3D printing in microscopy and science outreach

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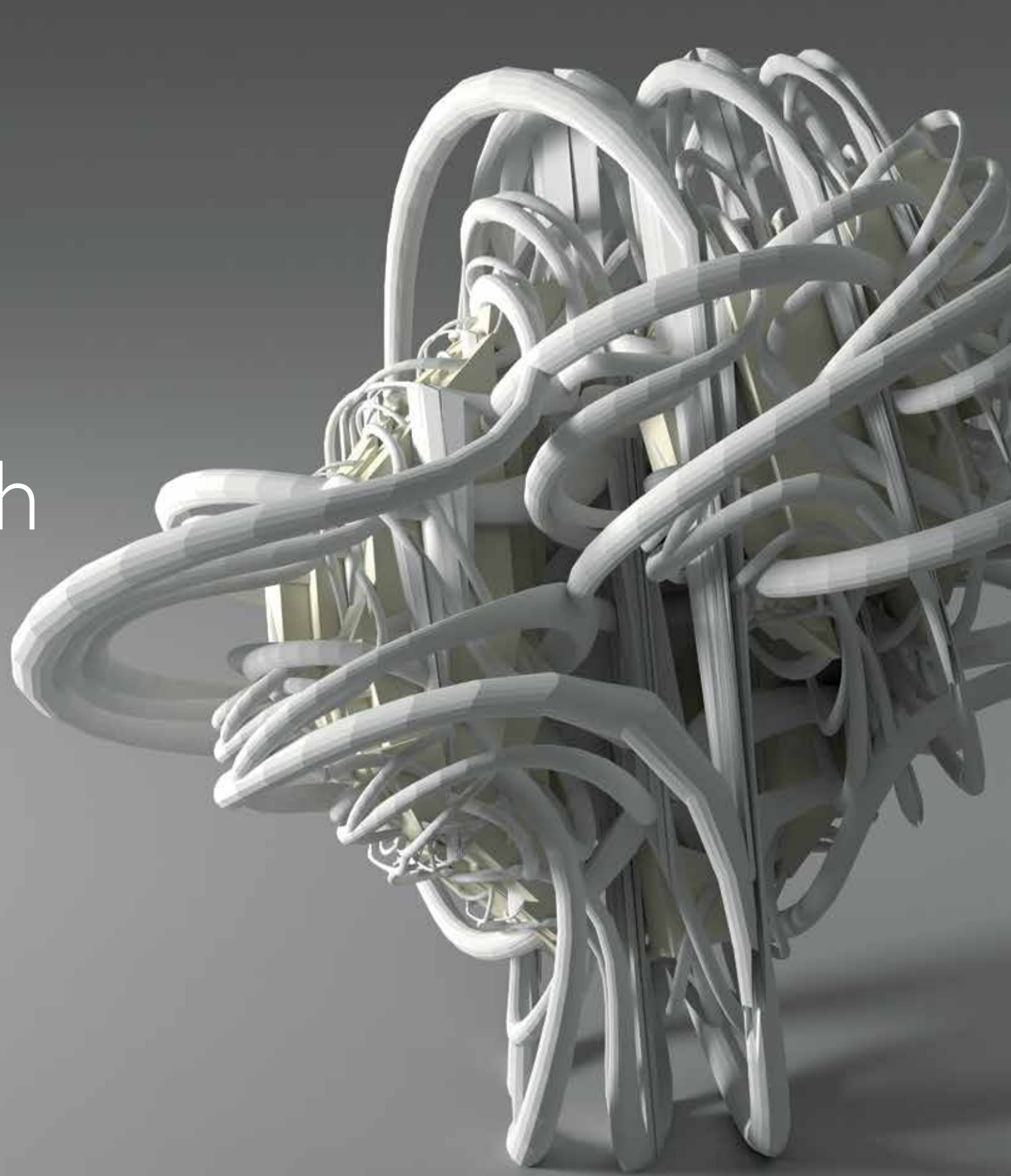

