

# Pollen image manipulation and projection using latent space

- 1 Ben Mills <sup>1</sup>, Michalis N. Zervas <sup>1</sup> and James A. Grant-Jacob <sup>1,\*</sup>
- <sup>1</sup> Optoelectronics Research Centre, University of Southampton, SO17 1BJ, UK;
- 3 bm602@orc.soton.ac.uk: mnz@soton.ac.uk
- 4 \* Correspondence:
- 5 James A. Grant-Jacob
- 6 J.A.Grant-Jacob@soton.ac.uk;
- 7 Keywords: Pollen, Latent space, Deep learning, Evolution, Imaging.
- 8 Abstract
- 9 Understanding the structure of pollen grains is crucial for the identification of plant taxa and
- understanding plant evolution. We employ a deep learning technique known as style transfer to
- investigate the manipulation of microscope images of these pollen to change the size and shape of
- pollen grains images. This methodology unveils the potential to identify distinctive structural
- features of pollen grains and decipher correlations, whilst the ability to generate images of pollen can
- enhance our capacity to analyse a larger variety of pollen types, thereby broadening our
- understanding of plant ecology. This could potentially lead to advancements in fields such as
- agriculture, botany, and climate science.

### 17 **1 Introduction**

- Pollen grains are essentially the male gametes of plants, carrying the necessary genetic material for
- 19 plant reproduction(Knox, Williams, and Dumas 1986). The role of pollen in plants is crucial as it
- allows plants to reproduce without relying on water for the transport of biological components
- 21 necessary for fertilization. Pollen grains come in a range of sizes and substructures at the nanometre
- scale (Halbritter et al. 2018). The morphology of these pollen grains such as shape, ornamentation,
- and aperturation (Mert 2009), play a crucial role in processes like germination (Matamoro-Vidal et
- 24 al. 2016). The surface of pollen grains can have unique features that help them cling to different
- 25 modes of transportation, such as bird feathers, bee legs, or animal fur, or help them sail through the
- air on appendages that resemble airplane wings or hot air balloons. A pollen grain's morphology can
- change due to dehydration (Fatmi et al. 2020), as dehydration can cause pollen to become more
- angular or irregular as the turgor pressure that maintained its shape is lost. Therefore, imaging of
- 29 pollen grains is a crucial technique as it provides information on the pollen's morphology in 2D and
- 30 3D, providing key insights into the health of crops and the environment (Lau et al. 1995; Fernandez-
- 31 Mensaque et al. 1998). Various imaging methods, including fluorescence microscopy (Atlagić,
- 32 Terzić, and Marjanović-Jeromela 2012), electron microscopy (Coutinho and Dinis 2009), and X-ray
- tomography (Wang et al. 2015; Li et al. 2016) have been used to discern the external and internal
- 34 structure of pollen grains. Despite their utility, these techniques have significant limitations.
- 35 Fluorescence microscopy relies on specific staining protocols that can obscure natural morphological
- details and requires precise sample preparation. Electron microscopy, though capable of high-
- 37 resolution imaging, is limited to surface morphology and necessitates labour-intensive preparation
- 38 steps and whilst X-ray tomography offers 3D imaging, is resource-intensive, involving costly

- 39 equipment and time-consuming data analysis. Additionally, these methods are unsuitable for high-
- 40 throughput analysis due to the extensive time and expertise required, making them impractical for
- 41 studying the vast diversity of pollen species on a large scale.
- 42 Analytical methods have also been employed to explore the creation of pollen grain apertures, such
- as the work by (Zhou and Dobritsa 2019), which used genetic and molecular biology approaches to
- 44 investigate the regulatory pathways controlling aperture formation in pollen grains. Their study
- 45 focused on the role of specific proteins and genes in determining the placement and structure of
- 46 apertures, which are critical for pollen function and viability. Whilst this research provides
- 47 fundamental insights into pollen development, it relies on labour-intensive experimental techniques
- and lacks scalability for analysing large numbers of pollen species.
- 49 Owing to the vast number of pollen species, additional methods of pollen analysis and identification
- have been sought to help understand pollen and thus plant ecology.
- Over the past 10 years, advancements in graphics processing units (GPUs) and deep learning
- algorithms have ushered in a new era of large-scale, data-driven research (LeCun, Bengio, and
- Hinton 2015). The convolutional neural network (CNN), which is inspired by the visual cortex (Serre
- et al. 2007), can be used to categorise images through outputting a label or value. CNNs have been
- applied across the field of palynology (Daood, Ribeiro, and Bush 2016; Grant-Jacob and Mills 2022;
- Romero et al., n.d.; Punyasena et al. 2012), with examples including pollen identification via visible
- 57 light microscopy of pollen grain types (Mahbod et al. 2021; Crouzy et al. 2016; Marcos et al. 2015;
- 58 Grant-Jacob et al. 2021), identification of 46 different pollen grain types (Sevillano, Holt, and
- Aznarte 2020), and for identification of pollen grains from scattering (Grant-Jacob et al. 2019; 2018)
- and holographic patterns (Sauvageat et al. 2020; Luo et al. 2022).
- In recent years, the application of deep learning models, CNNs have shown great promise in the field
- of palynology for pollen grain classification and analysis. However, existing studies primarily focus
- on classification tasks using real pollen images, often limited by the scarcity of high-quality and
- diverse datasets. Whilst CNN-based models have demonstrated impressive results in classifying
- pollen grains, such as the POLEN23E dataset for 23 pollen types with over 97% accuracy (Sevillano
- and Aznarte 2018), and the classification of 73 different pollen types with a higher than 90%
- accuracy (Astolfi et al. 2020) using the POLLEN73S dataset that comprises of 2523 images, these
- methods face challenges when dealing with underrepresented species or rare morphological features.
- These papers highlight the potential of deep learning in analysing and classifying pollen grains,
- which can significantly contribute to a range of fields such as agriculture, botany, and climate
- science. By automating the process of pollen identification, it becomes possible to analyse a larger
- variety of pollen types, thereby broadening our understanding of plant evolution and ecology.
- 73 Style Generative Adversarial Network (StyleGAN) (Karras et al. 2020), first introduced by NVIDIA
- researchers in 2018, is a type of generative neural network that has brought significant modifications
- 75 to the generator model by using an alternative generator architecture that is borrowed from the style
- transfer literature, which allows it to create and subsequently modify synthetic (generated) images.
- StyleGAN is more effective than other generative models in producing realistic images because it
- 78 introduces a unique style-based generator architecture that allows precise control over image
- 79 attributes at different levels of abstraction, from coarse to fine details. Additionally, its disentangled
- latent space enables the generation of high-quality, diverse, and photorealistic images with smooth
- 81 interpolation across variations. This makes it an ideal tool for creating synthetic pollen images that

