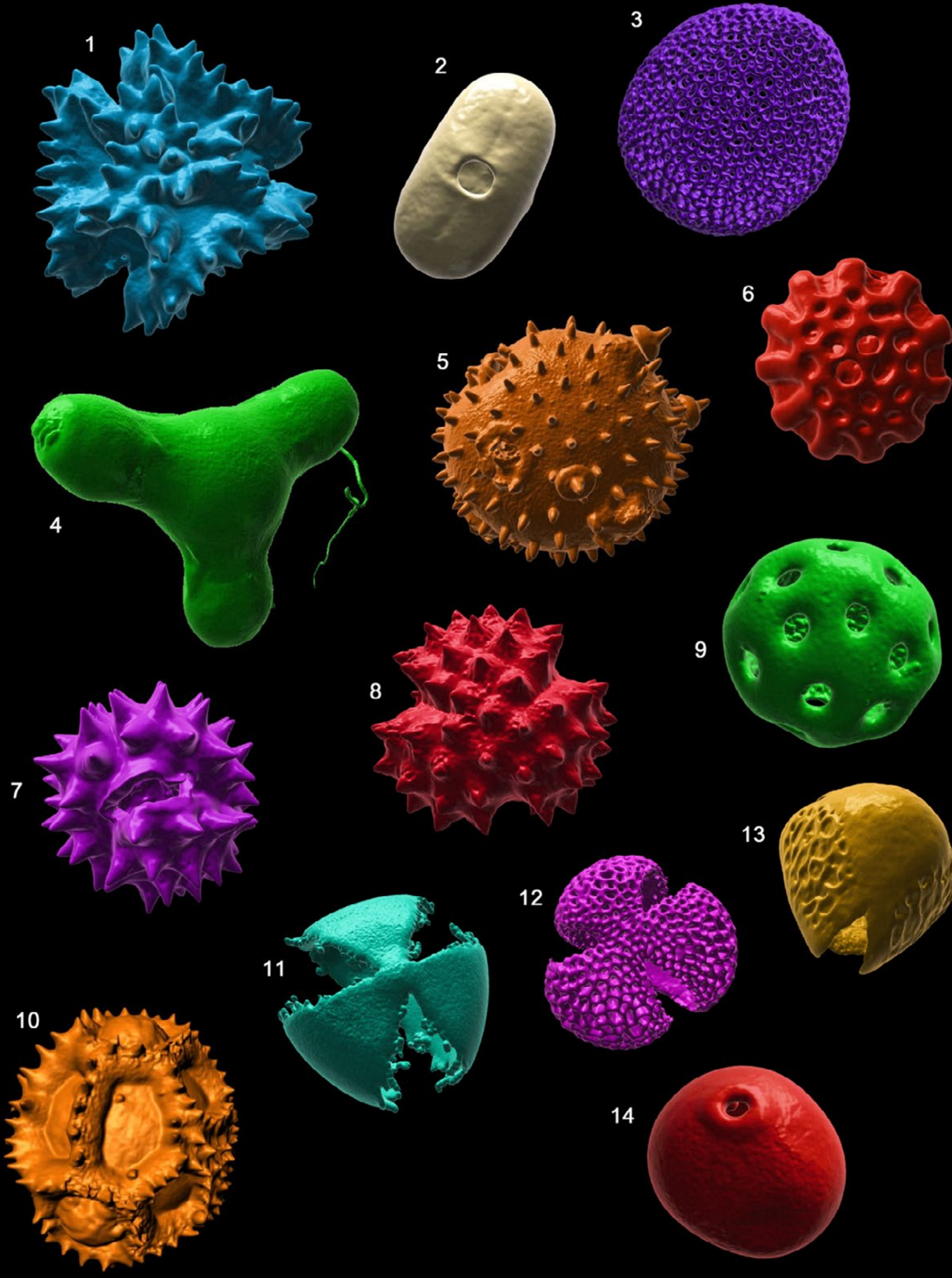
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The 3D Pollen Library Collection at NIH3D: From Humble Beginnings to the Largest Open-Source Collection of Online 3D Pollen Models Worldwide

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Pollen Counts: A selection of 3D pollen models from the Bioimaging Hub's NIH3D Pollen Library Collection curated by Dr Tony Hayes. The collection currently comprises 173 published, DOI-referenced surface-rendered 3D models of pollen grains and spores from 158 species of plant. All models are 3D printable and compatible with virtual reality and augmented reality immersive technologies. 1. meadow goat's beard (*Tragopogon pratensis*); 2. cow parsley (*Anthriscus sylvestris*); 3. seaside arrowgrass (*Triglochin maritima*); 5. pumpkin (*Cucurbita pepo*); 6. heath milkwort (*Polygala serpyllifolia*); 7. common daisy (*Bellis perennis*); 8. common groundsel (*Senecio vulgaris*); 9. thyme-leaf sandwort (*Arenaria serpyllifolia*); 10. common dandelion (*Taraxacum officinale*); 11. green field speedwell (*Veronica agrestis*); 12. cuckoo flower (*Cardamine pratensis*); 13. bastard-toadlax (*Thesium humifusum*); 14. creeping bentgrass (*Agrostis stolonifera*).

Keywords: pollen, palynology, 3D printing, virtual reality, augmented reality, pedagogy, science outreach.

Pollen Counts

A fine dust to the naked eye, exquisite in microscopic form and fundamentally important to life on earth. Pollen grains contain the male gametes (i.e., sperm cells) from a flower's anther that are essential for reproduction in seed-bearing plants thus are pivotal to the ecosystems and foods that we take for granted. As well as being critical to life, pollen records the evolutionary course of plant taxa in the geological strata, allowing scientists to understand and map environmental change; has relevance to forensic science, serving to establish links between objects, people and places; and represents a powerful allergen, causing hay fever in up to 30% of the world's population. Without overstating the obvious, pollen counts.

Pollen grains come in a staggering variety of shapes and sizes. Their outer surface, or exine, is often highly ornamented with unique structural features that allow palynologists – the scientists who study pollen and spores - to accurately identify the parent species.[1] Pollen identification is a highly skilled process requiring years of training and experience. Characterising and classifying pollen using a compound light microscope is not a trivial task. The shallow depth of field at high objective magnifications means having to focus through the volume of multiple grains in differing orientations to construct a mental picture of their overall 3D morphology. Conveying this information, particularly to a non-specialist, via a 2D photomicrograph or schematic presents further challenges as the brain is more adept at directly processing 3D spatial information than it is in having to extrapolate volume from flat 2D images.

Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) have emerged as powerful analytical tools in palynology as they allow an appraisal of 3D form at high resolution. Whilst SEM provides excellent depth of focus and extremely high (nanometre; nm) resolution of surface structure, CLSM has several practical advantages. First, CLSM is compatible with standard palynological slide preparations and does not require complicated preparatory procedures. As a fluorescence-based imaging technique, CLSM can exploit the intrinsic fluorescent properties (autofluorescence) of the pollen exine to image

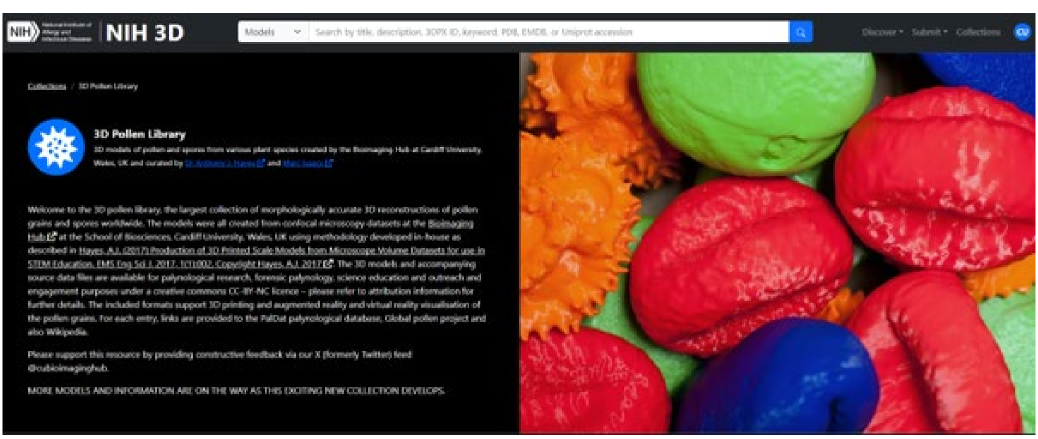

Figure 1. *The 3D Pollen Library collection at NIH3D. Screenshot of the web interface. NIH3D is the foremost open, community driven portal to download, share and create bioscientific and medical 3D models for 3D printing and interactive visualisation.*

1 The interested reader is directed to 'The Illustrated Pollen Terminology' (Halbritter et al., 2018) for comprehensive descriptions of pollen grain types and morphologies.

microstructure, thus readily facilitating taxonomic discrimination in a non-disruptive manner (Castro et al., 2010). Furthermore, many of the pollen stains routinely used in palynological research are also highly fluorescent (Atlagic et al., 2012), therefore archival pollen samples from herbarium collections are also amenable to this technique. Second, the optical sectioning capability of CLSM allows pollen grains to be sequentially sectioned through their volume at sub-micrometre (µm) intervals, or z-steps[2], yielding a dataset of image slices, or 'z-stack', that permits visualisation of both internal and external structure. Third, the volume information contained in the z-stack can be digitally reconstructed in any given 3D orientation, thus facilitating comprehensive appraisal of 3D form. Four, when surface rendered as a 3D polygon mesh and provided the correct file format, CLSM data is compatible with 3D printing and immersive technologies such as virtual reality and augmented reality (VR and AR, respectively), thus offering the potential for powerful 3D learning experiences within research and educational contexts.

The 3D Pollen Library: a palynology resource not to be sneezed at

We have developed and refined workflows as outlined above, to accurately 3D model pollen grains as well as other biological samples at various levels of scale. The resultant surface-rendered 3D pollen meshes have been curated into the largest publicly accessible collection for palynological research, educational purposes and scientific outreach worldwide. At the time of writing, the 3D Pollen Library Collection hosted via NIH3D contains 173 published, DOI-referenced 3D models of pollen grains and spores from 158 species of plant (Figure 1). All the 3D pollen models are free for download for non-commercial usage under a CC-BY-NC licence, together with source CLSM data, supporting methodology and associated image art. Each entry is linked to major palynological databases (PalDat, Global Pollen Project and PollenWiki) as well as the Wikipedia website for general information about individual plant species. The surface-rendered 3D digital meshes are readily scalable and can be used in STEM activities including 3D printing and both AR and VR, thus offering a multitude of sensory learning experiences within educational, research and science outreach and engagement contexts. The 3D printed outputs are effective pedagogic tools that facilitate haptic learning experiences with immediate relevance to blind or partially sighted individuals.

This article reviews the development and progress of this 3D pollen resource and provides examples of how it has been used in a multitude of collaborative projects, for education, public engagement and also for societal enrichment purposes.

Big journeys begin with small z-steps

Anyone who suffers from hay fever appreciates that pollen is literally everywhere. The sheer ubiquity of pollen, coupled with its striking diversity of form and prominent autofluorescence make it particularly attractive from a microscopical perspective. It was these properties that made pollen ideal for the methodology we were developing in 2015 aimed at creating tangible, physical 3D replica models of microscopic samples. The experimental approach we adopted was reasonably straightforward. The autofluorescent profile/fluorescent staining characteristics of the pollen exine can easily be mapped using lambda (λ; spectral) scanning (Donaldsen, 2020) thus CLSM acquisition parameters can readily be optimised for fluorescent signal collection. Z-stacks of optical sections are hence taken through grains at high

2 Z, refers to the focal axis of the microscope. The x-and y-axis represent the first two dimensions (width and height); the z-axis, the third dimension, denotes depth.