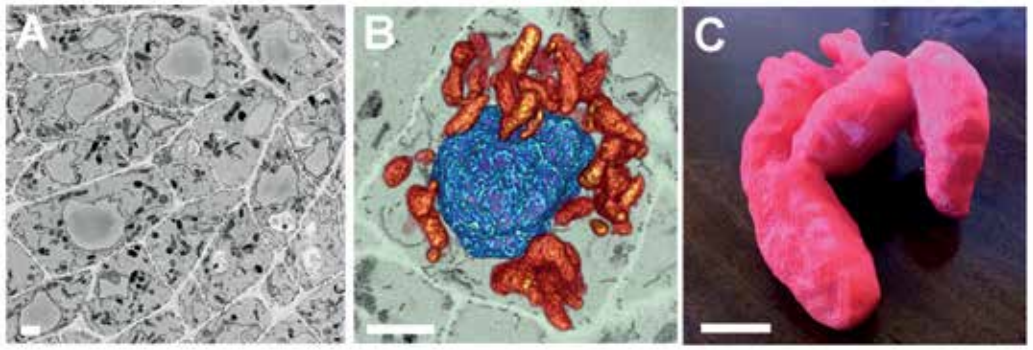

Figure 1 *(A) Single image from a 3D dataset from serial block face scanning electron microscopy showing cells from an Arabidopsis thaliana root tip. (B) Surface reconstruction of the nucleus (blue) and mitochondria (red) with the data in the background from the same data set shown (A). (C) 3D printed model of a cluster of mitochondria, from the same data shown in (A) and (B). Scale bars are approximately 2µm (A and B) and 2cm (C).*

3D printing is a useful tool in aiding the interpretation and dissemination of data. It is ideally suited to microscopy, producing tactile models of micrographs and models of structure imaged using 3D microscopy. Models of data are useful for research, beneficial in education and provide an excellent opportunity for outreach and widening participation. In particular, groups of visually impaired and blind people have responded positively to microscopy workshops, gaining access to the microscopic world in ways that have not previously been possible.

Illustration and outreach using microscopy

350 years ago, Robert Hooke published Micrographia, a book that brought microscopic structure to the attention of the world (Hooke, 1665). Since then, microscopy has changed drastically, going from relatively simple light microscopes with few lenses to an incredibly complex array of technologies that incorporates a range of electromagnetic radiation as well as physical detection of nanostructures. Microscopy has become an integral scientific instrument, facilitating our understanding and interpretation of the universe around us. But it also has the ability to engage audiences, providing attractive and visually appealing images that can be a powerful tool for science outreach (Araújo-Jorge et al., 2004). The close links between art and science in microscopy are well recognised (Orci and Pepper, 2002). This has never been more apparent than in recent years when scientific image competitions are popular and social media is transforming our communication into snappy, bite sized information accompanied by attention grabbing images. As microscopy technology evolves and changes, so should our methods for presenting it. One approach that has been used recently utilises three-dimensional (3D) printing to produce tactile models of microscopic data.

A brief history of 3D printing

3D printing is an additive manufacturing technique that has seen a meteoric rise in popularity over the past several years. Additive manufacturing is the process by which a product is built through the accumulation of layers of material, rather than the alternative of cutting material away (known as subtractive manufacturing). 3D printers are becoming increasingly accessible and their applications in both the home and work environment are escalating. 3D printing is utilised in art and fashion (Hoskins, 2013), building houses (Buswell et al., 2007), aircraft (Marks, 2011), dentistry and medicine (Michalski and Ross, 2014, Ventola, 2014) and even by NASA in space (Wall, 2014), naming only a fraction of the applications to date.

3D printing was originally referred to as rapid prototyping (RP) and even though wide-spread interest in this technology has been relatively recent, it was actually developed over 3 decades ago. An early and major development in 3D printing was by Hideo Kodama (Kodama, 1981) but the patent application was never completed (Venuvinod and Ma, 2013). Charles Hull developed a stereolithography apparatus (SLA) in 1983 and filed a patent for the technology in 1985 (Hull, 1999). While stereolithography (SL) was the first, other RP technologies were being developed at the same time. A patent was filed for selective laser sintering (SLS), developed by Carl Deckard, in 1987 and in 1989 one was filed for fused deposition modelling (FDM) by Scott Crump (Lipson and Kurman, 2013). The technology continued to advance and be developed throughout the 1990s and into the 2000s, with a great deal of success at high end manufacturing levels. It wasn't until much more recently, 2009, that 3D printing became commercially available to a wide audience at affordable prices and in 2012 3D printers were observed on the crowd-funding website Kickstarter (O'Brien, 2012).

