axiom INSIGHTS

NEW X-RAY FLUORESCENCE IMAGING

An advanced method for the deep in vivo tracking of immune cells, biomolecules, drug carriers, and antibodies

AXIOM INSIGHTS **TECHNOLOGY IN A NUTSHELL**

Imagine if you were ...

... able to directly support your preclinical research process with a new method by directly tracking your drugs, drug carriers and immune cells over time in vivo and without any restriction in terms of depth. Take one step further: What if you could find out using this same method how many of these entities are in which position - in the whole body? What new insights could you gain if you could see the immune response directly through the dynamics of the tracked immune cells? Or if it were possible to directly visualize the efficacy of an administered drug on the population of the relevant immune cells?

The use of X-ray Fluorescence Imaging (XFI) method from *axiom insights* GmbH makes all this possible!

XFI from axiom offers the simultaneous combination of all these advantages:

- It has no imaging depth limitations (in contrast to optical fluorescence/bioluminescence)
- XFI offers high sensitivity (higher than CT and MRI)
- XFI allows several time points and arbitrarily long diagnostic time windows in one single animal (in contrast to PET/SPECT and ICP-MS), because XFI-markers have no finite lifetime like radiotracers
- XFI allows high spatial resolution: in vivo typically 0.5mm-1.0mm, in special cases down to 0.25mm
- XFI is non-invasive and thus allows for time series (in contrast to ICP-MS, histology)
- Any chemical element between Zirconium and Neodym can be used as XFI-marker for small-animal imaging

AXIOM INSIGHTS **TECHNOLOGY**

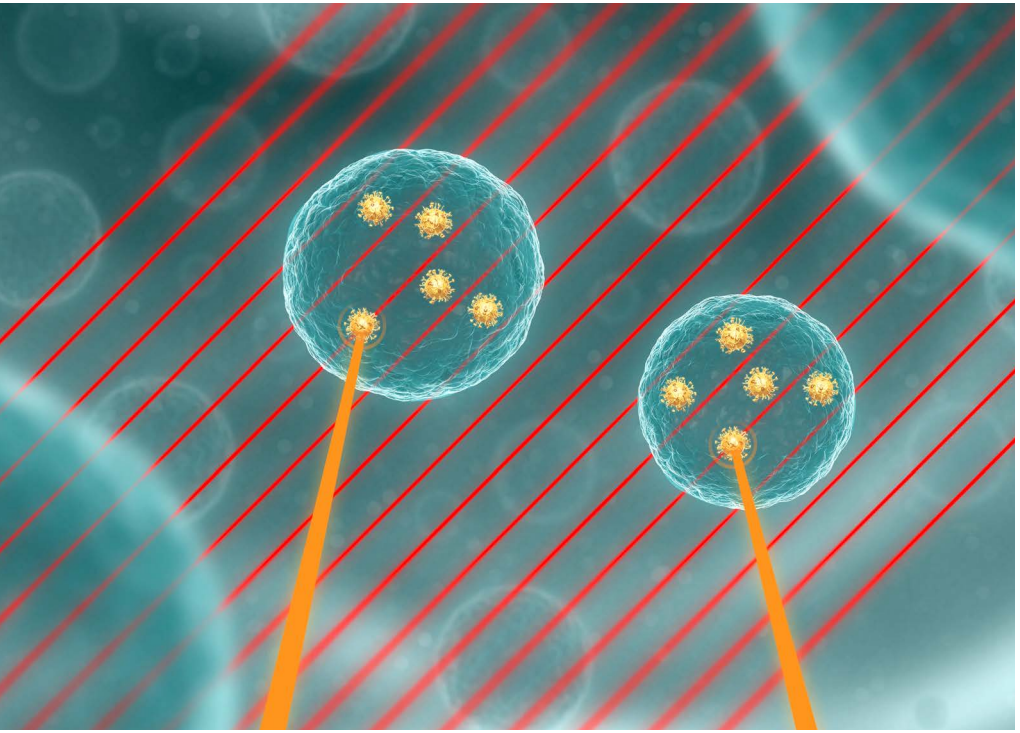


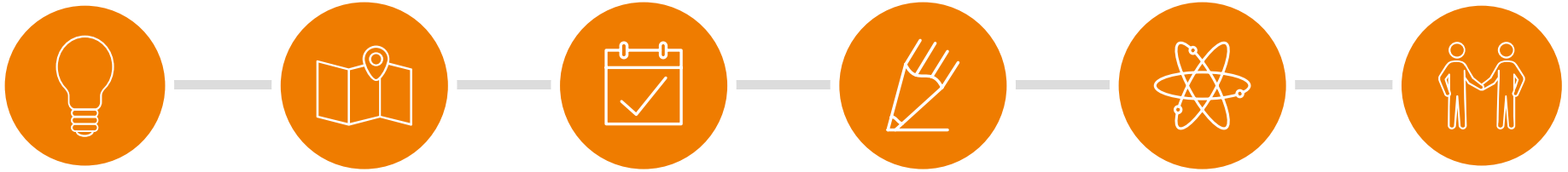
Figure: An X-ray pencil beam from the synchrotron scans along cells which are loaded with XFI makers such as gold nanoparticles (GNPs). A photon (red line) from the X-ray beam can remove an inner shell electron from a maker atom. This empty electron position is subsequently filled with an electron from a higher atomic shell. As a result of this event, an X-ray photon, the **"X-ray echo"**, is emitted into an arbitrary direction (orange line) where it can be recorded as a signal.

