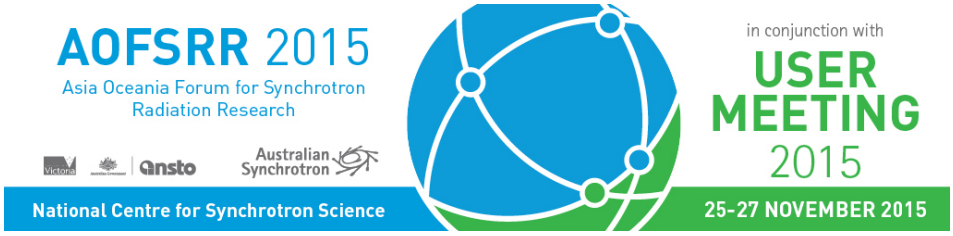

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Embryos in a synchrotron: revealing the internal structure of marsupial embryos and pouch young using synchrotron radiation

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The reconstruction, visualisation and interpretation of anatomy during the development of an embryo present significant difficulties, particularly when dealing with complex 3D structures. Historically, 3D reconstruction of embryonic structures requires alignment of histological slices, a destructive technique with inherent distortion of morphology, or the use of size-limited techniques such as optical projection tomography (OPT). The use of these techniques is highly limited or not possible when mineralised tissues are present. Here we report on the application of contrast-enhanced synchrotron X-ray microCT to investigate the development of teeth in embryos and pouch young of the tammar wallaby *Macropus eugenii*. Using Lugol's iodine as a contrast agent, we can identify key soft tissue and mineralised layers and structures in the developing tooth, including oral epithelium, dental lamina, enamel epithelium, dentine and enamel. We confirmed the identity of the features using comparative histological sections. Staining and X-ray imaging at high resolution enabled the reconstruction of 3D morphology for developing teeth during the entire development sequence from dental lamina and bud stage through to mineralisation and tooth eruption. We reconstructed the 3D position and orientation in the developing jaws of both tooth generations (deciduous and permanent, including vestigial tooth germs) for incisors, premolars and molars. Imaging at 34 keV, just above the K-edge of iodine, significantly improved contrast between iodine-stained tissues and surrounding material. This study demonstrates the major advantages over earlier techniques, making the most of rare specimens in embryonic or later stages of development.

Keywords

microCT, X-ray, development, marsupial, teeth, Macropus

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