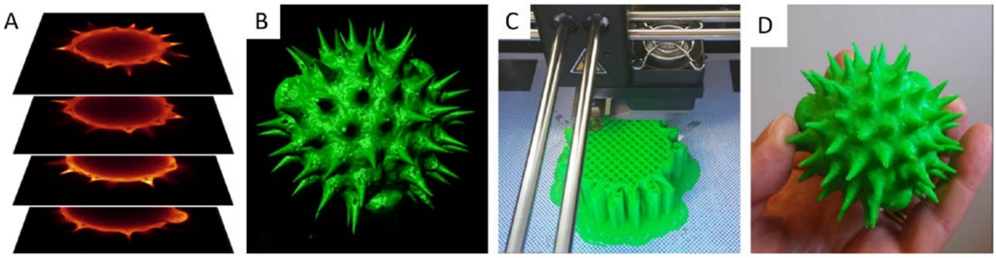

Figure 2. *Supersizing pollen: from microscopic grain to medieval mace ball. A. Pollen grain from sunflower (Helianthus annuus) is optically sectioned by confocal microscopy. B. Dataset is surface rendered as a 3D digital model; C. digital model is 3D printed (N.B., the crosshatch pattern is the internal scaffold, the outer struts are the external supports); D. the finished 3D print with external scaffold material removed. The choice of colour is entirely arbitrary.*

resolution, observing Nyquist sampling criterion to capture accurate volumetric information for 3D reconstruction. In our hands, a z-step of around 0.3µm (i.e. 300nm) is typically employed using a x63 Plan-Apochromat 63x/1.4 oil immersion objective of a Zeiss LSM880 Airyscan confocal microscope, however this value can vary depending upon the size of the pollen grain being imaged, with most grains occurring within a size range of 10-70µm in diameter. The resultant z-stack data is then surface rendered as a 3D digital polygon mesh, to reflect the surface topography of the pollen grain, and the digital output converted into a format conducive to 3D printing and AR/VR visualisation (e.g., the virtual reality modelling language world file format, wrl) using Bitplane's Imaris image analysis software (Oxford Instruments). The surface rendered output is further processed in MeshLab to reduce the size of the mesh and to remove visible scan lines via decimation and Laplacian smoothing, respectively, taking care to preserve topology. Digital meshes are highly reproducible and scalable thus the models can be 3D printed to specification within the hardware constraints of any 3D printer. We have used affordable fused filament deposition (FFD) 3D printing technology for our work; however, the meshes can be fabricated into physical models using other additive manufacturing techniques (see Zhou et al., 2024). Despite the underlying simplicity of the technique, it was a huge thrill when our first 3D print - a pollen grain from the common sunflower, *Helianthus annuus* - emerged from its supporting scaffold: a perfect doppelganger of a microscopic grain supersized from roughly 1/50th of a millimetre in diameter to the dimensions of a medieval mace ball! (Figure 2).

Kinaesthetic learning: getting in touch with palynology

After demonstrating the initial proof of principle, I wrote a short blog article on the Bioimaging Hub's news site to showcase the technique (Web link: IN-FOCUS: Bigging It Up: 3D Printing to Change the Shape of Microscopy) where I mused that a physical, tangible model would allow improved 3D conceptualisation of these microscopic structures and would have considerable relevance in an educational environment, particularly for the partially sighted or blind, by permitting kinaesthetic (i.e., tactile) learning experiences. Serendipitously, the article was read by a PhD student at the School of History, Archaeology and Religion at Cardiff University who then approached us for our help in generating 3D printed models of prehistoric pollen grains identified in soil strata sampled from archaeological sites in south Wales[3]. The research was part of an ongoing public engagement project, entitled 'Footprints in Time' that addressed climate change patterns in local coastal environments. This presented an exciting opportunity to

3 the study of ancient pollen grains is referred to as *paleopalynology;* whilst *palynostratigraphy* deals with the description and interpretation of vegetational successions based on the identification of pollen in geological strata.

further showcase the methodology and led to the establishment of a small 3D pollen reference collection (Web link: IN-FOCUS: Development of a 3D Printed Pollen Reference Collection) which was used for science outreach and public engagement activities both within and outside Cardiff University (Figure 3).

More interest followed from Cardiff University's School of Pharmacy with requests for 3D prints of pollen species identified in locally sourced honey as part of the Pharmabees nutraceutical research programme[4]. Buoyed by the interest and the growing potential for 3D printing, due to the increasing affordability and reliability of the technology, I published a short methodology paper in 2017 allowing researchers to create their own bespoke models of microscopic samples via optical sectioning microscopy (e.g., confocal, multiphoton and lightsheet imaging modalities).The paper utilised a variety of samples, including pollen, and exploited both fluorescence and reflectance microscopy to generate the 3D models. This helped to increase the profile of our work and was followed by several external collaborations with public organisations requiring 3D pollen models. These included the National Botanic Garden of Wales (Web link: IN FOCUS: Plastic Fantastic – Making Pollen for The National Botanic Garden of Wales), the UK Met Office (Web link: IN FOCUS: 3D Pollen Prints Not To Be Sniffed At - Printing Pollen for the Met Office), as well as the Smithsonian Institution's National Museum of Natural History in the US (Figure 4). In each instance, I created bespoke collections of 3D pollen prints based on the species and print specifications required. Typically, this meant sampling