- *axiom insights* has priority access to brilliant X-ray beams for scanning modalities at a synchrotron facility
- When one of these X-ray beams hits heavy elements such as gold nanoparticles, an "X-ray echo", i.e. fluorescence is generated; hence, the modality used is X-ray Fluorescence Imaging (XFI)
- Unlike optical fluorescence/bioluminescence, XFI has no imaging depth limitation
- In contrast to CT or MRI, XFI provides a significantly higher imaging sensitivity
- Unlike PET/SPECT, the diagnostic window period can be arbitrarily long, because XFI makers give their “X-ray echo” whenever excited, there is no half-life period as for radiotracers
- Unlike PET/SPECT, XFI provides a high spatial resolution (mm or sub-mm)

In addition:

- XFI allows for advanced in vivo tracking of immune cells, viruses and nanomedicine tools and/or medical drugs
- In order to make these objects XFI-visible, they need to be loaded with or bound to nanoparticles and/or XFI makers, such as iodine-based small molecules

AXIOM INSIGHTS **CONTRACT RESEARCH PROCESS**



Kick off

Kick off: *axiom* research team work out a high-level concept of the project with you

Check starting point

Check with research team from *axiom*, how to transfer your demand into an analytic study:

- NDA signed
- Entity of interest
- XFI maker
- Target region of interest
- Responsibility of action points
- Coordination of the logistical implementation for the measurements at DESY in Hamburg (Germany)

Define action & logistics

Define all responsibilities of actions and logistics:

- Setting the timetable
- Involved teams
- Delivery of Entities
- Preparation of XFI makers
- Preparation of probes or biological samples
- Expected timing

Sign deal & place order

Conclude a service contract, based on a specific elaborated study for contract research

Start research

axiom team prepares and performs measurements, followed by data analytics and writing final report.

Report out & handover

After finishing all research activities, axiom analytic team will present you a report out and hand over a study documentation

AXIOM INSIGHTS **PORTFOLIO**

axiom's Service Portfolio

axiom can provide you with analytical studies that can help you to reduce the costs of your R&D, shorten the time required for preclinical research, reduce the number of animal experiments, and increase the chances of obtaining approval by Regulatory Authorities. The studies are customized according to the requirements of the questions being asked. The focus of our work is as follows:

Distribution Study

- With our analysis, you will receive extended data from ex vivo (in situ) or in vivo measurements of which entities accumulate in what quantity at which location in the entire object under investigation.
- Track the dynamics of your drugs, drug carriers, antibodies, or immune cells over any diagnostic time window in vivo and without depth restriction in the same test object.
- Obtain data at different points in time on the population dynamics of tracked immune cells and visualize this information directly
- Make your biomolecules visible to imaging if it remained invisible so far

Safety study

- Obtain advanced data for better risk assessment regarding the deposition and degradation of your entities linked to XFI makers for your evaluation and for exchange with Regulatory Authorities such as the FDA and EMA.

Efficacy study

- Gain clarity about the achievement of objectives and efficacy of the ingredients or ingredient carrier systems
- Repeat in vivo measurements of efficacy in the same subject for longitudinal pharmacokinetics and pharmacodynamics purposes

XFI from *axiom* is a unique combination of technical imaging opportunities that is now available to you for your research.
Gain access to new data that were previously not accessible due to the limitations of other modalities.

If you have further requirements for analyses using *axiom* XFI or detailed questions about the method itself or our services, we would be happy to discuss them with you and your R&D team in person.

AXIOM INSIGHTS **APPLICATION**

***In vivo* small animal application**

In vivo cell tracking: axiom insights offers in vivo access to the spatio-temporal distribution of cells for monitoring cell therapies and/or immune reactions.

In vivo medical drugs tracking: axiom insights can assess the efficacy of drugs and drug carrier by studying their pharmacokinetics and in vivo pharmacodynamics when drugs are bound to XFI markers or nanoparticles. make your biomolecules visible to imaging if it remained invisible so far

The animal imaging is designed in collaboration with the customer; the induced radiation dose is below damage levels, so the anesthetized test animals will still be alive after the assessment. This allows for the same animals to be used for time series studies and a reduction in the number of test animals required.

