

## H138 Potential Applications of Micro-Computed Tomography (Micro-CT) in Forensic Casework: Evidence From Zebrafish

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**Learning Overview:** After attending this presentation, attendees will understand the differences in analytical power of traditional histology as compared with a 3D form of histology, X-ray histotomography, and be able to describe potential applications to forensics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence from zebrafish of the utility of X-ray histotomography and the potential value of this new imaging modality in future forensic casework.

Through zebrafish modeling, 3D tissue reconstruction data collected will support translation of utility of histotomography for use on human tissue in forensic casework. Histotomography is a form of micro-CT customized for tissue. Histology involves inspection of a small number of slabs of tissue that represent on the order of 1%–2% of the tissue volume, while histotomography potentially captures 100%. Potential applications of histotomography to forensic pathology casework will be considered, based on presented data from zebrafish pilot studies.

Traditional histology has been the standard tool for microscopic analysis of tissues in forensic pathology. The observed tissue architecture allows us to infer important aspects of cellular processes relevant to causes of death.<sup>1</sup> However, histology is destructive of the sample, limited in its value considering limited sampling and inability to provide volumetric data. 3D reconstructions from serial sections are impractical due to tissue distortion associated with cutting and floating sections on water.<sup>2,3</sup> Spatial patterns potentially relevant for inflammatory response or evaluation of vasculature are not fully discernable from a 2D image. In contrast, micro-CT does not require sectioning. Instead, minimally invasive punch biopsies can be rapidly collected, with or without guidance from lower resolution whole-body scans.

In this study, investigation of phenotypic detail with synchrotron based micro-CT was conducted using zebrafish as a model organism. Zebrafish were chosen because they are a small vertebrate with diverse tissues. Synchrotron, and potentially, institutional micro-CT, with the latest technologies, permits resolutions roughly equivalent to that obtained in digital slide scanning using a 20x lens, or ~1 micron optical resolution.<sup>3</sup> Whole zebrafish were stained in Phosphotungstic Acid (PTA) for contrast enhancement and embedded in polyamide tubing.<sup>4</sup> Samples were then scanned by synchrotron-based micro-CT. Projections collected from scanning were reconstructed that permitted observation of the whole organism through digital slices in three different planes. Reconstructions also allowed for 3D volume renderings or segmentation of different cell types, permitting discrimination of the larger, often branching structure of complex 3D tissue structures, such as vessels, that cannot be ascertained from just a few 2D histology images. Dynamic reslicing, particularly with technology like virtual reality, permits the analyst to study different cross sections of the sample without tissue destruction.

3D reconstructions obtained from experimental samples show detail across millimeters of tissue volume that is unattainable with traditional histology. Whole organism reconstructions can be viewed slice by slice on the web or with virtual reality. Such preliminary studies offer promise for translating this methodology to alternate sample specimens such as human tissue. Histotomography may be applied to forensic cases involving gunshot residue powder patterns, decomposition, trauma with sharp or blunt objects, or sudden death cases suspicious for structure-based or cellular-based disorders where total tissue architecture would be of value. Currently, a joint preliminary study is in development with the Dauphin County Coroner in Pennsylvania to scan human tissues sampled via punch biopsy from valid forensic casework.

### Reference(s):

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### Micro-CT, Forensic Pathology, Zebrafish