- 82 retain realistic qualities, which is vital for training deep learning models or enhancing datasets in
- 83 fields such as agriculture, botany, and climate science.
- The unique feature of such a style-based network is its ability to control specific aspects of the
- 85 generated image through the manipulation of the latent space, which describes a higher-abstraction
- 86 representation of the generated image. This allows for the generation of images with specific
- 87 characteristics, such as a particular style or feature. For instance, in the case of generating images of
- faces, the network can control aspects such as the pose, identity, and even details like freckles or hair.
- 89 Previous synthetic generation studies in palynology have focused on generating low-resolution
- 90 microscope images (Khanzhina et al. 2022) or using scanning electron microscope (SEM) images for
- 91 higher-resolution representations (Grant-Jacob, Zervas, and Mills 2022). Whilst these approaches
- have been useful for generating synthetic data, they do not offer the demonstrate the potential of
- 93 using StyleGAN for offering new insights into the relationship between these traits using multiple
- 94 vector manipulation, nor do they project real images into latent space for manipulation.
- 95 Unlike traditional approaches, this study introduces a novel application of StyleGAN for the
- 96 synthetic generation of pollen images, enabling manipulation of multiple latent space vectors to
- 97 control specific morphological features such as size, shape, and ornamentation to generate new
- 98 images of pollen. Furthermore, it allows for the exploration of feature relationships, such as the
- 99 correlation between size and ornamentation, which is often difficult to achieve through conventional
- imaging techniques.
- 101 This paper explores the potential of using StyleGAN for interpolating between microscope images of
- pollen grains in latent w-space to generate additional images of specific pollen taxa and to simulate
- transformations from one pollen taxa to another. It also demonstrates that w-space latent vectors can
- be identified that allow characteristics, such as pollen size and shape, to be manipulated in generated
- images, and that this technique could potentially unlock further understanding of the palynological
- relationships.
- 107 A block diagram concept of the study demonstrated in this manuscript is displayed in Figure 1,
- showing how 2070 microscope images are used to train a StyleGAN network, and then a single real
- image is then projected into the network and undergoes vector manipulation in latent space before
- being generated by the synthesis part of the StyleGAN network.

### 2 Materials and Methods

112 Data acquisition

111

- The image data used in this work is derived from the pollen dataset published by (Sevillano, Holt,
- and Aznarte 2020) and further detailed in (Holt 2020). This dataset comprises high-resolution
- microscope images of 46 different pollen taxa. The dataset is particularly well-suited for this research
- due to its diversity of taxa, which spans a wide range of morphological characteristics such as size,
- shape, ornamentation, and ornamentation. These variations are critical for training machine learning
- models to recognize and classify different pollen types accurately.
- To make sure that there was equal weighting in the training of the neural network, 45 images of each
- pollen taxa were used in training the neural networks (2070 total). The image files from the dataset
- were padded with zeros and then resized to  $256 \times 256$  pixels (RGB), to ensure all images had the
- same aspect ratio whilst preserving the relative size information, before being used as training data.

- 123 Neural networks
- This work used two separate neural networks, the StyleGAN network and the CNN. The StyleGAN
- network was used to generate images of pollen and to subsequently modify specific properties of
- these generated images. Owing to a CNN ability to classify pollen grains with great accuracy, a CNN
- was used to validate the generated images. The CNN was used as a classifier network, to identify the
- taxa of each image generated by the StyleGAN network. The neural networks underwent training on
- a workstation running Windows 10 and equipped with an AMD Ryzen Threadripper PRO 5975WX
- with 32 cores operating at 3.60 GHz, 128 GB RAM, and two NVIDIA A6000 GPUs (each with 48
- 131 GB memory).
- 132 StyleGAN, a generative neural network, was used to create synthetic microscope images of pollen
- grains, where the appearance of these images was based on the training data. This work used
- 134 StyleGAN2, which is available on GitHub (https://github.com/NVlabs/stylegan2-ada-pytorch.git). As
- shown in Figure 2, the StyleGAN network consists of two subnetworks, known as the mapping
- network and the synthesis network. The mapping network transformed a random noise vector  $\mathbf{z}$  (1 ×
- 137 512) into a vector  $\mathbf{w}$  (1 × 512), and the synthesis network transformed the vector  $\mathbf{w}$  into an RGB
- image  $(256 \times 256 \times 3)$  of a pollen grain. Therefore, either a **z** or a **w** vector could be used to generate
- a synthetic image of a pollen grain. The z vector is known as a latent space vector in z-space, and
- likewise the w vector exists in w-space. Critically, as the mapping network is designed to disentangle
- the properties (or 'style') of pollen grains in the generated images, vectors in w-space correspond to a
- higher abstraction of the pollen grains and hence offer the capability to unlock manipulations of
- specific features in the generated images. In this work, as discussed later, this capability enables
- properties such as the size or shape of pollen grains in the generated images to be modified, or for an
- image of a generated pollen grain to be gradually transformed into an image of a different pollen
- 146 grain.
- 147 The architecture of the StyleGAN network is shown in Figure 3. The StyleGAN architecture consists
- of three main components: the Mapping Network, Synthesis Network, and Discriminator Network.
- The Mapping Network (left panel) consists of fully connected layers and takes a latent vector (z)
- sampled from a distribution (e.g., Gaussian noise) and transforms it into an intermediate latent space
- (w), which helps disentangle features for better control during image generation. The Synthesis
- Network (middle panel) starts with a learned constant tensor at a low resolution (4×4) and
- progressively increases the resolution through up-sampling (e.g.,  $4\times4 \rightarrow 8\times8 \rightarrow 16\times16$ , up to the
- final resolution (n×n). Noise injections at each level add stochastic details, resulting in a synthesized
- image. The Discriminator (right panel) processes real or generated images, progressively reducing
- their resolution from high  $(n\times n)$  to low  $(4\times 4)$ , and outputs a prediction to differentiate between real
- and generated images. This network is connected to a loss function used to train both the generator
- and discriminator adversarially. The system is trained adversarially, with the generator aiming to
- produce images indistinguishable from real ones, whilst the discriminator tries to correctly classify
- them as real or fake.
- 161 The training for the StyleGAN was conducted using Python with CUDA enabled. The network was
- trained on 2070 images with 5000 kimg (5 million images processed), meaning that each of the
- training images was used approximately 2400 times each. The training process took about 2 days and
- 18 hours, averaging 47 seconds per kimg. The training parameters included a learning rate of 0.0025.
- a non-saturating logistic loss function, a batch size of 32, and ADAM (Adaptive Moment Estimation)
- was used as the optimizer (Kingma and Ba 2014). The Fréchet Inception Distance (FID) score
- 167 (Heusel et al. 2017) was used to measure the similarity between the generated images and the training