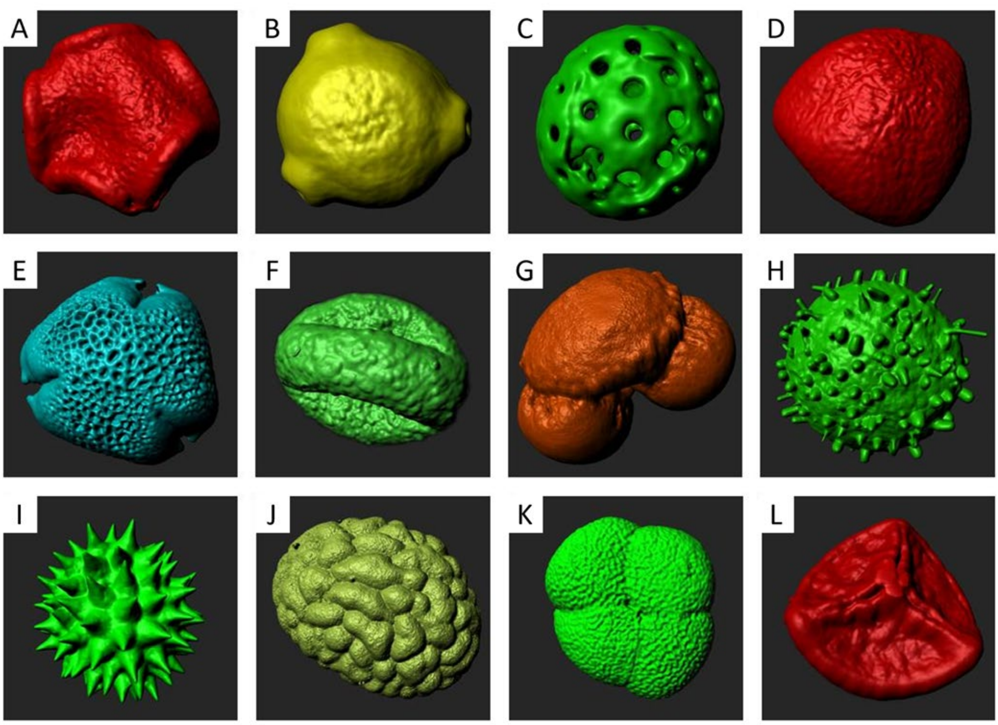

Figure 3. *Examples of some of the initial 3D pollen/spore reference collection created for the Footprints in Time project at Cardiff University. A. black alder (Alnus glutinosa); B. silver birch (Betula pendula); C. white goosefoot (Chenopodium album); D. common hazel (Corylus avellana); E common ivy (Hedera helix), F. common oak (Quercus robur); G. Scots pine (Pinus sylvestris); H. European white water lily (Nymphaea alba); I. common daisy (Bellis perennis); J. common fern (Polypodium vulgare); K. common bulrush (Typha latifolia); L. peat moss (Sphagnum sp.)*

4 *melissopalynology* is the field of palynology concerned with the study of pollen in honey to help identify the geographical location and genus of plants that honeybees have visited.

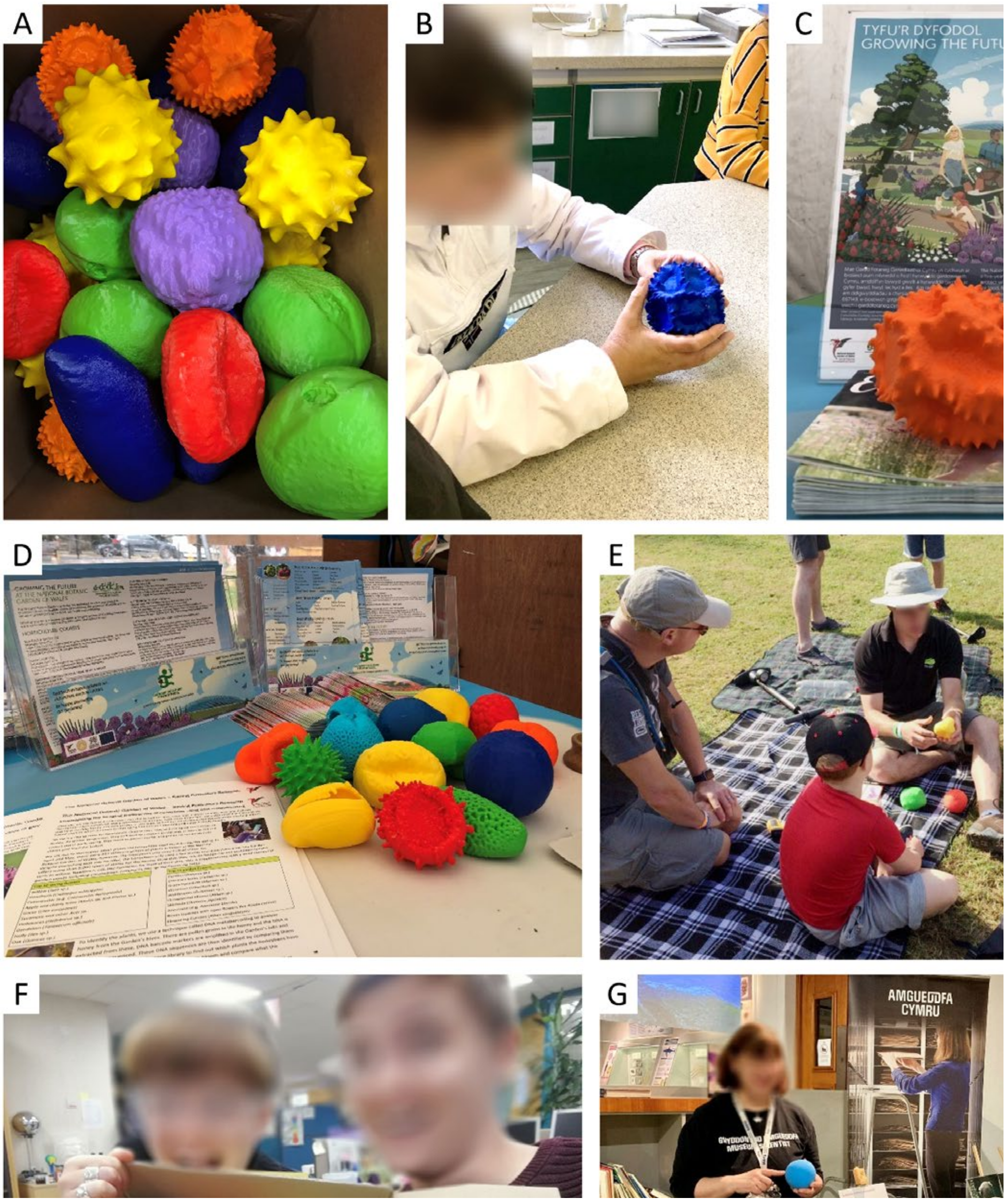

Figure 4. *The Bioimaging Hub's 3D pollen models out in the wild. Examples of how the 3D pollen prints have been used for pedagogy and science outreach and engagement. A. 3D models of assorted honey pollen species created for the PharmaBees programme at Cardiff University. B. 3D pollen model being used in a classroom environment (year 7) as part of the Pollen8 Cymru initiative. C-D. Pollen models created for the National Botanic Garden of Wales. C. Model of a dandelion (Taraxacum officinale) pollen grain being exhibited at the Royal Welsh Show. D. 3D pollen models used for public engagement at the Growing the Future Cymru Pollinator festival. E. The Met office explaining the link between pollen and hay fever at the Bluedot festival. F. Science Made Simple staff show off some of our 3D pollen models ahead of the Glastonbury festival. G. Heather Pardoe explains the importance of pollen at the After Dark science event at Amgueddfa Cymru (National Museum of Wales). Photographs courtesy of Mike Pascoe (B), Faye Watson (C, D), Felicity Liggins (E), Jack Laird (F) and Heather Pardoe (G).*

pollen in the field, performing the imaging and 3D modelling and then generating the finished 3D printed output on the facility's UltiMaker FFD 3D printer. The collections produced were used by each of the above organisations in their public outreach and education programmes and exhibited at various culturally significant national and international events such as the National Eisteddfod of Wales, The Royal Welsh Festival, Glastonbury, as well as smaller, local festivals, events and initiatives (e.g.,

