



Jake Gittlen Cancer Research Foundation

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Dear Dr. Singh:

We are pleased to submit our original research article entitled "Rigid embedding of fixed and stained, whole millimeter-scale specimens for section-free imaging by micro-CT" for consideration for publication in *The Journal of Visualized Experiments*. The potential for 3-dimensional imaging using micro-CT for morphometric studies in the life sciences is huge. The current practice of sample preparation, however, is subject to potential movement during acquisition, poor alignment between imaging sessions, and is ill-suited for long-term storage. This manuscript details a straightforward protocol to generate rigidly embedded samples that are refractory to damage, ideal for permanent preservation, and suitable for re-interrogation.

In this manuscript, we present an embedding methodology that combines the use of an acrylic resin and X-ray transparent polyimide tubing to immobilize specimens and minimize optical artifacts. We show that this technique accommodates a wide variety of biological samples that can be re-imaged over years and achieve comparable image quality. The capability for permanent preservation and re-imaging leads to multiple benefits including: (1) creation of long-term repositories of samples that are difficult to generate or prepare such as those from genetic or chemical phenome projects; (2) resistance to damage; (3) ease of sample transport; (4) image re-acquisition; (5) serial imaging across multiple imaging modalities; and (6) the generation of stable standards for calibration and technology development. We believe that this manuscript is appropriate for publication by JoVE because ease of sample preparation such as described will be necessary for the propagation of the application of micro-CT imaging for biological samples, particularly for scientists with limited access to this technology.

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to JoVE. We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,

Keith Cheng, MD, PhD
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Professor of Pathology, Biochemistry & Molecular Biology, and Pharmacology
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