The MERMAID Project: Multi-Emission Radioisotopes - Marine Animal Imaging Device

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ABSTRACT

MERMAID (Multi-Emission Radioisotopes – Marine Animal Imaging Device) is the first imaging concept fully devoted to non-invasive radioisotope imaging of small aquatic animals. In biomedical research, such an in-vivo functional imaging tool can provide new insights into the physiology of model organisms, e.g. zebrafish. Deepening knowledge of the metabolic processes in aquatic animals is also key to improving sustainable aquaculture. MERMAID includes a dedicated proof-of-concept prototype and new approaches for tracer application and animal handling. To date, a first proof-of-concept and various novel imaging chamber concepts have been developed and tested, and preliminary trials for alternative radiotracer transport have been successful.

Keywords: PET; small animal imaging; zebrafish.

1. INTRODUCTION

Positron Emission Tomography (PET) is a non-invasive molecular imaging technique that relies on the administration of positron-emitting radiotracers. PET provides information of physiological and biochemical functions through tomographic images of the radiotracer distribution. To date, PET scanners for humans and rodents are commercially available. The latter are a common tool for biomedical research, as rats and mice are well-established model animals. Certain fish species, e.g. zebrafish, have also become relevant as model organisms to study several human diseases, from obesity to neurological disorders. However, dedicated PET scanners are not available for fish. As PET is based on the radiotracer principle, dedicated fish PET can also provide unrevealed information about aquatic food chains and other relevant dynamic processes, e.g. within sustainable integrated multitrophic aquaculture. Unfortunately, several obstacles hinder the use of PET for fish imaging:

- PET scanners are not designed for animals smaller than mice;
- the need of imaging fish in water-filled imaging chambers is not contemplated;
- radiotracer administration is very challenging given the small size of the animals.

The MERMAID concept encompasses all steps necessary to make PET imaging of small aquatic animals possible, as the project includes:

- flexible proof-of-concept (PoC) PET prototype;
- dedicated imaging chamber;
- new ways of radiotracer administration;
- studies on imaging protocols.

MERMAID goes beyond constructing a PoC; it also aims to develop and establish imaging procedures for this novel application. The unparalleled combination of molecular tomographic imaging with novel methods of radiotracer transport will break new ground for life, biomedical and environmental sciences, and sustainable aquaculture industry.

A first PoC system has been built and characterised, including data processing and image reconstruction algorithms. First images with phantoms have been reconstructed and various concepts for animal handling and immobilisation have been developed and tested *ex-vivo*. Alternative approaches for tracer administration using labelled microalgae have shown very promising results.

2. STATE OF THE ART

To date, there is no dedicated PET system for fish. PET studies have been mainly restricted to larger fish species [1] using small animal or clinical scanners. First attempts on zebrafish with small animal scanners have been reported [2][3], but have not yet been able to provide precise information about the activity distribution. This is partly due to the limited spatial resolution of current

commercial small animal PET or PET/CT scanners (about 1.4 mm). Since there are no adequate imaging chambers yet, the scanning time to comply with animal welfare regulations is often too short to provide sufficient image quality. Alternatively, scans are carried out *post-mortem*.

Anatomical information of adult zebrafish has been obtained using MRI [4][5], and micro-CT [6]. In fact, the increasing interest in zebrafish imaging has led certain companies to advertise existing micro CT systems also for zebrafish[1]. Optical imaging methods are often used to study zebrafish larvae, as they are transparent; however, as adult zebrafish are opaque, optical imaging is less suitable due to the low penetration of light into tissues and water. In any case, none of these tomographic modalities can deliver the kind of information that PET provides.

Concerning PET, the tracer is ideally injected directly into the caudal vein. Whereas this is possible with large fish species [7][8], it becomes very difficult for small animals such as zebrafish; alternatively, it can be administered indirectly via the surrounding water, but at the cost of lower uptake and larger preparation times. Furthermore, the fixation of the fish during a measurement as well as the constant supply of oxygen-rich water and anaesthesia remains a problem. This affects not only PET imaging but other in-vivo imaging modalities. Current solutions are: short scans outside the water [9] and water-filled flow chambers [10][7], not yet optimized for zebrafish applications. However, these options might not comply with animal welfare regulation in several countries.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The MERMAID concept is unique: It encompasses the first tomographic device for small fish, as well as equipment and procedures fully conceived for fish imaging. MERMAID's main goal is to allow for PET imaging of zebrafish and other small aquatic animals (e.g. juvenile salmons). The realisation of this concept thus opens the door to unexplored paths in several disciplines. Such a dedicated device will be a valuable tool in biomedical research, where zebrafish is used as a model organism to study and develop new therapies for diabetes and other metabolism dysfunctions, Alzheimer disease, etc. Additionally, MERMAID will contribute to a better understanding of small aquatic animal physiology, including mussels and other species involved in sustainable aquaculture, environmental research, and biology.

