3D Imaging and Analysis

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3D X-ray microscopy for life sciences applications: high-resolution multiscale imaging of micro-anatomy, organismal diversity, and molecular expression

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Efforts in microscopy for life sciences research are driven by two opposing problems: the need for ever finer resolutions and the requirement to visualize tissues and structures in situ. At any given spatial scale, different imaging techniques have been developed for either direct 3D imaging or for reconstructing 3D information from 2D images. For organism-based biological research, the most relevant volumes of interest are in the sub-mm to cm range, with resolutions to around 1µm (or better). Several direct 3D imaging methods are effective in this size domain, particularly confocal microscopy, light sheet microscopy, and x-ray microtomography (microCT). The optical methods have clear advantages in available molecular markers and other visualization probes, but they require clearing of samples and have limits on sample size or thickness.

MicroCT has developed into a mature technology for applications in materials science and related fields, and it is now finding broader application in various areas of life sciences research, including microscopic analyses of unmineralized tissues at histological resolutions. The results obtainable with current lab-based microtomography systems can rival those commonly achieved by synchrotron facility users for whole organisms and other intact biological samples. Commercially available microCT systems can offer spatial resolutions down to less than 1µm in "micro" systems, which use projection-based imaging and microfocus x-ray sources, and to around 50nm in "nano" (or "ultra") systems, which employ x-ray focusing optics [1].

For animal embryos and other whole tissue samples, contrast-enhanced microCT imaging is a powerful complement to other imaging methods, including LM, TEM, and SEM. With simple contrast staining methods, high-resolution volume images of unsectioned specimens can be produced routinely for morphological, embryological, and even molecular studies. Because animal and plant tissues generally have very low opacities to x-rays above 1keV or so, contrast enhancement is a useful adjunct to microCT technology [2,3]. Stains based on elemental iodine are especially versatile (Figure 2B), and phosphotungstic acid imparts strong differential contrast to different animal tissues (Figure 1).

Using a modification to the usual antibody detection schemes that employ horseradish peroxidase-mediated chromogen reactions, we have developed a method for imparting x-ray density to immunoprobe staining [4]. By localized reduction of a soluble silver salt, the locations and quantities of a molecular probe can be visualized clearly in microCT images (fig 2C-E).

Because tomographic imaging records quantitative spatial and object density information, it naturally generates data suitable for various kinds of modeling and analysis. Current applications include functional studies in insects and quantitative modeling of various aspects of morphological development [5].

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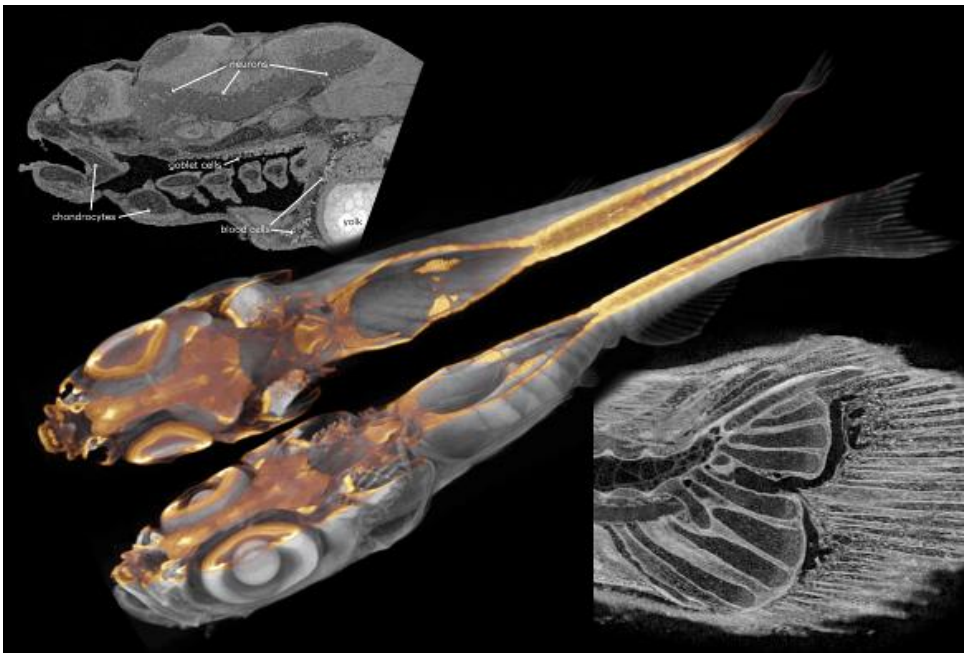