Types of 3D printing

As already indicated, there are several different types of 3D printing. There are several commonalities between the different types. 3D digital models are broken up into a series of 2D layers with X and Y co-ordinates, generally using a Standard Tessellation Language (STL) file format (Wong and Hernandez, 2012). Material is laid down in 2D layers

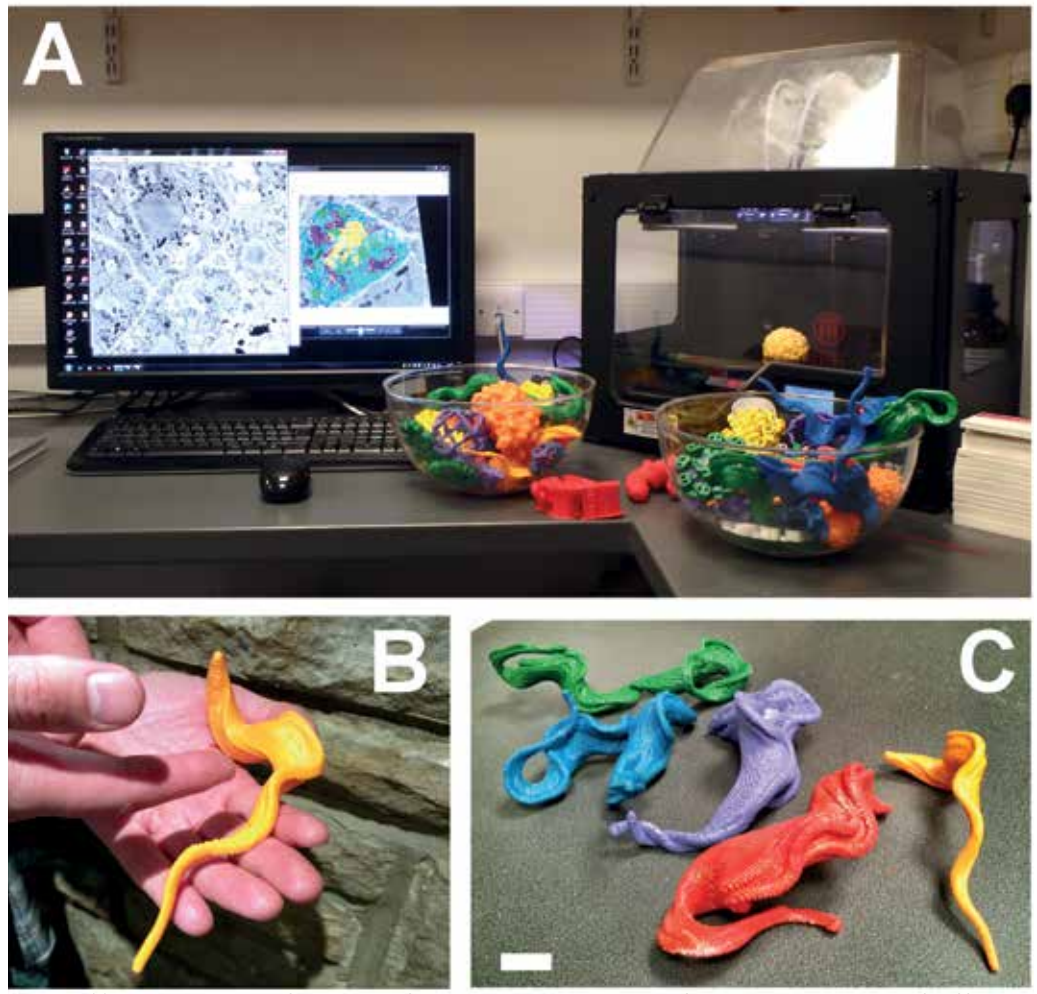

Figure 2 *(A) Photograph showing our 3D printer, one of our computer workstations with some 3D data on the screen and bowls full of 3D printed models. (B) A 3D print of a trypanosome cell being held. (C) Prints showing the trypanosome cell cycle. Scale bar is approximately 2cm.*

Figure 3 *(A) Photograph showing our stands at the Great British Bioscience Festival. 3D printed models are attached to the posters on the stands. (B) Photograph of our table with 3D prints and the 3D printers at Science Uncovered at the Natural History Museum, London. (C) A close up image of a tactile image and two 3D prints featuring a Golgi body and endoplasmic reticulum on an outreach poster.*

corresponding to the digital data. Each layer is fused to the one laid down before it. Some 3D printing techniques require a support structure to be produced at the same time as the model, to allow overhanging edges to be printed.

