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Multimodal Imaging Trials with Zebrafish Specimens

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ABSTRACT

Biomedical imaging is an important technique that can be used for several applications such as cancer research and cardiology. A range of imaging technology, such as PET, SPECT, micro-CT, and optical X-ray, is available for imaging. However, many research institutes use rats and mice for preclinical experiments. The purpose of this study was to determine if Zebrafish are compatible for use in pre-clinical imaging, and which modalities and probes work best. Different Zebrafish specimens were tested using five different modalities and four probes. In order to assess with two-dimensional modalities, both fluorescence and planar X-Ray were performed on the specimens. The fluorescence imaging was acquired using OsteoSense 750x and ProSense 750x as the probes. Through the results, we discovered that OsteoSense did not work as well as the ProSense. The next three modalities represented three-dimensional imaging. These modalities consist of X-ray Computed Tomography (CT), Positron Emission Tomography (PET), and Single Photon Emission Computed Tomography (SPECT). The standard settings of high dosage and low voltage were used to assess with X-ray Computed Tomography. Sodium Fluoride (NaF) and Fludeoxyglucose (FDG) were the two probes tested with PET imaging, while MDP with technetium-99 was used for SPECT. The majority of the probes were detected in the specimens, but not at the correct target.

INTRODUCTION

Over the last decades zebrafish have emerge as one of the leading vertebrate models for preclinical and biomedical research. Zebrafishes possess several different characteristic that make them a target for research. One of the most important feature is they share many similarities with human including their organ system. Since zebrafish, have optically transparent embryos and larvae imaging them to evaluate different diseases has become promising. They are very inexpensive to maintain, produce a large quantity of offspring and are constantly developing. In a recent study, Ignatius and Langenau has explore cancer in Zebrafish using fluorescent imaging. They have identify that because of the clarity of zebrafish features and the use of different fluorescent protein and dyes such as GFP/EGFP and RFP that Zebrafish are good model for imaging difficult tumors as well monitor growth development in diseases such as cancer. This data aligns with the result found in this study. Investigating what probes and modalities zebrafish are compatible with is important in understanding how beneficial this organism is as a model for different diseases in further research projects using two and three-dimensional imaging techniques.

MATERIALS & METHODS

Animal Handling

Each Zebrafish specimen was placed in a smaller petri dish with seven milliliters of water using a net. After the fish was in the dish, the different probes were added directly to the water so that the specimen can absorb the probes through their skin while they swim. The fish swim in the water with the probes for different time intervals for each probes. The time intervals vary between 45 minutes and 24 hours. Before the animals were imaged, they were euthanized using a 2-phenoxyethanol solution.

Imaging

Using the Bruker In-Vivo Xtreme, a Zebrafish was positioned in the center of the imaging platform to acquire a fluorescence and X-ray image. In the first fluorescence image, the fish swam in
200µL of Osteosense 750X for 24 hours. Following the Osteosense 750x probe, 100µL of ProSense 750x was used with a Zebrafish with a tumor using the same time interval. The fish fluorescence specimens were imaged under the following conditions: an excitation of 730 nm and emission of 830nm, 1.1 f-stop with 30 seconds exposure, binning of 2x2, field of view (FOV) of 7.2cm, and a focal plane of 0mm. The optical x-ray image had an exposure time of 60sec and binning of 1x1.

The three-dimensional modalities used the Bruker Albira. The Zebrafish specimens were laid in the center of the imaging bed. For the PET imaging there were two radioactive probes used, they included FDG, NaF and SPECT. FDG had a radioactivity of 428µCi and was injected into the fish tank for forty-five minutes. The NaF probe followed the same methods; the only difference was its radioactivity was 480µCi. The three-dimensional images were acquired using the following settings for PET image: the single best bed for twenty minutes. The CT scan used the standard CT setting with high dosage and low voltage.

**Imaging Analysis and Rendering**

Upon the acquisition of the images, three different types of software were used to analyze the results. They included Image J, Volview and PMOD. Image J was used to make the different montages. Volview and PMOD were used to analyze the three dimensional results.