Advantages:

- No imaging depth limitations
- High sensitivity
- Allows several time points and arbitrarily long diagnostic time windows
- High spatial resolution
- Non-invasive
- Flexibility in using XFI makers

AXIOM INSIGHTS **APPLICATION**

Ex vivo (in situ) / in vitro application

axiom insights provides tracking data on distribution of molecules of interest in probes like artificial tissues or biological samples for preclinical R&D in pharma

Advantages:

- Non-invasive, hence, samples are not damaged
- Abritrary tissue depth in contrast to laser ablation or optical fluorescence
- High spatial resolution in contrast to Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS)

Small design study package

We offer a simulation in order to predict the expected sensitivity and to design the optimal test setup and final decision-making process

Advantages:

- Fast feasibility check prior to beginning the major challenge of *in vivo* research
- Possibility to adjust settings for improved tracking sensitivity
- Upfront confidence with the *axiom insights* method

AXIOM INSIGHTS **DATA GAINED** & **VISUALISATION**

Data gained

- Since XFI is a scanning modality, the data gained is depicted as a map (typically on a two-dimensional projection plane). Each pixel refers to the amount of XFI makers located in this scan beam direction. The scan time for such a map depends on the size of the target area, resolution, and required sensitivity.
- If you require a full 3D map, we can apply XFI-CT or -tomosynthesis mode, however, this would require longer exposure time and a higher dose. Two-dimensional projection maps are usually sufficient because the objects of interest such as immune cells, are concentrated at the sites of interest. This means that the diffuse background does not overlay these areas and that it can be subtracted when using just a few different scanning angles (tomosynthesis).

Visualisation

- *axiom insights* maps typically have a spatial resolution of 1.0 mm and a temporal resolution on the scale of minutes. We can also repeat the scans at different time points to deliver 4D maps.

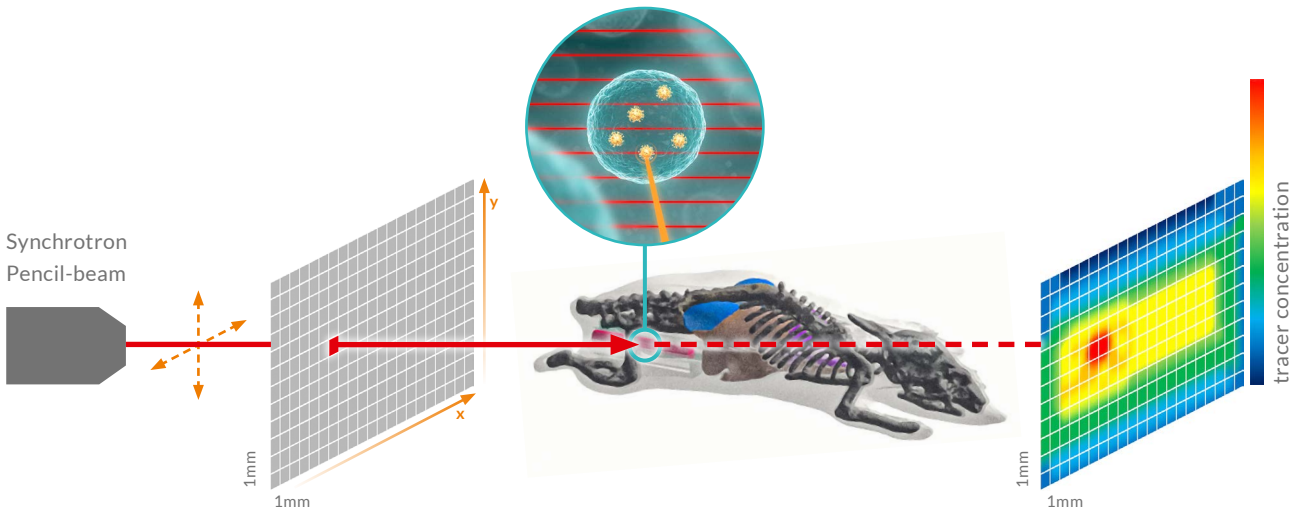


Figure: Small animal *in vivo* tracking scheme. When an XFI maker is hit by the beam, a scanning X-ray pencil beam (red) from the DESY PETRA-III synchrotron facility excites an “X-ray echo”, i.e. fluorescence. Scanning typically takes place across a projection plane (grey). For each scanning direction (red square), the concentration of the maker retrieved is depicted in a “heat map” (color map) after data analysis.

AXIOM INSIGHTS **ABOUT**

- *axiom insights* is a spin-off company of DESY (Deutsches Elektronen-Synchrotron), the large-scale research facility with one of the world's most brilliant X-ray light sources (PETRA-III synchrotron beamlines)
- Our office is based in the Innovation Village@DESY Research Campus in Hamburg-Bahrenfeld, Germany.
- *axiom insights* acquired IP-protected advanced XFI-technologies from the University of Hamburg (UHH) with whom we will provide our services as contract research. The UHH and the PETRA-III synchrotron facility are both on the same Research Campus as *axiom insights*, in Hamburg-Bahrenfeld.

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