- 168 images. This score represents the distance between the feature vectors of the two sets of images,
- 169 where a score of zero would mean that the distributions of the generated and training images are
- 170 identical. The FID score was computed every 200 kimg during the training process, and the score
- 171 was observed until it plateaued around a value of 29 after 5000 kimg.
- 172 The CNN was trained to identify the taxa of the images generated by the StyleGAN neural network,
- 173 and was trained using the same image data as the StyleGAN network. Therefore, an image of  $256 \times$
- 174  $256 \times 3$  size was used as the input to the CNN, and the network output was a prediction of the pollen
- 175 taxa. There were no other pre-processing steps beyond resizing and cropping. The training data for
- 176 the CNN was split into percentages of 70% for training, 25% for validation, and 5% for testing, and
- 177 the architecture was the Inception v3 (Szegedy et al. 2017) used in MATLAB
- 178 (https://uk.mathworks.com/help/deeplearning/ref/inceptionv3.html,
- 179 https://uk.mathworks.com/matlabcentral/fileexchange/65679-deep-learning-toolbox-model-for-
- 180 inception-v3-network). In this work, no augmentation was used on the dataset. The network was
- trained for 5 epochs, with an initial learning rate of 0.0002, a validation frequency of 200, a learn rate 181
- drop factor of 0.1, a minibatch size of 2, and took 2 days 21 hours 48m hours to train. The CNN 182
- 183 achieved a classification accuracy of 86% (see Figure 4 for training accuracy and validation accuracy
- 184 graph over 5 epochs (34580 iterations) when applied to the testing data and is labelled as the
- classification network in Figure 2. 185
- 186 Image generation
- 187 Figure 5 shows a schematic of the methodology for projecting an image into latent space, then
- 188 extracting relevant vector before manipulating the vector in latent space, either to increase or
- 189 decrease the size of the pollen or increase or decrease the spikiness. Compared to other work in
- 190 pollen image generation, we demonstrate not only manipulating latent space for image manipulation
- 191 in multiple ways, such as size and shape, but we also project experimentally obtained images into
- 192 latent space and manipulate them, potentially opening the opportunity to manipulate images and thus
- 193 species not present in the training data.
- 194 We generated 1000 synthetic images of pollen grains by using 1000 z vectors, where each of the 512
- 195 numbers in the vectors were randomly sampled from a normal distribution with  $\mu = 0$  and  $\sigma = 1$ . The
- 196 corresponding w-vectors for each of these generated images was also recorded, to support subsequent
- 197 latent space manipulations. Interpolation between two generated images with w-space vectors of wa
- 198 and wb could then be achieved by generation of an image using a w-space vector of wc = wa + k(wb)
- 199 - wa), where k is a scalar between 0 and 1, and we is the w-vector for the interpolated image. In this
- 200 case, the vector (wb - wa) therefore corresponds to a w-space vector that describes the structural
- 201 change between the two images. If, for example, wa corresponded to a small pollen grain and wb
- 202 corresponded to a large pollen grain then (wb - wa) would correspond to a w-space vector for
- 203 increasing the size of the pollen grain. However, this w-space vector would also correspond to the
- 204
- changes in other features, such as the difference in the shape of the two pollen grains. Therefore, by
- 205 averaging over many such vectors, a vector for increasing pollen grain size was identified that
- 206 encapsulates the visual information contained in the training dataset. This 'size' w-space vector could
- 207 then be added to (or subtracted from) any w-space vector corresponding to a generated image, to
- 208 increase (or decrease) the size of the pollen grain in the generated image. To achieve this, a folder of
- 209 synthetic images of 'small' pollen grains was created, and a folder of 'large' pollen grains was
- created, from which the w-space vectors were obtained. Similarly, an 'spike' w-space vector for 210
- 211 pollen transitioning from no spikes to spikes, and a 'round' w-space vector for pollen transitioning
- from triangular to round was identified. As shown later in this work, this allows the generation of a 212

wide variety of images representing different types and sizes of pollen grains, along with bespoke

214 morphological changes to the pollen grains in these images.