SL (Stereolithography), the first process that was commercially available (Ventola, 2014, Schubert et al., 2014), involves the deposition of resin onto a movable platform using a laser. The platform is submerged in a vat of photo-curable resin. A laser is applied in a 2D pattern, determined by the STL file, onto the resin surface, hardening it. The platform is lowered a set amount, which determines the z height of each deposition, and a second layer is created on top of the first. This is repeated until the object has been built, whereupon the platform is raised and the printed model is detached. Post-processing is necessary with SL to remove support structures, clean the model and for a final curing of the resin. A similar 3D printing process is Digital Light Processing (DLP) (Dean et al., 2012), which uses a different light source and exposes the entire surface of resin at once rather than scanning over small areas. The platform for this technique lifts out of the resin rather than lowers into it, reducing waste and resin costs.

SLS (selective laser sintering) and SLM (selective laser melting) (Ventola, 2014, Hoskins, 2013) also uses lasers to deposit layers. This technique uses powder laid down using a roller. Each layer is fused or melted upon application of the beam. The platform is lowered after each layer, as with SL. A distinct advantage with this technique is that the remaining powder is only removed at the end of the build process, providing a support for the structure and making it an ideal technique for complex objects that might not otherwise be possible to build. This technique can be applied to a variety of materials and is often used with metals and plastics. A related technique, electron beam melting (EBM)(Wong and Hernandez, 2012), uses an electron beam as the heat source.

Fused deposition modelling (FDM) (Stratasys, Eden Praire, MN, USA) or Freeform Fabrication (FFF) printers (Wong and Hernandez, 2012) are available both as open source and commercial machines and are probably the most recognisable types of 3D printer. FFF works by extruding melted plastic, usually acrylonitrile butadiene styrene (ABS) or poly lactic acid (PLA). The plastic is supplied as a filament and is pushed or extruded through a hot nozzle. The hot end of the extruder is moved over the build platform, depositing the soft plastic in layers onto the build platform. The plastic hardens as it cools and a solid plastic object is formed by the end of the

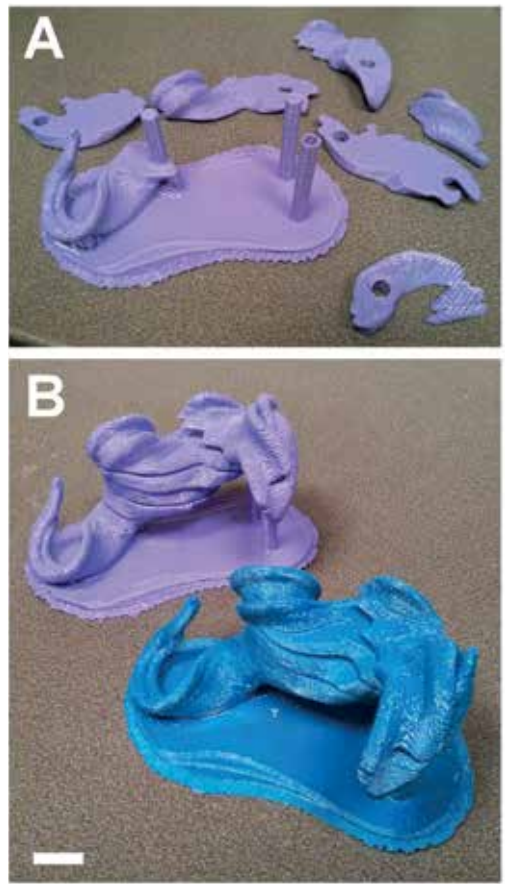

Figure 4 *Photographs of 3D printed trypanosome cell jigsaw puzzles highlighting the difficulty in working out 3D structure using 2D slices. (A) The puzzle is disassembled into thick "2D" slices. (B) Two trypanosome puzzles fully assembled. Scale bar is approximately 2 cm.*

print process. Support scaffolding is necessary and post processing to remove the scaffold and smooth the models may be required. It is possible to have more than one extruder and thus different colours or materials, including a soluble filament for the scaffold, can be used to make the object.

