X-ray micro-tomography for observation of three-dimensional structure of the zebrafish brain with single cell resolution

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Vertebrate brain is subdivided into histologically distinct small area, termed as brain nuclei, and they are also distinct functionally. Traditionally, histological sections have been utilized to observe such structures. However, it is difficult to understand three-dimensional organization of the brain from histological sections because they contain only two-dimensional information. Recently high-resolution MRI and X-ray CT scan are utilized but their resolution is not sufficient in general to visualize single cells as in histological sections. Here we introduce our current challenges with refraction-enhanced X-ray micro-tomography at SPring-8 of the zebrafish brain. Fixed tissues were embedded in the paraffin, and scanned with the beamline BL20XU in SPring-8. From reconstructed coronal plane images, distinct layer structures and brain nuclei could be recognized due to visualization of individual neurons even with unstained samples. This result could be confirmed both with larval (10 days old) and old juvenile (around 45 days old) brains. Simple staining with phosphotungstic acid emphasized the contrast between cell bodies and background in the brain resulting increase of image quality. This new methodology allows us to understand spatial distribution of individual small brain nuclei in the whole brain and quantitative analysis of three-dimensional structure of the vertebrate brain.