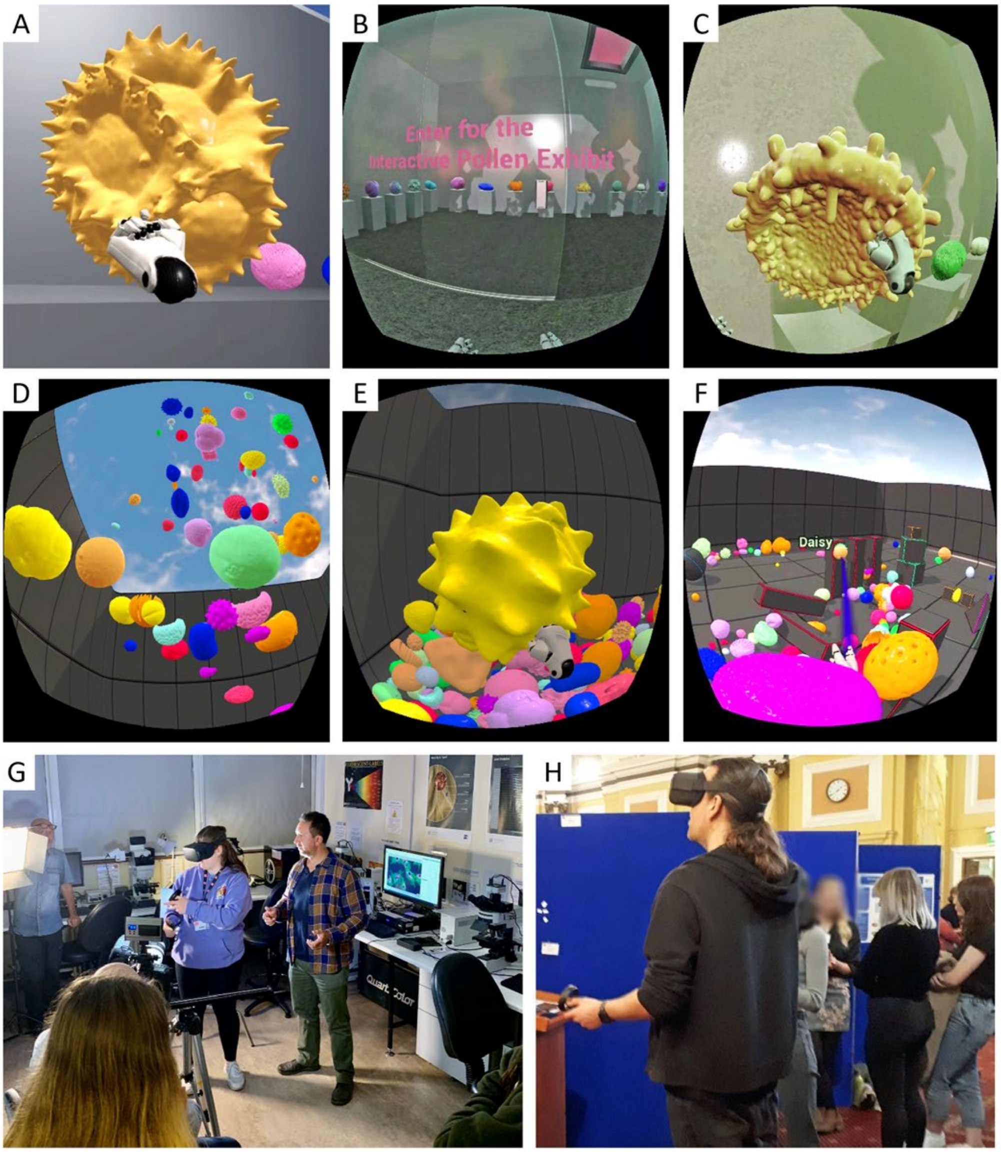

Figure 5. *Virtual reality (VR) pollen resource developed by the Bioimaging Research Hub. The VR resource allows users to interact with pollen grains and other 3D objects in different virtual learning environments. A-C. Interactive 3D pollen exhibit. A. Working prototype using the first-generation Oculus Go headset (2019) – one hand and three degrees of freedom movement (pitch, yaw and roll). B-F. updated and improved version using the second-generation Oculus Quest headset (2020) – two handed and six degrees of movement (also includes surge, heave, sway). D, E. Fun VR learning environments designed for children: 3D pollen pit, F. 3D pollen cannon. Unreal engine coding by Marc Isaacs and Angelina Murphy. G. An undergraduate student gets to grips with the VR controls during filming of a promotional video for Cardiff University at the Bioimaging Hub. H. Testing the VR at a research day at City Hall, Cardiff. Figure 4G courtesy of Rhys Jones, Cardiff University.*

Between the Trees, Pollen8 Cymru, etc) generating significant interest from the public. Feedback from these was shared on the Bioimaging Hub's X (formerly twitter) feed @cubioimaginghub.

Immersive learning: probing the reality of nature

In 2019, the Bioimaging Research Hub began developing workflows that would allow virtual reality (VR) interaction with our surface rendered

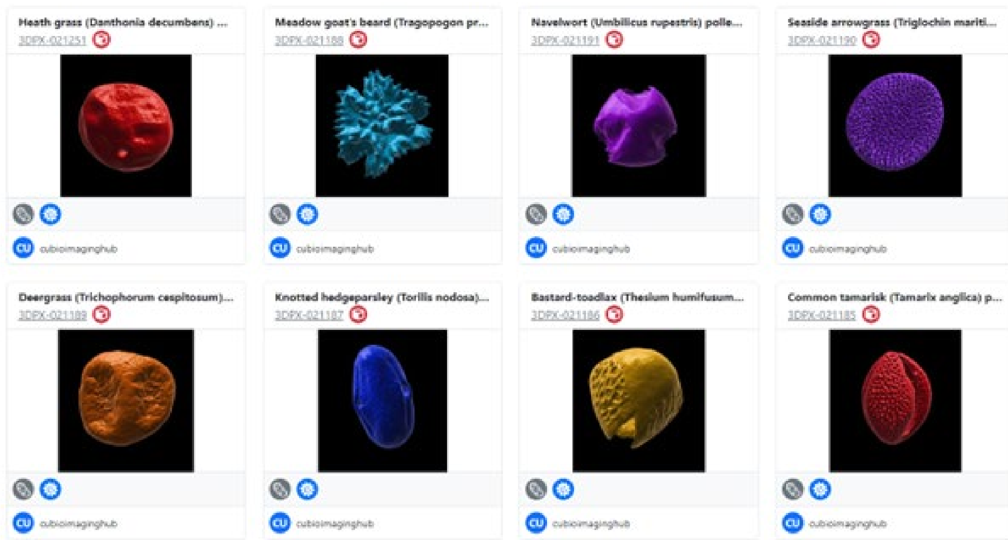

Figure. 6. *Screenshot from the 3D Pollen Library collection showing a small selection of thumbnails of 3D pollen models available for download. The collection currently holds 173 published, DOI-referenced models of pollen grains and spores from 158 species of plant, together with source data (confocal z-stacks), published supporting methodology, and links to major palynological databases for cross-referencing. The models and supporting data are free to download and to use for non-commercial purposes under a CC-BY-NC copyright licence.*