Selective Deposition Lamination (SDL) (Mcor Technologies Ltd., Ireland) and Laminated Object Manufacturing (LOM) (Wong and Hernandez, 2012) involve layers of material being glued onto one another. A laser is employed to cut each layer of the material and the excess is later removed. Both metal and paper can be used in this 3D printing process. An advantage with using paper is that it can create accurate colour CMYK reproduction as part of the build process, providing a full colour model. However, a disadvantage is that it cannot produce the same complex geometry that is possibly with other types of 3D printers.

Inject technology is also utilised in 3D printing (Wong and Hernandez, 2012), known as binder jetting and material jetting (Bak, 2003). Binder jetting is similar to SLS as it uses layers of powder. Instead of a laser or electron beam fusing layers of material together, a binding agent is applied to hold layers of powder together. This may need further processing to create a stronger lasting bond after the printing process is finished. Colour can be added to the binder. 3D printed ceramics are created using this technology (Sachs et al., 1993). Material jetting involves the deposition of liquid or molten material directly onto the build platform. Several materials can be combined to produce complex prints. The most common materials are photopolymers that are set using UV light following deposition. Material jetting is also used to create objects in wax, which can then be replaced with a metal using a lost wax casting method (Singh, 2010).

3D printing from micrographs

The process of creating a 3D object occurs via a series of steps. A digital model is first created using a variety of different software programs. This is converted into an STL file format, processed for 3D printing and is then sent to the 3D printer (Hughes, 2015). For microscopy, the greatest hurdle is generating the 3D digital model in the first place. Prints of micrographs to produce 3D tactile images for blind or visually impaired students utilise a system for converting 2D images into 3D based on greyscale values (Kolitsky, 2014a). This is a simple but effective technique to quickly produce 3D images. However, there have been significant advances in microscopy over the past few decades and a volumetric data set can be produced using any 3D microscopy technique. For example, confocal laser scanning microscopy (CLSM), light sheet microscopy, single particle analysis, serial section transmission electron microscopy (TEM), electron tomography (ET), focused ion beam

scanning electron microscopy (FIBSEM), serial block face scanning electron microscopy (SBFSEM) and array tomography can all produce data that can be segmented and reconstructed into a surface model. These type of surface reconstructions may need some refinement in order to overcome potential issues with converting it into a format for 3D printing (Hughes, 2015). Some of the issues that arise when converting data include resolution of the model (too high and the printer software may not be able to handle it), size (microscopy data is generally measured in microns or nanometres, 3D printers assume measurements are in millimetres) and the integrity of the surface model (Hughes, 2015). These conversion techniques are not restricted to microscopy. CT scans of living and fossilised organisms and models of protein structure can also be converted into objects for printing using similar methods. Software and the techniques involved in generating digital models can involve a steep learning curve, but initiatives such as the NIH 3D print exchange (NIH, 2015) and the Howard Hughes Medical Institute (HHMI, 2015) provide open access to ready-made biological models that can be downloaded and printed with little to no processing involved.

Why would you want to print microscopy data? There are several reasons for wanting a physical model of your data including benefits for interpretation, dissemination and demonstration. Microscopists are trained to interpret 3D information from 2D images, whether from micrographs, digital data or a computer models. It can pose a challenge for others to interpret data in the same way. There is also an issue with data orientation. It is easy with many of the volumetric data collection techniques to accidently invert z slices, creating a structure that does not have the correct chirality. This is less obvious in a 2D image than with a 3D model. Since printing models of EM data, many hours have been saved when orientation has been indicated using a printed structure as an aid rather than attempting to do this by description only. Printed models are also more visceral than data in a presentation, poster or computer screen. This facilitates rapid data interpretation and in my experience makes it an ideal accessory for poster presentations or discussions at conferences where there access to computing facilities capable of displaying the data in 3D may be problematic. The use of 3D printing in an academic environment is shown to be beneficial for students studying engineering (Kolitsky, 2014b) and has also been applied to diverse range of subjects from archaeology (Rahman et al., 2012), anatomy (McMenamin et al., 2014) and maths (Aboufadel et al., 2013). It is also beneficial for visually impaired or blind students and researchers (Leander, 2012) and is a more inclusive approach to disseminating data.