Figure 1. MicroCT images of 5-day zebrafish stained with phosphotungstic acid. Sagittal virtual sections through head (Xradia Versa) and tail (Xradia MicroXCT-200) showing histological details, and a volume-rendered cutaway of a mosaic image of a whole fish.

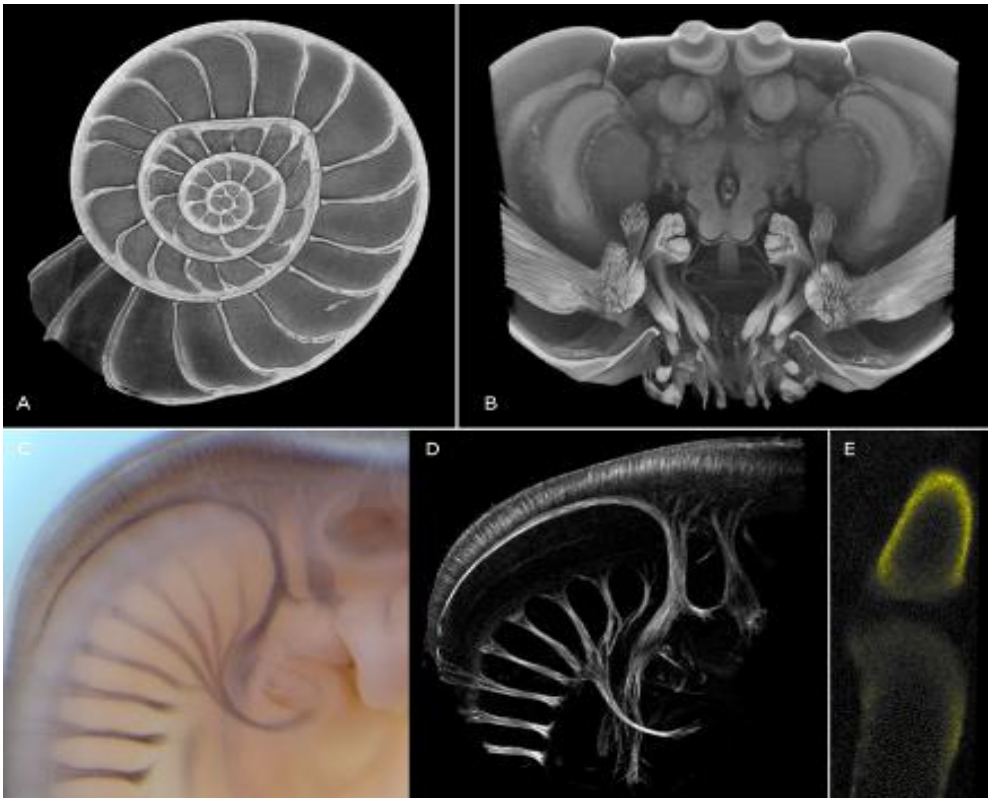

Figure 2. MicroCT imaging in diverse applications. A: fossil foraminiferan; B: neuropteran insect (*Pison*), iodine stained, cutaway of head; C, D: chick embryo immunostained for acetylated tubulin showing detail of developing nerves, LM (C) and microCT (D); E: chick hindlimb digit immunostained for type II collagen, microCT virtual section. Xradia MicroXCT-200 images.