3D printing of custom sample holders as a responsive and cost-effective method of sample holder generation for electron microscopy.

Scanning electron microscopy (SEM) has become more accessible owing to advances in technology and reductions in instrument cost, and its use as a combined image acquisition and analytical tool across multiple sectors including outreach & education efforts using low-cost, benchtop systems. Whilst instruments themselves are extremely versatile in their application for research and development, interdisciplinary use of SEMs can be hampered by transferability of component parts, such as sample holders that are often produced with subject-specific users in mind [1; 2; 3]. Interdisciplinary laboratories, or those that support a variety of commercial contracts where instrumentation is not owned directly, face an increasingly diverse requirement for a variety of holders to cover a wide range of sample applications, often across both life and physical science applications. This can be further hampered by variations in fittings between different microscope manufacturers, with few sample holders are designed to be interchangeable between manufacturer's instruments, which can be prohibitive for some facilities. Whilst both manufacturers and third-party suppliers can provide an array of specified sample holders, holding a large selection in-house can prove to be both expensive and limited by the standardization of their design. 3D printing of conductive materials may provide a solution to this.

Fused Deposition Modelling (FDM) printers are rapidly becoming commonplace in laboratories owing to their simplicity and adaptability. FDM or 3D printing of sample holders for light-sheet and electron microscopy has previously been suggested to be a quick and cost-effective method of custom sample holder fabrication [4; 5], presenting a methodology that can potentially overcome limitations faced by the more traditionally manufactured holders. To emulate their traditionally engineered counterparts, however, 3D printed holders need to be stable, secure, and conductive. The application of extensive analytical techniques such as large area energy-dispersive X-ray spectroscopy mapping [6], or 3D acquisition through focused ion beam scanning electron microscopy and tomography [7], highlight the importance of the requirement for stability alongside conductivity in any new design and manufacture.

3D printing introduces a versatility in responsiveness to the individual users' requirements in terms of specific specimen size, shape, angle of interest etc., which is not available with traditional sample holders. These designs can also be optimized for specific instrument configurations and layouts, i.e. chamber geometry, improving access and minimizing the risk of contact with surrounding detectors and apparatus. They can also incorporate specific angles to allow for more specialist techniques such as electron backscattered diffraction (EBSD), or serial block face imaging (SBFI). Finally, by introducing standardized adaptors into sample holder design as removable parts, samples can be transferred between multiple instruments from various manufacturers, minimizing risk to the sample due to excessive loading and unloading from the holders themselves.

Our method utilizes a conductive polyethylene terephthalate glycol (PET-G) filament as opposed to the traditional polylactic acid (PLA) filaments used previously, which retains superior structural stability, not only demonstrating increased resistance to beam damage but also long-duration stability under typical beam and chamber conditions. Such holders have been used within variable-pressure, field-emission, and focused ion beam systems, without any degradation or quantifiable impact on data quality. Furthermore, these holders have also been developed for both cryo-SEM and 3D reconstructions via FIB-SEM, testing the limits of stability under extreme environments without issue.

This comparatively rapid, low-cost method of design, following through to fabrication and utilization, will benefit academic research facilities as well as industry-facing laboratories by increasing responsiveness in a sample specific and cost-effective manner. In addition, the use of 3D printed holders in low-cost SEM systems allows for a quick and versatile addition to education and outreach initiatives. All of which has the potential to reach a larger audience with minimal financial outset, increasing the scope and implementation of microscopy more widely.

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Figure 1. (A) Schematic of 12 mm stub holder generated using Autodesk Fusion 360 v 2.0.6045; (B) photograph of the resulting 3D printed holder ready for use.

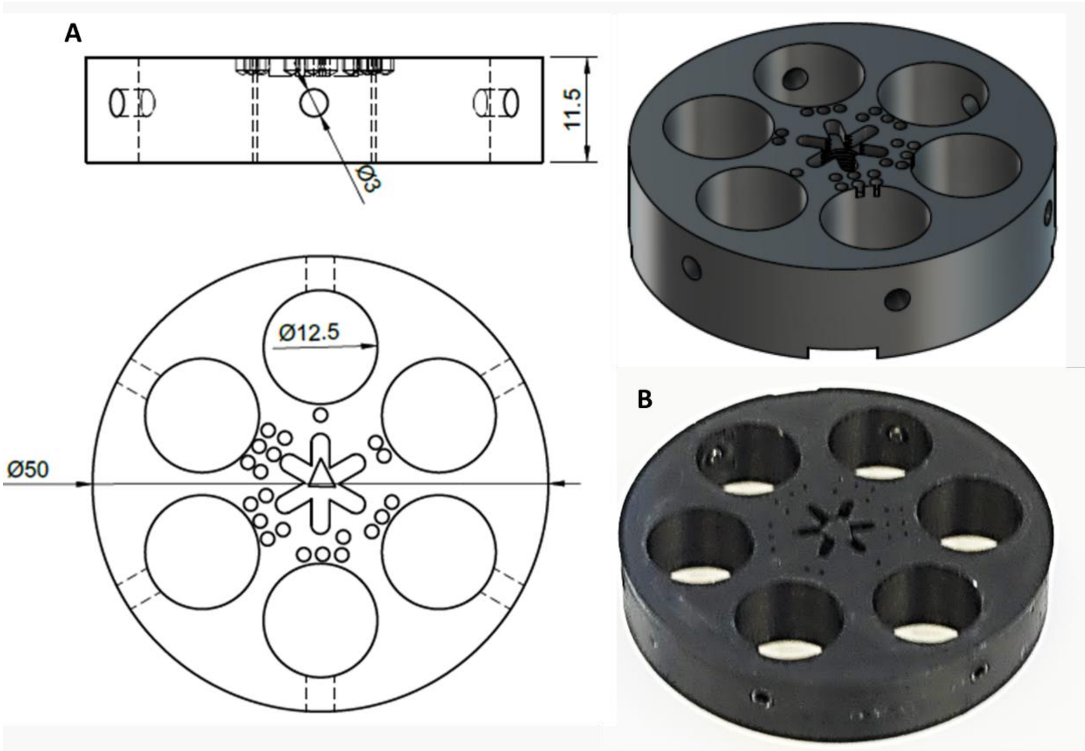

Figure 2 Image of a pollen grain collected with the sample held in a (A) conventional metal holder and (B) the custom 3D printed holder.