### 3 Results

215

- Following training of all the two neural networks, 1000 z-space vectors were used to create 1000
- 217 images using the StyleGAN neural network, and the corresponding 1000 w-space vectors were also
- recorded. The CNN was then used to predict the taxa for each of these generated pollen grains.
- The selection of 1000 z-space vectors was guided by the need to balance computational feasibility
- with adequate coverage of the latent space's variability. The latent space of StyleGAN is inherently
- high-dimensional, and z-space vectors are typically modelled as a standard Gaussian distribution,
- meaning random sampling spans a representative subset of the space. Generating 1000 synthetic
- images is a practical compromise, manageable in terms of resources whilst sufficiently capturing
- variability for downstream tasks. To ensure the generated images reflected the training dataset's
- diversity, the Fréchet Inception Distance (FID) was used for validation, giving a value to 6.281 for
- 226 5000 kimg. Lower scores indicate higher similarity between the feature distributions of the real and
- 227 synthetic datasets. Scores below 10 are considered very good in most generative model tasks,
- 228 indicating higher similarity between the feature distributions of the experimental training and
- 229 generated datasets. In addition, we compared the distribution of taxa labels in the training data with
- the 1000 predicted labels of the generated data, as shown in Figure 6.
- The Jensen-Shannon (JS) divergence (Nielsen 2019) is a symmetric and bounded metric that
- measures the similarity between two probability distributions, making it interpretable and robust. A
- value of 0 indicates identical distributions, and the low divergence observed here suggests that the
- synthetic data closely approximates the real dataset, capturing much of its variability with only minor
- 235 differences.
- 236 The JS divergence is calculated using,

237 
$$D_{JS}(P||Q) = \frac{1}{2}D_{KL}(P||M) + \frac{1}{2}D_{KL}(Q||M)$$

- where P and Q are the two distributions being compared, M=1/2(P+Q) is the average distribution,
- and  $D_{KL}$  is the Kullback-Leibler divergence. The JS divergence is bounded between 0 and 1 (or
- 240 log(2)) for certain bases) and symmetrically averages over both distributions. The JS divergence was
- calculated to be 0.073. This, along with the histogram comparison, indicates a relatively low
- 242 divergence between the real and synthetic datasets.
- 243 The low JS divergence highlights that the synthetic dataset does an accurate job of replicating the
- variability of the real dataset. However, the histogram reveals that some specific categories might
- still benefit from refinement.
- The calculated w-space vectors of 'size', 'spike' and 'round' were added (or subtracted) from a range
- of generated images to visualise the predicted changes in morphology of the generated pollen. As
- 248 displayed in Figure 7, -100%, -50%, 0%, +50%, +100% of the 'size' and 'spike' vectors were added
- onto the w-space vector corresponding to a generated image of (a) *Knightia* and (b) *Coriaria*. The
- central image shows the generated images with no additional w-space space vectors, and the change
- in the horizontal direction shows addition (or subtraction) of the 'size' vector and the change in the
- vertical direction shows addition (or subtraction) of the 'spike' vector. The classification CNN was

- 253 applied to these generated images, and the predicted pollen taxa, along with prediction confidence, is
- 254 shown on each generated image. Each generated image also includes the area of the pollen grain,
- 255 calculated via summing the number of image pixels corresponding to the pollen grain. However,
- 256 labelling is omitted from of images without any visible grains.
- 257 In Figure 8, the same methodology was also applied to generated images of (a) *Metrosideros* and (b)
- 258 Disphyma, with a the 'size' vector applied in the horizontal direction and the 'round' vector applied
- 259 in the vertical direction. The roundness value is also labelled on the figure, which quantifies how
- closely the shape resembles a perfect circle, with higher roundness values indicating shapes that are 260
- more circular, and lower values corresponding to more irregular shapes, where this value was 261
- determined  $((4\pi \text{Area/Perimeter}^2) \times (1 0.5/r)^2$ , where  $r = \text{Perimeter}/(2\pi) + 0.5$ ). 262
- 263 The ability to use images of the real-world for interpolation could allow previously unseen pollen
- 264 grains to be examined. As such, by projecting an experimental image into the latent space, an
- 265 equivalent latent space image can be found and be used in the vector manipulation. The process of
- 266 mapping a real-world image into the latent space of the model is known as "projection". An initial
- latent vector is created, usually either at random or based on the average latent vector of the model. 267
- 268 This latent vector is then progressively refined using gradient descent to reduce the disparity between
- 269 the image produced from the latent vector and the original real-world image. The outcome of this
- 270 iterative optimization is a fine-tuned latent vector that encapsulates the real-world image in the
- 271 model's latent space. This vector can be further manipulated or analysed as needed. Overall, this
- procedure enables the model to effectively translate real-world images into its own latent space. In 272
- 273 Figure 9, we interpolate between two different taxa in (a) *Knightia* (LHS) and *Kunzea* (RHS), and (b)
- 274 Brachyglottis repanda (LHS) and Citrus (RHS) to demonstrate the capability of such a technique.
- 275 We also use the CNN to predict the generated pollen taxa. As seen in the images, it is possible to
- 276 interpolate between two taxa, generating what appear to be other taxa in the process. Discussion
- 277 The generated images shown in Figure 7 demonstrate that when the 'spike' vector is added (or
- 278 subtracted), not only does the appearance of the spikes on the generated pollen grains change, but the
- 279 taxa also changes. Likewise, as the 'size' vector is added (or subtracted), the generated pollen
- 280 changes size and taxa. When the size or spike vector is too negative, there is no image generation,
- 281 meaning the latent space vectors may not be mapped to the features that generate an image of pollen.
- When both vectors are applied, such as +100% 'size' and +100% 'spike', a generated image that 282
- 283 resembles Lycopodium pollen is created. It should be noted that whilst the CNN predicts
- 284 Lycopodium, it is limited to the dataset in which it has been trained on, and the actual Lycopodium
- 285 pollen contains more ornamentation on its surface. Interestingly overall, when the 'spike' is added,
- 286 the size of the pollen grain increases, perhaps implying correlation between size and spikes (or
- 287 ornamentation), based on the dataset and calculated vectors used in this work. Indeed, this can be
- 288 observed in Figures 7(a) and (b), where only the edge of the pollen grains was visible in the smaller
- 289 grains, and as the spike vector is increased, the pollen changes taxa to that which are not only larger,
- 290 but have more ornamentation over the whole pollen as viewed by the microscope.
- 291 The ability to manipulate images in latent space is also demonstrated by changing the roundness in
- 292 Figure 8. It can be seen that the 'size' vector increases the size of the generated pollen grain, and
- 293 'round' vector generally increases the circularity. In Figure 8(a), there is little evidence that the
- 294 roundness of the pollen has correlation with the size, though for some taxa (e.g., Griselinia (0%,
- 295 50%) and *Ixerbia* (0%, 100%)) increasing the roundness does increase the size, due to how the neural
- 296 network has positioned such pollen in the multidimensional latent space. It is evident that increasing
- 297 the size or roundness does not merely at spikes or make the pollen rounder, but changes the structure