3D digital models (Figure 5). This coincided with the release of the Oculus Go VR headset, which was the first affordable untethered headset to take VR mainstream. We used this device, and the later Oculus Quest headset, in conjunction with the Unreal Engine to create and populate VR environments with our entire range of 3D digital models (Web link: IN FOCUS: Immersive microscopy – 3D Visualisation and Manipulation of Microscopic Samples Through Virtual Reality). In addition to the 3D pollen models, we were also experimenting with 3D photogrammetry techniques in collaboration with the Wales Centre for Anatomical Education to create highly realistic 3D models of anatomical samples for use in anatomy teaching (example here).

The VR pollen resource, along with the 3D prints, were employed for public engagement activities within the School of Biosciences during Cardiff University open days, UCAS visits etc until March 2020. And then covid happened.

The consequence of covid: the 3D pollen library collection and augmented reality

The covid pandemic had enormous societal impact worldwide, profoundly affecting human interaction and resulting in a huge shift to the digital realm. The lockdowns and subsequent social distancing measures introduced by the Welsh Government over this period effectively prevented us from utilising the 3D printed pollen models or the VR headset for public engagement or pedagogy in group-based learning environments due to the infection risks posed. A different approach was therefore necessary. Up until this point, all our 3D digital pollen models were held on the Bioimaging Hub's internal server, thus it made sense to now make them freely available for non-commercial usage, under a Creative Commons CC-NY-NC licence, via the recently redeveloped NIH3D website (formerly the NIH3D print exchange). The NIH3D website is the foremost open, community driven portal to download, share and create bioscientific and medical 3D models for 3D printing and interactive and immersive visualisation. This decision made the collection truly international in its scope. Interested parties could download the models, together with supporting metadata, directly to their computers or personal devices for 3D visualisation, 3D printing or immersive experiences from anywhere in the world without the infection risk associated with a shared physical resource. We were also able to work collaboratively with the NIH3D development team and contribute to the look and feel of the new site. This partnership led to the curation of our datasets into a special *3D Pollen Library* collection (Figures 6 and 7).

A second consequence of covid was that it pushed us into developing new workflows to allow AR visualisation of our 3D pollen models (Figure 8). Critically, unlike VR, this technology does not require a head set and hand controller(s), only a personal smartphone or tablet, thus overcoming the potential risks associated with equipment sharing. AR also allows users to experience a photorealistic environment via the smart device's camera output, with generated perceptual information overlaid upon it, thus providing a more natural immersive experience than the artificial environments of VR. Furthermore, freed from the physical encumbrance of a VR headset, the user has a greater awareness of

Figure 7. *The 3D engine for model visualisation on the NIH3D website***.** *This example shows a pollen grain from thyme-leaf sandwort (Arenaria serpyllifolia). The display can be toggled between 2D and 3D modes. The 3D display presents the option of viewing the pollen grain as a surface rendered model (green) or wireframe (polygon mesh). Both views are shown here. The 3D model can be manipulated on screen into any desired orientation via rotate, drag, and zoom controls allowing visualisation of both external and internal structure (internal detail shown in lower figures). Description and links shown top right; download options, bottom right.*

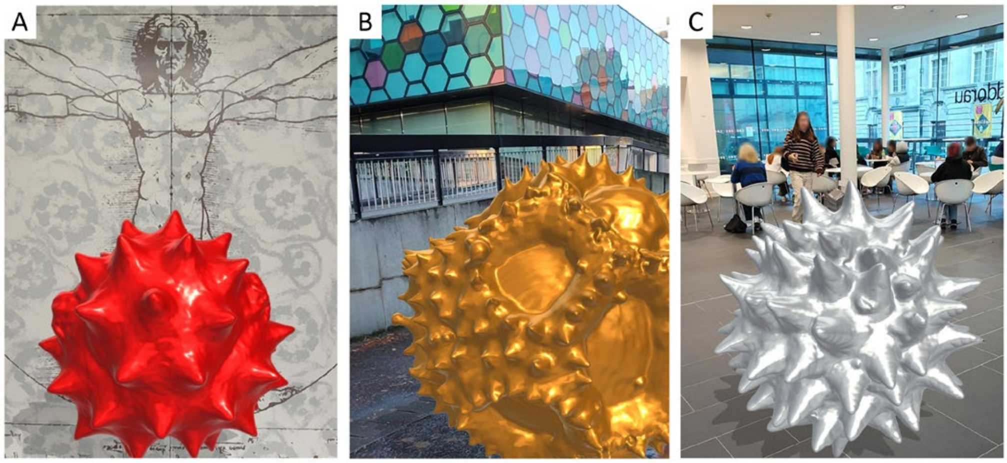

Figure 8. *Augmented reality (AR) visualisation of pollen grains. A-C. Snapshots taken from an android smartphone running a free AR app. Ambient lighting and shadow effects make for a highly realistic immersive experience. The 3D pollen models can be freely moved, rotated and zoomed via the smartphone's touchscreen interface and also circumnavigated and explored - both externally and internally - via directional information from the device's sensors (i.e., accelerometer, gyroscope and compass). A. a pollen grain from ox-eye daisy (Leucanthemum vulgare) skews the proportions – whilst protecting the modesty - of Leonardo's Vitruvian man. B. a dandelion (Taraxacum officinale) pollen grain, supersized as a gilded colossus. C. a pollen grain from common daisy (Bellis perennis) skinned in metallic silver.*