The first step is the development of a dedicated custom scanner prototype with high spatial resolution and a flexible geometry. The latter is conceived to allow fish of various sizes to be examined, as well as to accommodate further imaging modalities, such as Computer Tomography (CT) or a Compton Camera (CC). Using a CC and a dedicated reconstruction software, visualisation of non-pure positron emitters will be enhanced. Such radioisotopes are becoming more and more relevant in medical diagnostics and theranostics. Besides the application, our approach differs from other PET/CC concepts in terms of configuration and software.

In-vivo imaging of fish, in particular for long scans and dynamic studies, requires a dedicated imaging chamber. The MERMAID imaging chamber is conceived to gently immobilize the fish and provide constant supply of fresh, oxygen-rich water and anaesthetics over the measuring period, as well as removal of excretion products and monitoring of vital functions. Although some few approaches have been proposed, neither do they include all aspects covered by ours nor are they commercially available.

Regarding tracer administration, MERMAID aims to fully exploit radiotracer techniques and thus go beyond conventional nuclear imaging. In addition to direct administration procedures, we also propose to use radiolabelled micro-organisms as radioactivity carriers. This is an unexplored approach for PET imaging which expands PET applications. Together with our dedicated imaging device, this procedure will allow for tracking food and nutrients through relevant species present in closed aquatic environments.

4. PROJECT RESULTS

The results obtained during the first phase of ATTRACT are described in the following.

Scanner Prototype

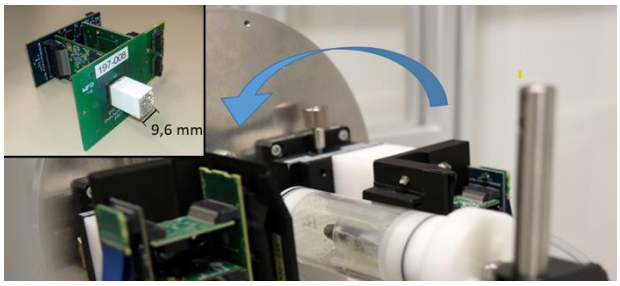

Fig. 1. PoC Prototype with two detector modules. The modules can rotate around the object (imaging chamber with fish dummy inside). Upper left: One detector module consisting of crystal matrix, SiPM and ASIC.

[1] https://www.blue-scientific.com/imaging-zebrafish-micro-ct/

A first PET prototype has been built and characterised. It includes two detector modules with a variable separation. Each module consists of an 8x8 LYSO crystal matrix, directly coupled to a 64 channel SiPM. The crystal size is 1.2 x 1.2 x 15 mm^3. To digitise the signals, a TOFPET2 ASIC [9] working in charge integration mode is used. The two modules can rotate in user-defined steps around the object to measure a full dataset (Fig.1). This setup allows for imaging a Field-of-View (FoV) of 9 mm diameter without data truncation. CT components (X-ray tube and detector) have been integrated in this setting but not yet put into operation.

Detector Calibration and Characterisation

The 128 channels of the first prototype were characterised with the aim of clear photopeak identification, good energy resolution and channel homogeneity. The accuracy of peak identification and energy resolution mainly depended on the operating voltage and the ASIC energy thresholds. For characterisation, the ratio between photopeak events to the total number of events was also used. This ratio decreases up to 50% for increasing operation voltage (2.5 to 6 V) where the rate goes up with higher energy thresholds. The mean Full Width at Half Maximum (FWHM) energy resolution at the photopeak (Fig. 2) amounts to approx. 8% for operation voltages higher than 3 V, whereby individual channels vary between 5.5 and 11.5%. The standard deviation of the detector efficiency was measured to be approx. 2%. In terms of count rate performance, a linear behaviour was found for activities between 14.5 to 35.3 MBq which translates to 3.4 to 8.2 MBq/ml. The best coincidence time resolution (CTR) of an individual channel pair was approx. 300 ps, whereas the average CTR lies at around 400 ps after time alignment. It is to be noted that the system has not been optimised for timing.