- or ornamentation as well. For example, increase the roundness of *Carpodetus* removes the lobe
- 299 structure as it transitions to *Citrus*. The conclusions drawn from these figures may be limited to the
- training dataset, and hence additional training data might provide additional insights. If this approach
- was applied to a much more varied dataset, perhaps containing thousands of taxa, latent w-space
- 302 vector arithmetic could have potential in helping understand the relationship between features and
- traits in pollen grains, and to predict taxa changes (and results of these changes) due to environmental
- and evolutionary factors.
- The ability to essentially upload an image into latent space for manipulation could provide a power
- 306 tool in understanding relationships and behaviour of pollen. In Figure 9, which shows the transition
- between two pairs of pollen images not present in training, we can see a transition between one taxa
- 308 to another, not by only straight pixel interpolation, but through different taxa. In (a) the image
- transition occurs through 7 different taxa, and in (b) the transition occurs through 6 different taxa, as
- 310 classified by the CNN. The transition between pollen is dependent on how latent space has
- distributed the data, as in (a) the transition is not simple a large triangular shape to a small triangular
- shape. Although pollen grain taxa have been identified, the identification is limited to the training of
- 46 taxa, meaning that although a pollen grain maybe identified as *Laurelia* at 99%, it may not
- 314 necessarily look like it due to the limited data used in the CNN training. Owing to such as vast
- number of pollen taxa present in the world, being able to train a latent space neural network on every
- 316 type would be extremely difficult. As such, this methodology demonstrates the possibility of using
- pollen taxa not used in training so that it could be explored in latent space and manipulated to
- 318 understand it morphology in different environments, such as undergoing dehydration (Grant-Jacob et
- al. 2022), or understand the pollen in the context of their phylogenetic relationships. This
- 320 methodology could be used in addition to work on using CNNs to analyse pollen morphology and
- 321 place extinct pollen morphotypes within a phylogenetic framework using Bayesian inference
- 322 (Adaïmé, Kong, and Punyasena 2024).
- A key challenge in isolating specific features, such as size versus shape, through w-space
- manipulation in StyleGAN is the potential overlap between latent vector representations for different
- 325 characteristics. The latent space in StyleGAN is highly compressed and abstract, meaning that
- features like size, shape, ornamentation, and colour are not always entirely independent. As a result,
- 327 adjusting one feature may inadvertently affect others, complicating the process of isolating and
- 328 controlling a specific characteristic independently.
- For example, when manipulating the latent vector to adjust the size of a pollen grain, the shape or
- ornamentation of the grain may also change. This is because these features might share latent
- dimensions in the vector space, and the model may not perfectly separate them. As seen in Figure 7,
- when the 'size' vector is altered, the taxa of the pollen grain can change, along with its size,
- indicating a correlation between size and taxa or other features like ornamentation. This overlap in
- latent vector representations makes it difficult to manipulate one feature without influencing others.
- Furthermore, the model's ability to separate features effectively depends on the richness and diversity
- of the training dataset. If the dataset lacks sufficient variation in certain attributes, the model may
- struggle to create distinct latent representations for each feature. This could lead to challenges in fine-
- tuning or generating high-quality, realistic images where individual characteristics are clearly
- separated, as seen in the generated images where manipulating the 'spike' vector also influenced the
- size and ornamentation of the pollen. These issues highlight the complexity of manipulating specific
- 341 features in StyleGAN and the need for a more refined approach to disentangling latent
- representations.

- 343 Synthetic images generated by StyleGAN can be highly valuable for augmenting real-world datasets,
- especially in areas like pollen classification, where obtaining a diverse and high-quality dataset may
- be challenging. By generating realistic and controlled synthetic images, StyleGAN can help fill gaps
- in datasets, increase their size, and improve the diversity of training examples available for deep
- learning models. This can enhance the robustness and generalization ability of classifiers, making
- them more effective in real-world applications.
- For example, in the context of pollen classification, obtaining images of all possible pollen types with
- varying characteristics (e.g., size, shape, ornamentation) under different imaging conditions can be
- difficult. Augmentation of images could be achieved by carefully manipulating latent vectors,
- specific features such as spike density, size, and ornamentation can be adjusted, enabling the
- 353 generation of images for underrepresented or difficult-to-capture species or scenarios. This synthetic
- data can thus act as a supplement to the real dataset, improving classifier performance on less
- 355 common or poorly represented pollen types.
- 356 Moreover, synthetic images can be particularly useful in cases where real-world data is scarce due to
- privacy concerns, cost, or limited access to expert annotation. For example, in clinical or
- environmental settings where data collection is expensive or time-consuming, synthetic images can
- fill in the gaps, allowing deep learning models to be trained on a more diverse set of examples.
- Additionally, StyleGAN-generated images could be used to simulate edge cases or rare occurrences
- that may not be adequately captured in real-world datasets, further enhancing the model's ability to
- 362 handle a wide range of real-world conditions.

