What Lies Beneath: 3D vs 2D Correlative Imaging challenges and how to overcome them

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Correlative imaging provides a novel means of studying diverse sample types in both 2D and 3D at varying length scales (i.e., macro to nano) and via various modes (including, but not limited to, optical light microscopy, X-ray microscopy (tomography), scanning electron microscopy, and focused ion beam scanning electron microscopy). The correlation of datasets generated from these (and other) techniques enables multiple data types to be investigated at the same time (e.g., 2D surface properties, 3D structural properties, crystallographic information via EBSD, surface and sub-surface chemistry), thereby developing an interpretation which considers all sample properties, not just one alone. It is possible to target specific representative sample regions rather than at random, which may not be representative of the conditions. This improved method of correlating data has been applied to varied sample types from materials (e.g., creep in stainless steels [1]), to geological (e.g., reservoir shales [2]), to biological (e.g., bio-inspiration from barnacles [3]), and others, therefore illustrating the versatility of the method.

The correlative imaging method is further improved by utilizing specialized correlative software; for example, Zeiss ZEN Connect and Atlas 5 (3D). These relatively new methods of correlation, in combination with data derived from Zeiss instruments within the Advanced Imaging of Materials (AIM) Facility at Swansea University (UK), enables 3D tomography data to be used in tandem with 2D data, allowing the pinpointing of internal subsurface objects of interest that would otherwise be unknown (Figure 1). X-ray microscopy imaging can provide accurate measurements (depths) to objects of interest, and consequently specific slices in X-ray image stacks can be targeted. Some of these objects may be near surface, but some may be hundreds of µm or even mm deep, therefore requiring specific amounts of sample surface material to be removed to expose them (Figure 1). Currently there is not a single, ideal method for obtaining this over large sample surface areas. Once this surface material is removed, then the object of interest is exposed and may be further examined via a number of conventional 2D imaging and analytical methods (e.g., SEM-EDS, EBSD, nanotomography via FIB-SEM) to continue the correlative process.

Here, we provide and compare example manual procedures (manual grinding and polishing) and automated systems (e.g., Struers Targetmaster, Microtome) which are capable of efficiently removing such material. We also highlight some of the results pertaining to subsurface objects of interest across varied sample types, which would otherwise remain unknown without this intermediate step in the correlative imaging workflow. Manual polishing and grinding is time consuming, however results indicate it is possible to get within ~20 µm of the targeted image slice. The microtome, although common in universities across the globe, is often limited by the cutting ability of the blades used and is mostly restricted to soft specimens (e.g., biological). Limitations to these contrasting methods include the voxel resolution of the original 3D tomographic data, however by using a high-resolution XRM (e.g., Zeiss

Xradia Versa system) this problem should be overcome. Additionally, there is a question of accessibility to these techniques, where they can be limited in number globally and so not practical for the wider community.

Undertaking this important step in the correlative imaging workflow has bettered our understanding of biologically-induced weathering in soils, steel/slag formation, and iron-rich phases in aluminum-silica alloys. This work highlights the importance of the intermediate sample preparation stage currently missing from the correlative imaging workflow; additionally, we hope the assortment of methods discussed here can provide other researchers with a suitable option to complete their correlative imaging research.

References:

[1] TJA Slater et al, Scientific Reports **7:7332** (2017).
[2] L Ma et al, Geological Society of London Special Publication **454** (2017).
[3] RL Mitchell et al, Proceedings of Microscopy & Microanalysis **v. 24 Supp. S1** (2018), p. 376-377.

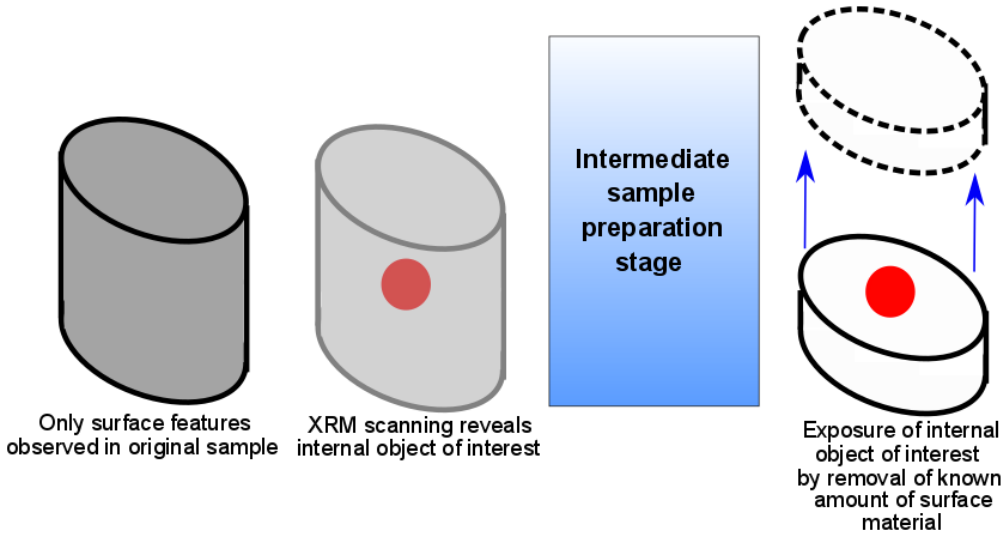

Figure 1. Intermediate sample preparation stage needed to reveal subsurface objects of interest during correlative imaging.