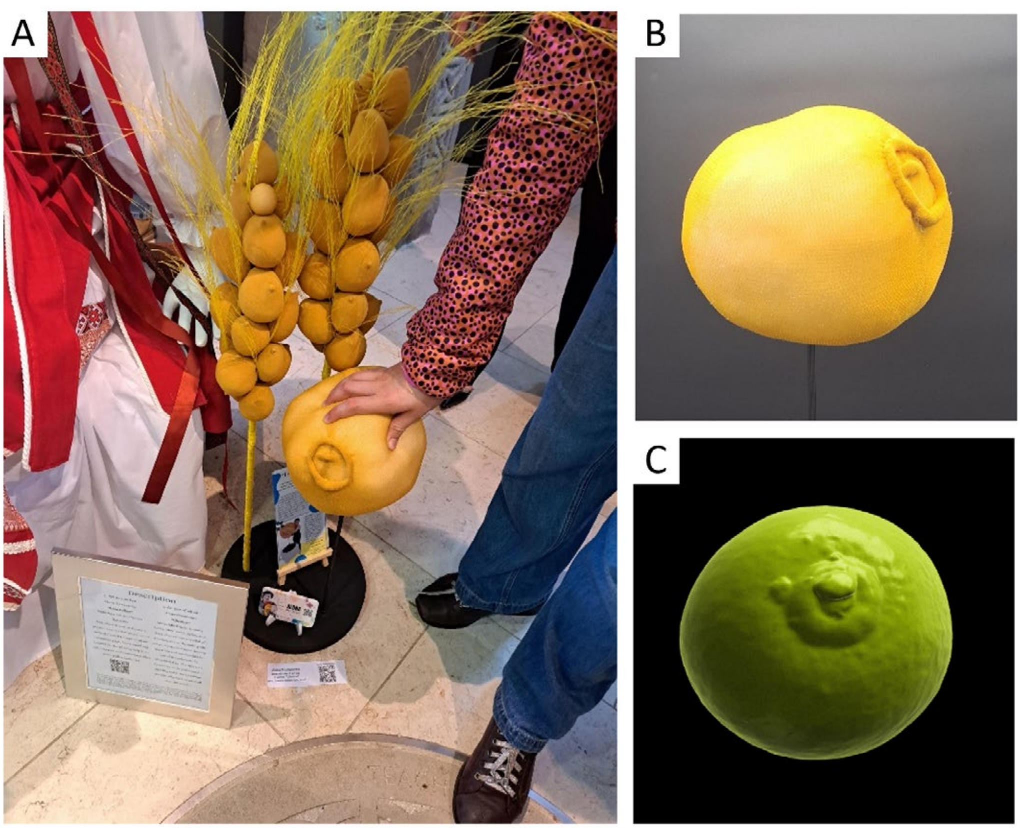

Figure 9. *A, B. Exhibit showing wheat pollen plush for visually impaired and blind. The exhibit, created by Alona Nesterenko, was part of the Ukrainian refugee art exhibition at the These3Streams Art festival at Llantwit Major. C. Surface rendered digital model of pollen grain from wheat (Triticum aestivan) which the plush was based upon. The pollen plush is approximately 20cm in diameter, whilst the pollen grain is approximately 50µm in diameter. Photographs A & B courtesy of Alona Nesterenko.*