## 4 Conclusion and future scope

363

- The results presented in this work demonstrate the significant potential of leveraging StyleGAN's
- latent space manipulation to explore and understand pollen grain morphology. Through the
- projection, generation and manipulation of synthetic pollen images, we have shown that adjusting
- latent vectors such as 'size', 'spike', and 'roundness' not only alters the appearance of pollen grains
- but can also lead to changes in their taxa classification. These findings suggest that latent space
- manipulation offers a powerful method for studying the relationships between different features of
- pollen grains, such as size, ornamentation, and shape, which are crucial for understanding both
- and evolutionary influences on pollen morphology.
- However, this approach is not without limitations. The results observed in this study are inherently
- 373 tied to the training dataset, which restricts the generalizability of the findings. The manipulation of
- latent vectors is influenced by how the data is distributed in the multidimensional latent space, and
- 375 this may result in unexpected transitions, particularly when working with pollen taxa not included in
- 376 the training set. Further research into expanding the training dataset to include a broader range of
- pollen taxa, and perhaps even thousands of species, could reveal deeper insights into the underlying
- 378 relationships between pollen traits and environmental factors.
- 379 The future scope of this work involves expanding the training dataset to include a broader range of
- pollen taxa, which would improve the accuracy and reliability of generated images and enable more
- 381 precise feature manipulation. A larger, more diverse dataset would offer deeper insights into the
- relationships between pollen traits and environmental factors. Additionally, the ability to manipulate
- pollen images in latent space could be utilised to study the effects of environmental influences, such
- as dehydration or climate change, on pollen morphology, and to simulate evolutionary changes in
- pollen structures. This approach could also be integrated with phylogenetic frameworks to better

- understand the evolutionary relationships between different pollen taxa, as seen in work that places
- extinct pollen morphotypes within a phylogenetic context. Furthermore, insights gained from latent
- space manipulation could enhance predictive models for pollen identification and classification,
- particularly for taxa not included in the training dataset. Ultimately, with continued refinement and
- 390 expansion, this methodology holds the potential to improve our understanding of pollen grain
- morphology and its implications in areas such as agriculture, climate science, and botany.

### 392 **5** Conflict of Interest

- 393 The authors declare that the research was conducted in the absence of any commercial or financial
- relationships that could be construed as a potential conflict of interest.

## 395 **6 Data Availability Statement**

- 396 The original dataset used in training the neural network in the current study is available in the
- 397 <a href="https://doi.org/10.6084/m9.figshare.12370307.v1">https://doi.org/10.6084/m9.figshare.12370307.v1</a> repository. The datasets generated and/or analysed
- during the current study are available at https://doi.org/10.5258/SOTON/D3108.

### 399 **7 Author Contributions**

- 400 **BM**: Writing review & editing, Resources. **MNZ**: Writing review & editing, Funding acquisition.
- 401 **JAGJ**: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation,
- Writing original draft, Writing review & editing, Visualization, Project administration.

## **403 8 Funding**

- 404 This research was funded by Engineering & Physical Sciences Research Council, grant numbers
- 405 EP/W028786/1 and EP/T026197/1.

### 406 **9** Acknowledgments

- This is a short text to acknowledge the contributions of specific colleagues, institutions, or agencies
- 408 that aided the efforts of the authors.

### 409 **10 References**

- 410 Adaïmé, Marc-Élie, Shu Kong, and Surangi W Punyasena. 2024. "Deep Learning Approaches to the
- Phylogenetic Placement of Extinct Pollen Morphotypes." *PNAS Nexus* 3 (1): pgad419.
- https://doi.org/10.1093/pnasnexus/pgad419.
- 413 Astolfi, Gilberto, Ariadne Barbosa Gonçalves, Geazy Vilharva Menezes, Felipe Silveira Brito
- Borges, Angelica Christina Melo Nunes Astolfi, Edson Takashi Matsubara, Marco Alvarez, and
- Hemerson Pistori. 2020. "POLLEN73S: An Image Dataset for Pollen Grains Classification."
- 416 *Ecological Informatics* 60:101165. https://doi.org/https://doi.org/10.1016/j.ecoinf.2020.101165.
- 417 Atlagić, Jovanka, Sreten Terzić, and Ana Marjanović-Jeromela. 2012. "Staining and Fluorescent
- 418 Microscopy Methods for Pollen Viability Determination in Sunflower and Other Plant Species."
- 419 *Industrial Crops and Products* 35 (1): 88–91. https://doi.org/10.1016/J.INDCROP.2011.06.012.
- 420 Coutinho, Antonio Pereira, and Augusto M. Dinis. 2009. "A Light, Scanning Electron, and
- Transmission Electron Microscopic Study of Pollen Wall Architecture in the Subtribe

422 423	Gnaphaliinae (Gnaphalieae, Asteraceae)." <i>Plant Systematics and Evolution</i> 283 (1): 79–92. https://doi.org/10.1007/S00606-009-0216-2/TABLES/2.
424 425 426	Crouzy, Benoît, Michelle Stella, Thomas Konzelmann, Bertrand Calpini, and Bernard Clot. 2016. "All-Optical Automatic Pollen Identification: Towards an Operational System." <i>Atmospheric Environment</i> 140:202–12. https://doi.org/10.1016/j.atmosenv.2016.05.062.
427 428	Daood, Amar, Eraldo Ribeiro, and Mark Bush. 2016. "Pollen Grain Recognition Using Deep Learning." In <i>International Symposium on Visual Computing</i> , 321–30.
429 430 431	Fatmi, H, S Mâalem, B Harsa, A Dekak, and H Chenchouni. 2020. "Pollen Morphological Variability Correlates with a Large-Scale Gradient of Aridity." <i>Web Ecology</i> 20 (1): 19–32. https://doi.org/10.5194/we-20-19-2020.
432 433 434	Fernandez-Mensaque, Pilar Candau, Francisco José González Minero, Julia Morales, and Carmen Tomas. 1998. "Forecasting Olive (Olea Europaea) Crop Production by Monitoring Airborne Pollen." <i>Aerobiologia</i> 14 (2): 185–90.
435 436 437 438	Grant-Jacob, James A, Saurabh Jain, Yunhui Xie, Benita S Mackay, Michael D T McDonnell, Matthew Praeger, Matthew Loxham, David J Richardson, Robert W Eason, and Ben Mills. 2019. "Fibre-Optic Based Particle Sensing via Deep Learning." <i>Journal of Physics: Photonics</i> 1 (4): 44004.
439 440 441	Grant-Jacob, James A, Benita S Mackay, James A G Baker, Daniel J Heath, Yunhui Xie, Matthew Loxham, Robert W Eason, and Ben Mills. 2018. "Real-Time Particle Pollution Sensing Using Machine Learning." <i>Opt. Express</i> 26 (21): 27237–46. https://doi.org/10.1364/OE.26.027237.
442 443 444	Grant-Jacob, James A, and Ben Mills. 2022. "Deep Learning in Airborne Particulate Matter Sensing: A Review." <i>Journal of Physics Communications</i> 6 (12): 122001. https://doi.org/10.1088/2399-6528/aca45e.
445 446 447	Grant-Jacob, James A, Matthew Praeger, R W Eason, and Benjamin Mills. 2021. "Pollen Grain Identification Using Semantic Image Segmentation." In <i>IOP Physics Meets Biology</i> 2021, p01. Virtual.
448 449 450	Grant-Jacob, James A, Matthew Praeger, Robert W Eason, and Ben Mills. 2022. "Generating Images of Hydrated Pollen Grains Using Deep Learning." <i>IOP SciNotes</i> 3 (2): 24001. https://doi.org/10.1088/2633-1357/ac6780.
451 452 453	Grant-Jacob, James A, Michalis N Zervas, and Ben Mills. 2022. "Morphology Exploration of Pollen Using Deep Learning Latent Space." <i>IOP SciNotes</i> 3 (4): 044602. https://doi.org/10.1088/2633-1357/acadb9.
454 455 456	Halbritter, Heidemarie, Silvia Ulrich, Fri Gr\'\imsson, Martina Weber, Reinhard Zetter, Michael Hesse, Ralf Buchner, Matthias Svojtka, and Andrea Frosch-Radivo. 2018. "Methods in Palynology." In <i>Illustrated Pollen Terminology</i> , 97–127. Springer.
457 458 459	Heusel, Martin, Hubert Ramsauer, Thomas Unterthiner, Bernhard Nessler, and Sepp Hochreiter. 2017. "GANs Trained by a Two Time-Scale Update Rule Converge to a Local Nash Equilibrium." <i>Advances in Neural Information Processing Systems</i> 30.

