USING FIB/SEMs TO INVESTIGATE BIOLOGICAL SAMPLES

Annalena Wolff^{1*}, Yinghong Zhou^{2,4}, Jinying Lin^{3,4}, Yong Y. Peng⁵, John A.M. Ramshaw^{5,6},

Yin Xiao^{2,4}

- 1 Central Analytical Research Facility, Institute for Future Environments, Queensland University of Technology (QUT), Brisbane QLD 4000, Australia
- 2 Institute of Health and Biomedical Innovation, Queensland University of Technology (QUT), Brisbane QLD 4000, Australia
- 3 Department of Implantology, Xiamen Stomatological Research Institute, Xiamen Stomatological Hospital, Fujan, China
- 4 The Australia-China Centre for Tissue Engineering and Regenerative Medicine (ACCTERM), Queensland University of Technology, Brisbane QLD 4000, Australia
- ⁵ CSIRO Manufacturing, Bayview Avenue, Clayton VIC 3168, Australia
- ⁶ Department of Surgery, St. Vincent's Hospital, University of Melbourne, VIC 3065, Australia
- * e-mail: Annalena.wolff@qut.edu.au

FIB/SEMs, which combine a Scanning Electron Microscope (SEM) and a Focused Ion Beam (FIB) in a single device have become the "go to" tool in the materials sciences and semiconductor industry. Their unique capability set - revealing a sample's subsurface structures at high resolution, preparing TEM-lamellas, as well as reconstructing 3D sample models at precisely selected points within the sample (slice and view process) cannot be achieved by other technique. This tool has recently found increasing interest in the life sciences (1-8). However, appropriate milling and imaging parameters for soft materials like polymers and non-resin-embedded biological samples are not yet well known. Generic parameters which are used for hard materials cause heat damage and produce undesired artefacts in the samples.

This study focuses on gallium ion-solid interactions to derive sets of suitable operational parameters and a technique, based on Fourier's law of conductive heat transfer and Monte Carlo simulations, which prevents heat damage in soft materials. The technique is successfully demonstrated on non-resin embedded collagen, a biomaterial which serves as a case study for other soft tissues.

- [1] Drobne et al., 'Surface Damage Induced by FIB Milling and Imaging of Biological Samples is Controllable'; Microscopy Research and Technique 70; 895-903 (2007)
- [2] Leser et al., 'Focused ion beam (FOB) / scanning electron microscopy (SEM) in tissue structural research'; Protoplasma 246 (1-4); 41-48 (2010)
- [3] Earl et al., 'Characterization of dentine structure in three dimensions using FIB-SEM'; Journal of Microscopy 240, Pt 1, 1-5 (2010)
- [4] Milani et al.; 'How to study biological samples by FIB/SEM?'; Modern Research and Educational Topics in Microscopy, FORMATEX; 148-154 (2007)
- [5] Villinger et al.; 'FIB/SEM tomography with TEM-like resolution for 3D imaging of high-pressure frozen cells'; Histochem Cell Biol, 138; 549-556 (2012)
- [6] Schneider et al.; 'Serial FIB/SEM imaging for quantitative 3D assessment of the osteocyte lacuna-canalicular network'; Bone 49, 304-311 (2011)
- [7] Stokes et al.; 'A New Approach to Study Biological and Soft Materials Using Focused Ion Beam Scanning Electron Microscopy (FIB/SEM)'; Journal of Physics: Conference Series 26; 50-53 (2006)
- [8] Chaturanga D. Bandara, Sanjleena Singh, Isaac O. Afara, Annalena Wolff, Tuquabo Tesfamichael, Kostya Ostrikov, and Adekunle Oloyede 'Bactericidal Effects of Natural Nanotopography of Dragonfly Wing on Escherichia coli' *ACS Applied Materials & Interfaces* 2017 9 (8), 6746-6760 DOI: 10.1021/acsami.6b13666

The authors acknowledge the facilities and the scientific and technical assistance of Peter Hines, Jamie Riches, Rachel Hancock, and Ning Liu at the Australian Microscopy & Microanalysis Research Facility (AMMRF) at the Central Analytical Research Facility (CARF), Queensland University of Technology, Brisbane, Australia, the facilities (FEI Scios), and the scientific and technical assistance of and discussions with Hui Diao and Rick Webb, of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy and Microanalysis, The University of Queensland