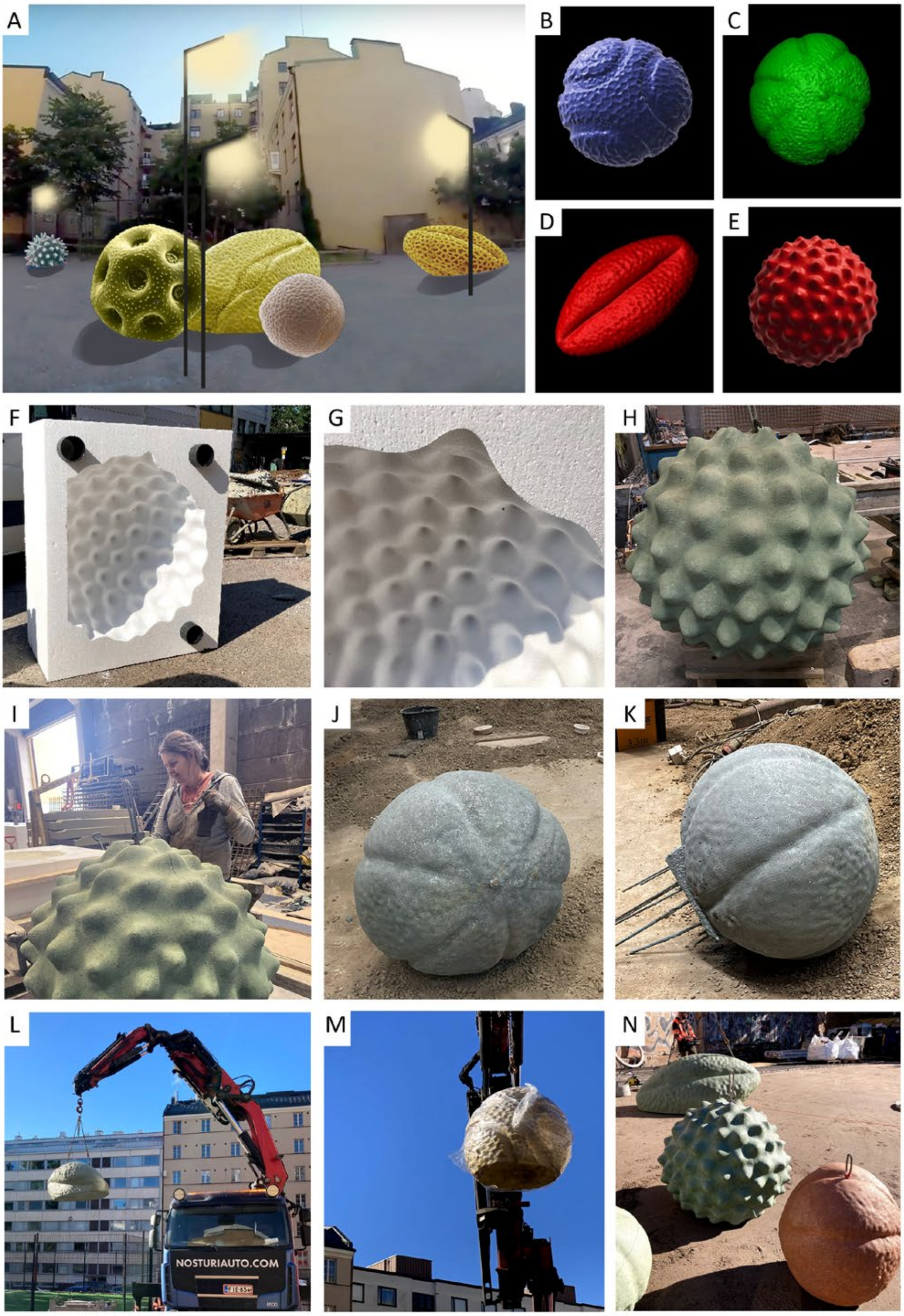

Figure 10. *Breaking the mould: supersizing pollen grains into gigantic public art sculptures. A. Concept art for the 3D pollen sculptures, copyright Saara Ekström. B-D. 3D pollen models from the NIH3D pollen collection to be made into concrete sculptures: B. blue passionflower (Passiflora caerulea), C. thyme (Thymus vulgaris), D. black elderberry (Sambucus nigra), E. ragweed (Ambrosia sp.). F, G. Examples of the casting moulds for the ragweed pollen sculpture. CAD (computer Aided Design) software is used to program a sculpting tool that mechanically carves a negative form of the pollen grain out of high density styrofoam blocks to create the moulds. A flat base, visible in F, is introduced into the mould to ensure that the sculpture remains stable. Eco-friendly natural concrete is poured into the moulds for each of the pollen species. When cured, the moulds are removed from the concrete sculptures. H-K concrete sculptures of ragweed (H, I) and thyme (J, K) pollen. The ragweed pollen sculpture (H-K) is one metre in diameter weighing approximately one and a quarter tonne. The individual shown in I demonstrates the enormous scale of the sculpture (approximately 500,000x larger than the source grain). Steel reinforcing rebar is visible in K. After the sculptures are freed from their moulds, they are hand finished and a coloured protective coating is added. L-N. The sculptures are then transported to the exhibition site and carefully lowered into place under ambient lighting, as demonstrated in concept art. Photographs A, F-N courtesy of Saara Ekström.*

their surroundings and is less likely to experience motion/simulation sickness (Web link: IN FOCUS: AR Palynology: Probing the Reality of Nature/Nature of Reality).

Amgueddfa Cymru: digitising the National Museum's palynology collection.

Over the last year, the Bioimaging Hub has collaborated with Heather Pardoe, the principal botany curator and senior palynologist at Amgueddfa Cymru (the National Museum of Wales), to digitise their archival pollen collection. This successful collaboration has significantly increased the number of pollen models that are now available through the 3D pollen library collection, almost doubling our content, and these have been incorporated into a subcollection of the library, Amgueddfa Cymru, National Museum of Wales, palynology collection. I have also curated a small selection of models to highlight the range of interesting pollen morphologies present within the broader collection, 3D Pollen Library highlights.

Usage of the NIH3D Pollen Library

Following the establishment of the 3D Pollen library collection at NIH3D, the supported models and their supplementary metadata (confocal z-stacks etc) have been viewed and downloaded many hundreds of times. Other than the number of views and downloads per 3D mesh, we rely on direct feedback from the user community to inform where and how our models are being used. Feedback from users indicate that the resource is being used worldwide for a variety of purposes. Two recent usage examples include the University of Medellin, Columbia who have utilised some of our 3D pollen models for their palaeoecology classes, and the Beringia Interpretive Centre, Yukon, Canada, who have exhibited our models as part of an interactive display featuring pollen from pre- and post-glacial periods. Recently we have supported local artist, Alona Nesterenko, in her studies at Cardiff College Art Academy, who has drawn inspiration from our 3D pollen models to create sensory plush models for the visually impaired and blind. One of these, a wheat pollen plush, dedicated to Ukraine, was recently exhibited at a local arts festival (Figure 9).

Currently, we are collaborating with Finnish visual artist and film maker, Saara Ekström, who is supersizing four of our 3D pollen models into gigantic concrete public art sculptures for an elementary school in Helsinki (Figure 10) – a brilliant way to enrich communal spaces, stimulate young (and old) minds and to increase awareness of the unseen natural world. This work was commissioned by the City of Helsinki and will be administered by the Helsinki Art Museum who are responsible for the city's public art collection (Web link: IN FOCUS: Breaking the mould: Supersizing Pollen Grains as Gigantic Concrete Public Art Sculptures).

Current and future directions

The 3D pollen library collection at NIH3D has been an effective mechanism for dispersal of our 3D pollen models at an international level and we hope to improve and develop the site going forward. We are continuing with our ongoing collaboration with Amgueddfa Cymru and are open to new partnerships to exploit and expand the 3D pollen resource for novel applications, activities and events in research, teaching and science engagement, as well as for societal enrichment purposes (e.g., public art, exhibitions etc). As a microscopist, unveiling the unseen beauty of the microscopic world and stimulating and promoting scientific enquiry remains a passion. There are now many publicly accessible science discovery centres in the UK dedicated to STEM learning, with the longest established centre, Techniquest, being based at Cardiff Bay. These discovery centres are united by a national charity, the Association for Science and Discovery Centres (ASDC), which plays an important strategic role in the nation's engagement with science. I am currently looking into collaborative opportunities and partnerships that would allow the 3D pollen models to be utilised in novel STEM activities in these public learning environments. One exciting area might be the development of new workflows to facilitate mixed reality immersive imaging of pollen grains. For example, the new Meta Quest 3 mixed reality VR headset has opened the potential for next level immersive experiences. The headset combines the advantages of VR and AR, with full colour, high-resolution 'passthrough' technology which enables mixed reality (i.e., the blending of the real and computer-generated worlds) with enhanced hand tracking features, thus reducing the need for a hand controller and allowing a more natural immersive experience. New additive manufacturing techniques also offer the potential to 3D print at large scale at much higher print resolutions, and advances in computer aided design (CAD) open up new possibilities in the analysis, modification and optimisation of 3D design for STEM in the development of scientific learning resources, and also for public engagement and societal enrichment through 'Sci-Art'. Lastly, new 3D imaging modalities providing faster speeds, higher throughput and greater resolution, coupled with AI deep learning will provide a step change in palynology research, allowing high throughput screening and rapid phenotyping of pollen samples across multiple scientific disciplines. It's fair to say that the pollen forecast is looking good!

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