460	Holt, Katherine.	2020.	"New	Zealand	Pollen	Dataset."	Figshare.

- 461 https://doi.org/https://doi.org/10.6084/m9.figshare.12370307.v1.
- Karras, Tero, Samuli Laine, Miika Aittala, Janne Hellsten, Jaakko Lehtinen, and Timo Aila. 2020.
- "Analyzing and Improving the Image Quality of Stylegan." *Proceedings of the IEEE Computer*
- Society Conference on Computer Vision and Pattern Recognition, 8107–16.
- https://doi.org/10.1109/CVPR42600.2020.00813.
- 466 Khanzhina, Natalia, Andrey Filchenkov, Natalia Minaeva, Larisa Novoselova, Maxim Petukhov,
- 467 Irina Kharisova, Julia Pinaeva, et al. 2022. "Combating Data Incompetence in Pollen Images
- Detection and Classification for Pollinosis Prevention." *Computers in Biology and Medicine* 140
- 469 (January):105064. https://doi.org/10.1016/J.COMPBIOMED.2021.105064.
- Kingma, Diederik P, and Jimmy Ba. 2014. "Adam: A Method for Stochastic Optimization." ArXiv
- 471 *Preprint ArXiv:1412.6980.*
- Knox, R Bruce, Elizabeth G Williams, and Christian Dumas. 1986. "Pollen, Pistil, and Reproductive
- 473 Function in Crop Plants." *Plant Breeding Reviews* 4:9–79.
- Lau, T-C, X Lu, R T Koide, and A G Stephenson. 1995. "Effects of Soil Fertility and Mycorrhizal
- Infection on Pollen Production and Pollen Grain Size of Cucurbita Pepo (Cucurbitaceae)."
- 476 Plant, Cell & Environment 18 (2): 169–77.
- 477 LeCun, Yann, Yoshua Bengio, and Geoffrey Hinton. 2015. "Deep Learning." *Nature* 521 (May):436.
- Li, Qiong, Jürgen Gluch, Peter Krüger, Martin Gall, Christoph Neinhuis, and Ehrenfried Zschech.
- 479 2016. "Pollen Structure Visualization Using High-Resolution Laboratory-Based Hard X-Ray
- Tomography." *Biochemical and Biophysical Research Communications* 479 (2): 272–76.
- 481 https://doi.org/https://doi.org/10.1016/j.bbrc.2016.09.058.
- 482 Luo, Yi, Yijie Zhang, Tairan Liu, Alan Yu, Yichen Wu, and Aydogan Ozcan. 2022. "Virtual
- 483 Impactor-Based Label-Free Pollen Detection Using Holography and Deep Learning." ACS
- 484 *Sensors* 7 (12): 3885–94. https://doi.org/10.1021/acssensors.2c01890.
- 485 Mahbod, Amirreza, Gerald Schaefer, Rupert Ecker, and Isabella Ellinger. 2021. "Pollen Grain
- 486 Microscopic Image Classification Using an Ensemble of Fine-Tuned Deep Convolutional
- Neural Networks." In Pattern Recognition. ICPR International Workshops and Challenges,
- edited by Alberto Del Bimbo, Rita Cucchiara, Stan Sclaroff, Giovanni Maria Farinella, Tao Mei,
- 489 Marco Bertini, Hugo Jair Escalante, and Roberto Vezzani, 344–56. Cham: Springer
- 490 International Publishing.
- 491 Marcos, J. Víctor, Rodrigo Nava, Gabriel Cristóbal, Rafael Redondo, Boris Escalante-Ramírez,
- 492 Gloria Bueno, Óscar Déniz, et al. 2015. "Automated Pollen Identification Using Microscopic
- 493 Imaging and Texture Analysis." *Micron* 68 (January):36–46.
- 494 https://doi.org/10.1016/J.MICRON.2014.09.002.
- 495 Matamoro-Vidal, Alexis, Charlotte Prieu, Carol A. Furness, Béatrice Albert, and Pierre Henri
- Gouyon. 2016. "Evolutionary Stasis in Pollen Morphogenesis Due to Natural Selection." New
- 497 *Phytologist* 209 (1): 376–94. https://doi.org/10.1111/NPH.13578.