3D printed models and scientific outreach

While academic dissemination of data is the lifeblood of research, it should be remembered that scientific outreach is becoming increasingly important, especially when the degree of publicly funded research is taken into account. Science outreach can have a significant impact and free resources made available to the public enhance their scientific understanding (Falk and Dierking, 2010). 3D printing has provided a fascinating tool that readily engages the general public with research (Rahman et al., 2012). It can be especially important to incorporate 3D prints and models when teaching scientific concepts (Ferk et al., 2003) and children in particular benefit from tactile aids as part of their learning process (Dunn and Dunn, 1992).

Several science outreach exhibitions have featured 3D printed models. Palaeontology is an example where 3D printing can enable public interaction with fossils in a way that would not otherwise be possible and one such event is described in detail by Rahman and colleagues (Rahman et al., 2012). Another example is a rare fossil exhibition at the Natural History Museum in London (Amos, 2015). Exhibitions featuring our 3D prints of microscopy data have been held at several venues, Pegasus theatre in Oxford (Watson, 2014), Science Uncovered at the Natural History Museum in 2014, the Great British Bioscience festival (Keown, 2014, Thimmesch, 2014, BBSRC, 2014), Giant Germs (Keown, 2014) and Zoom; Worlds through a microscope (RMS, 2015). The exhibits have been interactive workshops (Giant Germs), manned exhibitions with public interaction (the Great British Bioscience Festival and Science Uncovered) and unmanned gallery exhibits (Zoom and the Pegasus theatre exhibition). The set-up was slightly different for each exhibit but all contained a variety of media to tell the exhibition story and included 3D microscopy prints, micrographs, images of modelled data and coloured micrographs to highlight key features, stereoscopic images with 3D glasses, text to accompany the images or models and videos of the 3D datasets on TV screens or laptops. The 3D printer itself and microscopes for the public to use were included in several of the exhibitions. All of the exhibits had some level of interactivity and visitors were encouraged to handle 3D models and use other interactive elements. For example, at Science Uncovered a 3D jigsaw of a cell was used to highlight how difficult it is to interpret and reconstruct a 3D cell from 2D slices.

The demographic of visitors ranged from small children through to pensioners, from educated professionals to individuals with little or no scientific background. Unsurprisingly, the manned exhibitions provided an opportunity to engage with visitors to a greater extent than with the unmanned exhibitions and had more emphasis on scientific outreach. The unmanned exhibitions were more artistic, although text was available for those interested in exploring the science behind the exhibit.

Widening participation is something that researchers need to be aware for science communication and outreach. Microscopy is a completely visual field and fundamental to our understanding of biology. There is ongoing work around the world to improve access to this type of data with 3D printing (Leander, 2012, Kolitsky, 2014b). Microscopy events for individuals that are blind and partially sighted were held in the run up to the Giant Germs event located in Tower Hamlets (London), in collaboration with the Biotechnology and Biological Sciences Research Council. Giant Germs was specifically aimed at visually impaired and blind adults. The event featured hands on exploration of a basic compound microscope, explaining individual parts, before progressing onto an exploration of cell biology, parasites and viruses using 3D printed models. As participants handled the models and equipment, lecturers talked through what they were holding and the biological implications as well as explaining relative scales and magnifications. Enough models had to be produced for each group of adults to hold and examine examples of viruses and parasites. The sessions were wrapped up with an examination of the 3D printer and processes involved in producing a model. Feedback from the event was excellent with participants saying "..it is so tangible and tactile my concentration was kept, so I think this is a fantastic way of conveying a science lesson" and "to have the opportunity to get the tactile impression was fantastic" (Hamper, 2014). The event was very successful and it is hoped that similar events can be held in the future.

As 3D printing technology becomes more available, outreach possibilities will continue to improve. Models and data to allow schools and other institutions to print their own models is certainly the way forward in addition to providing models as kits to school groups and science outreach events.

In summary

3D printing is rapidly becoming an accessible technology that can be applied to microscopy data. In addition to informing research and facilitating data dissemination, it can also be used for scientific outreach in combination with other forms of media. 3D printing may well be beneficial for science education in schools. Of particular note is the application of 3D printing in widening participation to blind and partially sighted individuals that would not otherwise be able to access microscopy data,

not only for outreach but also in education and research environments.

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