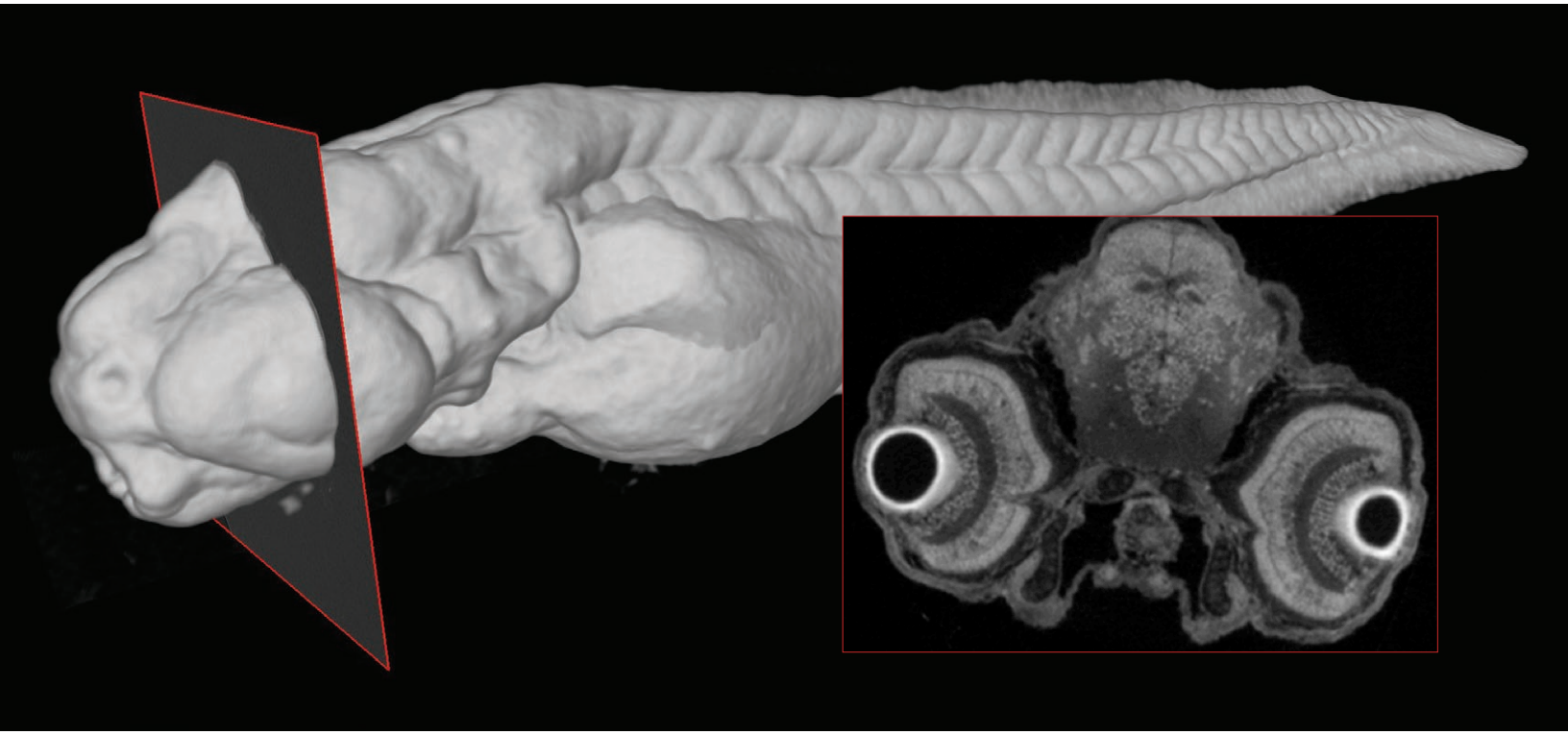
Application Note



3D Quantitative Histology of Zebrafish

ZEISS Xradia Versa X-ray microscopes

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3D Quantitative Histology of Zebrafish

ZEISS Xradia Versa X-ray microscopes

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Research in developmental biology often begins by observing phenotype differences in control and mutants in the embryos and larvae of model organisms such as mice and zebrafish. Traditionally, these embryos have been imaged with 2D techniques such as optical microscopy or with low resolution (~10 µm and above) techniques such as conventional X-ray micro-computed tomography (micro-CT) and optical projection tomography (OPT).

The recent introduction of 3D X-ray microscopy (XRM) offers cellular resolution at <1 µm to provide an unprecedented capability for quantitative 3D phenotyping. ZEISS Xradia Versa submicron microscopes (<700 nm) deliver 3D volumetric and distribution measurements of anatomical and cellular features including eye, nerve, brain, and heart cells. In addition, the non-destructive nature of X-rays and complementary sample preparation techniques enable correlative studies between X-ray microscopy and TEM or serial-sectioning SEM imaging techniques.