498 499	Mert, Cevriye. 2009. "Pollen Morphology and Anatomy of Cornelian Cherry (Cornus Mas L.) Cultivars." <i>HortScience</i> 44 (2): 519–22. https://doi.org/10.21273/HORTSCI.44.2.519.
500 501	Nielsen, Frank. 2019. "On the Jensen–Shannon Symmetrization of Distances Relying on Abstract Means." <i>Entropy</i> 21 (5). https://doi.org/10.3390/e21050485.
502 503 504	Punyasena, Surangi W, David K Tcheng, Cassandra Wesseln, and Pietra G Mueller. 2012. "Classifying Black and White Spruce Pollen Using Layered Machine Learning." <i>New Phytologist</i> 196 (3): 937–44. https://doi.org/https://doi.org/10.1111/j.1469-8137.2012.04291.x.
304	Phylologist 196 (3): 937–44. https://doi.org/https://doi.org/10.1111/j.1469-8137.2012.04291.x.
505 506 507 508	Romero, Ingrid C, Shu Kong, Charless C Fowlkes, Carlos Jaramillo, Michael A Urban, Francisca Oboh-Ikuenobe, Carlos D'apolito, and Surangi W Punyasena. n.d. "Improving the Taxonomy of Fossil Pollen Using Convolutional Neural Networks and Superresolution Microscopy." https://doi.org/10.1073/pnas.2007324117/-/DCSupplemental.
509 510 511 512	Sauvageat, Eric, Yanick Zeder, Kevin Auderset, Bertrand Calpini, Bernard Clot, Benoit Crouzy, Thomas Konzelmann, Gian Lieberherr, Fiona Tummon, and Konstantina Vasilatou. 2020. "Real-Time Pollen Monitoring Using Digital Holography." <i>Atmospheric Measurement Techniques</i> 13 (3): 1539–50. https://doi.org/10.5194/AMT-13-1539-2020.
513 514 515	Serre, Thomas, Lior Wolf, Stanley Bileschi, Maximilian Riesenhuber, and Tomaso Poggio. 2007. "Robust Object Recognition with Cortex-like Mechanisms." <i>IEEE Transactions on Pattern Analysis &amp; Machine Intelligence</i> , no. 3, 411–26.
516 517 518	Sevillano, Víctor, and José L Aznarte. 2018. "Improving Classification of Pollen Grain Images of the POLEN23E Dataset through Three Different Applications of Deep Learning Convolutional Neural Networks." <i>PLOS ONE</i> 13 (9).
519 520 521	Sevillano, Víctor, Katherine Holt, and José L. Aznarte. 2020. "Precise Automatic Classification of 46 Different Pollen Types with Convolutional Neural Networks." <i>PLOS ONE</i> 15 (6): e0229751. https://doi.org/10.1371/JOURNAL.PONE.0229751.
522 523 524	Szegedy, Christian, Sergey Ioffe, Vincent Vanhoucke, and Alexander A Alemi. 2017. "Inception-v4, Inception-Resnet and the Impact of Residual Connections on Learning." In <i>Thirty-First AAAI Conference on Artificial Intelligence</i> .
525 526 527	Wang, Shengxiang, Dajiang Wang, Qiao Wu, Kun Gao, Zhili Wang, and Ziyu Wu. 2015. "3D Imaging of a Rice Pollen Grain Using Transmission X-Ray Microscopy." <i>Journal of Synchrotron Radiation</i> 22 (4): 1091–95. https://doi.org/10.1107/S1600577515009716.
528 529 530	Zhou, Yuan, and Anna A. Dobritsa. 2019. "Formation of Aperture Sites on the Pollen Surface as a Model for Development of Distinct Cellular Domains." <i>Plant Science</i> 288 (November):110222. https://doi.org/10.1016/J.PLANTSCI.2019.110222.
531	

Figure 1. Block diagram concept of the study

- Figure 2. Schematic of the StyleGAN neural network (formed of the mapping and synthesis
- networks) for generating images, and of then the use of a CNN for subsequent classification of the
- 535 generated images.
- Figure 3. Diagram of StyleGAN with 3 networks (Left) The mapping network transforms a random
- input into a style signal, controlling various aspects of image generation. (Middle) The synthesis
- (generator) network uses the information (A) from the mapping network to generate images from low
- to high resolution. It also incorporates random noise (B) to introduce variations and fine details.
- (Right) The discriminator network compares real and generated images, updating the weights of all
- networks through adversarial training to enhance performance.
- Figure 4. Graph showing the accuracy of training and validation progress during training of the CNN.
- Figure 5. Schematic of methodology of projecting an image into latent space, by generating random z
- vector, generating an image then comparing that image with the projected to obtain the suitable
- vector in latent space. The vector is then manipulated via adding or subtracting a vector before the
- 546 synthesis network generates a new image.
- 547 Figure 6 Histogram of distribution of taxa in training dataset and generated dataset (as predicted by
- 548 CNN).
- Figure 7. Generated images of pollen grains created through latent w-space vector manipulation,
- showing the addition of a 'size' vector (-100%, -50%, 0%, +50%, +100%) added in the horizontal
- direction and a 'spike' vector (-100%, -50%, 0%, +50%, +100%) in the vertical direction, to
- generated images of (a) Knightia and (b) Coriaria. Each generated image also shows the predicted
- pollen taxa and predicted confidence, as well as the pollen size in pixels. Labelling is omitted from of
- images without any visible grains.
- Figure 8. Generated images of pollen grains created through latent w-space vector manipulation,
- showing the addition of a 'size' vector (-100%, -50%, 0%, +50%, +100%) added in the horizontal
- direction and a 'round' vector (-100%, -50%, 0%, +50%, +100%) in the vertical direction, to
- generated images of (a) *Metrosideros* and (b) *Disphyma*. Each generated image also shows the
- predicted pollen taxa and predicted confidence, as well as the circularity of the pollen grain.
- Labelling is omitted from of images without any visible grains.
- Figure 9. Generated images of pollen grains created through latent w-space vector manipulation,
- showing the interpolation of projected images between (a) *Knightia* and *Kunzea* (b) *Brachyglottis*
- 563 repanda and Citrus. Each generated image also shows the predicted pollen taxa and predicted
- 564 confidence.