**RESULTS**

As stated above, planar X-ray and fluorescence were imaged using the In Vivo Xtreme. In Figure 1, an optical X-ray of Zebrafish with a tumor can be seen. There are different images taken: the first one shows the whole fish, while the other three are enlarged on different features of the specimen. Figure 2 is a montage of Zebrafish specimen using the ProSense750X probe. The first image in that montage is just an X-ray followed by the fluorescence and then the overlay of the two. In this image, most of the fluorescent dye is seen in the upper part of the fish body close to the gills and mouth. The last figures 3-5 all demonstrate images from the three-dimensional modalities. Figure 3 shows X-ray Computed Tomography of a Zebrafish with the anatomy labeled. Figure 4 and 5 are both PET images with Radioisotopic probes. Figure 4 shows the FDG probe and NaF in figure 5. The montage shows the specimens rotated 90°, 60°, and 30° from the left and right.

![Figure 1: Optical X-ray Imaging acquisition of Zebrafish with a tumor. Images were acquired at different positions with the following settings: 7.2cm FOV, focal plane of 0 mm, Mag stage 10 and 60 seconds exposure time.](image-url)
**Figure 2:** Fluorescence imaging acquisition of Zebrafish with ProSense 750x. Images were acquired with the following settings: an excitation of 730 nm and emission of 830 nm, 1.1 f-stop with 30 seconds exposure, 2x2 bin, 7.2 FOV, and 0 mm Focal plane.

**Figure 3:** Computed Tomography Image of Zebrafish with labeled anatomy using the CT Standard setting of high dosage and low voltage.

**Figure 4:** PET Image of Zebrafish with a FDG using the following conditions of a single best bed for twenty minutes. The image shows from left to right where the specimen was rotated 90°, 60°, and 30°.
**DISCUSSION**

The two-dimensional modalities both seem to work with Zebrafish imaging. Fluorescence, the first modality used, did show that the specimen can be imaged, but one of the probes did not work as well. OsteoSense 750X is a fluorescent in vivo bisphosphonate imaging dye that targets bone. However, the dye was not clear enough to distinguish where it was detected in the body. The second fluorescent dye, ProSense 750X, is a protease in vivo imaging agent activated by important disease markers. It is used as an indicator for disease developments in animal tumor models. This is why this probe was used for the Zebrafish specimens with tumors. By looking at Figure 2, it is shown that the probe was able to detect some of the features in the body. Most of the imaging agent is shown near the swim bladder and the spine. The intensity shown to be around 127 in this area. However, the tumor was not clearly differentiated by the probe. The next modality, planar x-ray, did show that image works with the specimen, which was expected.

Both PET and CT, the three-dimensional modalities, were able to successfully acquire images. Through the CT scans, more features can be distinguished than with the planar x-ray. In Figure 3, one can see the anatomy of a Zebrafish specimen labeled. FDG and NaF were the two radioactive probes experimented with PET. FDG is a glucose probe that is uptake by high metabolism using cells such as the brain and kidney. In Figure 4, it is shown that most of the probe was detected in the gills and around the mouth. Through this, it could be concluded that probe did not identify the correct target. The last probe used was NaF, which is similar to OsteoSense in that it is supposed to detect bones. However, it was shown in Figure 5 that the probe was identified in the gills, as with the FDG probe, and in the area near the spine. This area can be part of the digestive tract of the Zebrafish, but it cannot be confirmed by the image.

**CONCLUSION**

Two-dimensional and three-dimensional imaging of Zebrafish specimens were successfully acquired using the Bruker In-Vivo Xtreme and Albira. A clear X-ray image, in both planar and tomographic modalities can be captured using the Xtreme and Albira. The Fluorescence image can also be obtained; however, it was shown that ProSense shows a more distinct image than OsteoSense. FDG and NaF both seem to work with Zebrafish. It shows that most activity occurs in the specimen gills. These results and methods shows that further pre-clinical imaging studies can be obtained using Zebrafish.

**REFERENCES**


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