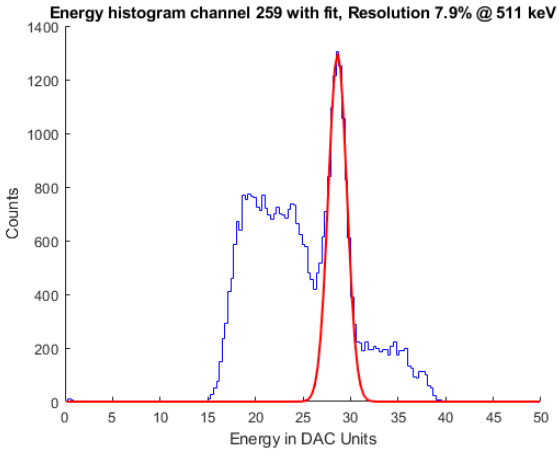

Fig. 2. Energy Spectrum of a measured sodium-22 two-point-source for one detector channel. Energy Resolution of fitted photopeak (red) is approx. 8% at 511 keV.

Reconstructed Images

Data from two point sources of sodium-22 (1 mm diameter each) were reconstructed using a custom MLEM algorithm (Fig. 3). The current algorithm includes a system model which takes into account the detector geometry and the small FoV of the scanner, but still neglects crystal penetration and inter-crystal scattering. The reconstructed distance between the two point sources corresponded to the true one. The current system FWHM, determined from these images, is ~1.4 mm. Further improvement of the reconstruction algorithm and related system model are planned to improve the resolution and compensate for parallax effects. The latter are particularly relevant for the current configurations, due to the small distance between source and detectors compared to the length of the individual crystals. To further improve the system and support reconstruction developments, a digital phantom of a zebrafish was further developed (Fig. 4). The phantom is based on micro-CT scans (16 µm resolution) and will be used for Monte-Carlo simulations of the scanner [11].

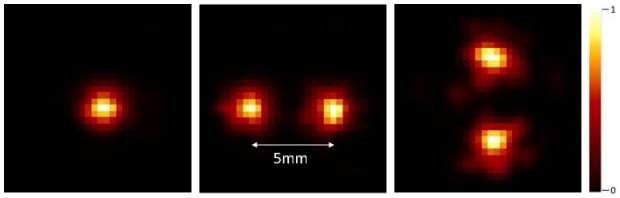

Fig. 3. Reconstructed images of a sodium-22 two-point source with approx. 400 kBq per point. From left to right: x-z plane, y-z plane and x-y plane.

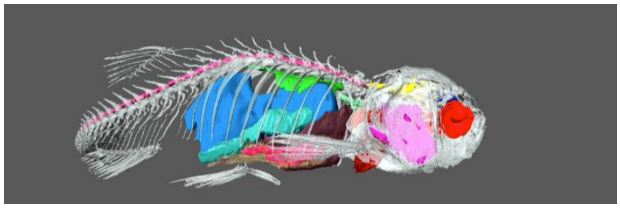

Fig. 4. Digital phantom of a zebrafish based on segmentation from micro-CT images. Different organs are assigned with different colours [11].

Imaging Chamber

To gently immobilise the fish during measurements and to assure fresh water and anaesthetics supply, a dedicated imaging chamber was constructed (Fig.1). This chamber consists of a flow-through chamber and an inner holder that immobilises the fish. The latter is flexible enough to accommodate various fish sizes without injuring the animals. The amount of incoming water and anaesthetics can be regulated using peristaltic pumps. The flow velocity determined not only the amount of fresh water and anaesthetics but also the drainage of possible tracer

residues excreted by the fish, which could deteriorate the image quality.

Tracer application

Several micro-organisms have been compared as potential radioactivity carriers. To this aim, the radioisotopes ^{18}F-FDG and ^{125}I were used for labelling and first tests were performed with mussels. The results point out to the microalgae *chlorella sorokiniana* (size of ~10 µm) as an adequate carrier. The labelling efficiency was between 40-45% of the original activity. Compared to the direct administration of ^{18}F-FDG into water, feeding the mussels with radiolabelled *chlorella sorokiniana* increased the amount of tracer uptake and it was up to 16 times faster than direct administration in the surrounding water. Further studies with zebrafish and other radiotracers are envisioned.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

For commercialisation, we will first focus on the flow-through imaging chamber, including a fish immobilisation setup. We envision a plugin system for multi-modal imaging that can easily be integrated in commercial small-animal PET, CT or MRI scanners. This chamber will incorporate equipment for water quality and animal monitoring, as well as a feedback control system for fresh water and anaesthesia supply. This chamber is now on a Technology Readiness Level (TRL) of 3-4. For scaling it to level 5-7, several steps are necessary: The single components need to be improved, and a reliable continuous operation must be ensured. As the current setup has only been tested *ex-vivo*, animal trials are necessary. According to the results, further refinements might be necessary. After the development phase, this device is relatively easy to be produced in larger quantities.