Methodology

High resolution tomographies of a stained zebrafish were performed on an Xradia Versa microscope without destroying and slicing through the samples. The data sets were segmented to provide quantitative, volumetric information.

A four-day old zebrafish was fixed in formalin, stained with Phosphotungstic acid (PTA) and then embedded in JB4+. The sample was then placed in plastic tubing of about 1 mm diameter for X-ray computed microtomography data collection using Xradia Versa. The system was operated at 40 kV at 0.8 µm per voxel. The results demonstrated Xradia Versa capabilities for non-destructive 3D imaging with far superior resolution and contrast than conventional X-ray methods. Visualization software was used to visualize and analyze the final reconstructions.

The images captured include virtual cross-sections (A1, 2)

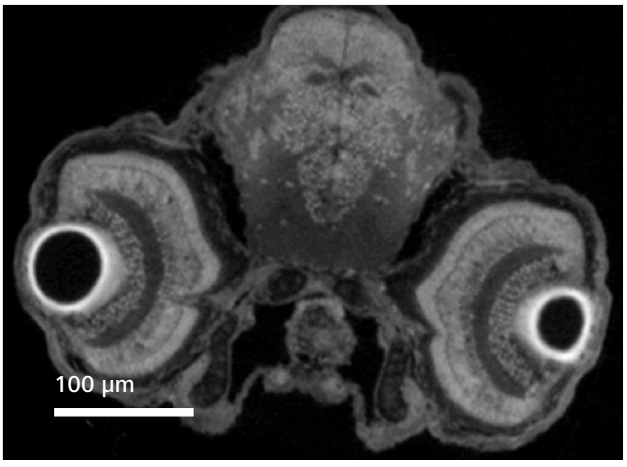

Figure A1

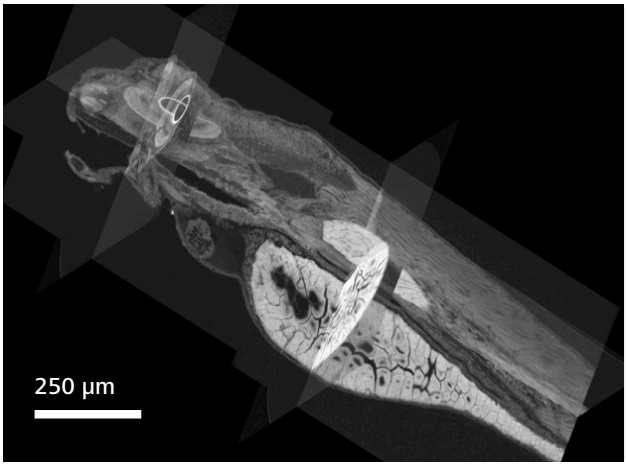

Figure A2

Cellular resolution for 3D phenotyping 2D virtual cross-section of a zebrafish head, showing eye and nerve bundle distribution. Imaged at 0.8 µm pixel.

and 3D volume rendering (B) of zebrafish that clearly show the heart cells (C), brain and eyes of the zebrafish. Such images prove highly valuable to researchers in following 3D structural developments under different conditions.

This review demonstrates the value of X-ray microscopy and Xradia Versa systems from ZEISS in achieving insightful three-dimensional measurements of cells and tissue to advance the emerging field of quantitative biology. With their unique architectural design, Xradia Versa can acquire high resolution, artifact-free images of zebrafish that can be correlated with 2D or 3D information from other microscopy methods such as electron or light microscopy. The volumetric analysis delivered by X-ray microscopy may be used to advance 3D structural analysis of stained soft tissue for phenotyping mutants and major developmental biology studies.

Figure B

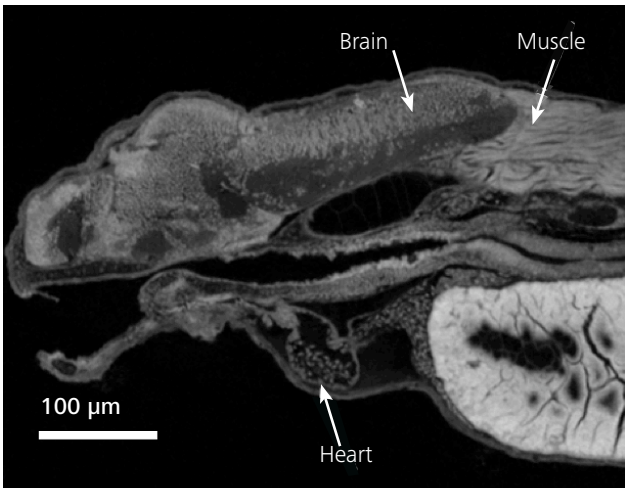

Figure C

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