can be combined with BioSpecs ranging from 3 to 15.2 Tesla.

**PET/CT Si78:** The PET/CT Si78 features homogeneous, high-resolution, and quantitative PET/CT imaging with a large field of view of 80 x up to 142 mm. The unique low dose x-ray technology, combined with ultra-fast full body 3D CT scanning, the familiar ParaVision 360 software and a high-precision motorized animal transport system, simplifies laboratory workflows.

## Conclusion

Lung imaging approaches have provided health professionals with a better understanding of how the lung is affected by a range of disorders so treatments can be optimized. It plays a crucial role in understanding how the body works in both healthy and disease states and describing responses to physiological or environmental change. The use of imaging techniques in preclinical studies has enhanced understanding of disease mechanisms by observing changes at systemic, organ, tissue, cell, and molecular level, in response to different physiological or environmental conditions and disease states, under prescribed experimental conditions.

Preclinical imaging techniques are central to evaluating the effectiveness and safety of new treatments and describing drug distribution patterns before clinical use. Additionally, their use in clinical trials assists with the translation from animal models to human subjects by minimizing changes in experimental paradigms while the model organism is changed. Preclinical small animal imaging has provided valuable insights into the mechanisms of lung disease and the effects of treatment. Some imaging techniques like MRI also can be used to observe organs without euthanizing the animals, reducing the numbers of animals required in the experiments, thus making them both more humane and more cost efficient.

Innovation in imaging technology has helped researchers to better understand the underlying causes of many lung disorders. Such research has identified key features for confirming the diagnosis of lung disorders, such as pneumonia, pulmonary embolism or pulmonary hypertension, early detection of lung tumors and staging bronchial carcinoma. This knowledge informs the development of novel therapeutic strategies, enabling pharmaceutical companies to evaluate drug candidates more efficiently, efforts that ultimately can improve patient outcomes and save lives. Dr. Beckmann describes the impact of these technological advances on drug development:

*"Pharmaceutical research is an extremely dynamic process, in which projects have specific timelines and objectives are constantly changing. Researchers need to cope with that. The versatility of imaging techniques is one of their biggest advantages. The advancement of imaging technology has helped researchers keep pace with rapid changes in pharmaceutical drug development, and I look forward to seeing where the field progresses in the future."*

Dr. Beckmann describes his collaboration with Bruker:

*"For more than 25 years I've been using Bruker imaging scanners. The performance and stability of the instruments are remarkable and an invaluable element in my research, as reproducibility of measurements and speed are central to my activities. The electronics and software evolved over time, and techniques used in the clinics became available at high quality for small rodent imaging. These techniques can be easily adapted to the particular questions to be addressed. I received professional technical support from Bruker whenever required, also at some critical moments of my career. I appreciate that I can call my colleagues at Bruker when necessary. This dialogue is extremely important and despite the growth of Bruker over the years, I continue to value the openness of the company."*

For more information about Bruker's preclinical imaging solutions, please visit

https://www.bruker.com/products/preclinical-imaging.html

To watch our lung imaging webinar series on demand, please click https://www.bruker.com/events/webinars.html

## About Dr Nicolau Beckmann

Nicolau Beckmann has an MSc in Physics from the University of São Paulo in Brazil and a PhD in Biophysics from the University of Basel in Switzerland. Currently he is the head of an imaging and histology group at the Novartis Institutes for BioMedical Research, Basel, and an assistant professor in Biophysics at the University of Basel. His research interests center around the use of imaging techniques in pharmacological research, in different areas as musculoskeletal diseases, neurological disorders, respiratory diseases, arthritis, metabolism and transplantation. He has co-authored more than 100 publications and edited three books. Nicolau serves as reviewer for several scientific journals, research agencies and the European Commission, and is in the editorial board of the Journal of Magnetic Resonance Imaging and Frontiers in Pharmacology.

For more information about the Novartis Institutes for BioMedical Research, please visit

https://www.novartis.com/our-science/novartis-institutes-biomedical-research

## About Bruker Corporation

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

For more information, please visit: www.bruker.com.

## References


1. Counting the cost of failure in drug development. Pharmaceutical Technology. 19 June 2017. https://www.pharmaceutical-technology.com/features/featurecounting-the-cost-of-failure-in-drug-development-5813046/
2. Van Echteld CAJ and Beckmann N (2011) A View on Imaging in Drug Research and Development for Respiratory Diseases, J. Pharmacol. Exper. Ther., 337(2): 335-349.
3. Beckmann N, Cannet C, Karmouty-Quintana H, Tigani B, Zurbruegg S, Blé FX, Crémillieux Y and Trifilieff A (2007) Lung MRI for experimental drug research, Eur. J. Radiol., 64:381-396.
4. Babin A, Cannet C, Gérard C, Wyss D, Page CP and Beckmann N (2011) Noninvasive Assessment of Bleomycin-Induced Lung Injury and the Effects of Short-Term Glucocorticosteroid Treatment in Rats Using MRI. J. Magn. Reson. Imaging, 33:603–614.
5. Beckmann N and Ledermann B (2017) Noninvasive Small Rodent Imaging: Significance for the 3R Principles. In: Kiessling F, Pichler B, Hauff P (eds) Small Animal Imaging. Springer, Cham.



**Bruker BioSpin**
info@bruker.com
bruker.com