Additionally, our imaging concept and associated methodology can be offered as a service for molecular imaging of aquatic animals in core facilities. To this end, the MERMAID prototype needs to be scaled from a TRL of currently 4 to level 7, analogously to the steps above. In parallel, the novel methodology for tracer application needs to be tested and stablished in animal trials.

5.2. Project Synergies and Outreach

Our current Consortium offers a twofold expertise in marine biotechnology and biomedical imaging. For the development of the flow-through imaging chamber, further partners within the University of Lübeck and nearby centres of the *Fraunhofer Gesellschaft* have been identified, e.g. in the domains of feedback control systems, and 3D printing technology. For the fabrication of the single components, liaisons with an SME in the domain of medical imaging are planned, and we are already in close contact with a regional SME. To establish our imaging methodology as a service, co-operations with research institutes working with zebrafish, as well as the European Zebrafish Resource Centre will be desirable to identify further specific fields of application and use cases.

We have started collaborating with the ATTRACT Phase I project H2I2 within the field of PET software. Valuable information exchange, mainly related to detection and readout electronics, has also taken place with other researchers involved in ATTRACT Phase I projects, and will be maintained in Phase II.

In terms of dissemination for the public, we will further exploit channels such as social media, and online videos. Because of the COVID-19 pandemic and related restrictions, more events like open days and student tours will take place virtually, so that these activities can be further exploited to reach a broad audience beyond Lübeck.

5.3. Technology application and demonstration cases

MERMAID aims to further consolidate fish as a model for human diseases. In the long term, this goal translates into reducing research costs and fostering the translation of the outcomes from animal to human, with subsequent benefits for drug discovery and therapy development.

Our second long-term goal is to improve the existing knowledge of aquatic animals' physiology and aquatic food chains, thus supporting the optimization of sustainable aquaculture. Finally, expanding functional imaging of small aquatic can also benefit environmental research, given that several aquatic animals are used to monitor water pollution. Additionally, some of the approaches to be developed are not restricted to aquatic animal imaging.

Since we aim at developing a novel imaging paradigm, once it has been established, European research infrastructures devoted to either biomedical research with zebrafish or aquaculture can take profit from both the nuclear imaging methodology as well as the multimodal plugin imaging chamber. We believe that there is a growing market for imaging equipment of zebrafish and other aquatic animals, and a lack of adequate products.

5.4. Technology commercialization

We are currently preparing a patent application for the flow-through chamber. The future product could be commercialised via a spin-off company, with the support of the University dedicated advice centre that also helps

with several financing instruments. Alternatively, an SME in the field of medical imaging could take over the commercialisation.

A dedicated aquatic imaging service could be established at the Campus Lübeck, for instance within the SAIL facility, currently under construction[2]. In combination with the neighbouring Fraunhofer institute with its fish husbandry, the Campus Lübeck offers an ideal environment for this purpose.

5.5. Envisioned risks

The components and methods for the imaging chamber have been tested and validated *ex-vivo*, and the components themselves are well established and readily available. No significant risks have been identified so far. The main obstacle is the small size of the market, even if the product should be suitable not only for PET but also for other imaging modalities. On the other hand, the costs are relatively low. Scaling the product for larger fish species and scanners is also an alternative.

Because of its breakthrough character, the establishment of the MERMAID imaging methodology involves a relatively high risk; further research is still necessary to determine the limits of technology (e.g. spatial resolution) and specify the kind of studies and scientific questions which can be better solved with this approach. However, the high risk in this application is accompanied with a potentially high gain.

5.6. Liaison with Student Teams and Socio-Economic Study

As part of the University of Lübeck, we regularly contribute to educational and training activities of students, including supervision of research work. The MERMAID project has already hosted several students of various University programmes and levels. As Phase 1 was much focused on technical and research aspects, a liaison with Management student teams seemed more appropriate for Phase 2. For the latter, a design thinking pilot will be helpful to identify additional applications and markets.

6. ACKNOWLEDGEMENT

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