

1 Learning from Synthetic Dataset for Crop 2 Seed Instance Segmentation

3
4 Yosuke Toda^{1,2*}, Fumio Okura^{1,3}, Jun Ito⁴, Satoshi Okada⁵, Toshinori Kinoshita², Hiroyuki Tsuji⁴, and
5 Daisuke Saisho⁵

6
7 ¹Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
8 ²Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Chikusa, Nagoya 464-8602, Japan
9 ³Department of Intelligent Media, Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047,
10 Japan
11 ⁴Kihara Institute for Biological Research, Yokohama City University, Maioka 641-12, Totsuka, Yokohama, 244-0813 Japan
12 ⁵Institute of Plant Science and Resources, Okayama University, Chuo 2-20-1, Kurashiki, Okayama 710-0046, Japan

13
14 *Correspondence should be addressed to Yosuke Toda; tyosuke@aquaseerser.com

15
16 Incorporating deep learning in the image analysis pipeline has opened the possibility of introducing precision
17 phenotyping in the field of agriculture. However, to train the neural network, a sufficient amount of training
18 data must be prepared, which requires a time-consuming manual data annotation process that often becomes
19 the limiting step. Here, we show that an instance segmentation neural network (Mask R-CNN) aimed to
20 phenotype the barley seed morphology of various cultivars, can be sufficiently trained purely by a
21 synthetically generated dataset. Our attempt is based on the concept of *domain randomization*, where a large
22 amount of image is generated by randomly orienting the seed object to a virtual canvas. After training with
23 such a dataset, performance based on recall and the average Precision of the real-world test dataset achieved
24 96% and 95%, respectively. Applying our pipeline enables extraction of morphological parameters at a large
25 scale, enabling precise characterization of the natural variation of barley from a multivariate perspective.
26 Importantly, we show that our approach is effective not only for barley seeds but also for various crops
27 including rice, lettuce, oat, and wheat, and thus supporting the fact that the performance benefits of this
28 technique is generic. We propose that constructing and utilizing such synthetic data can be a powerful method
29 to alleviate human labor costs needed to prepare the training dataset for deep learning in the agricultural
30 domain.

31

32 Introduction

33 Deep learning is a process that involves neural network parameter optimization to solve a specific task of
34 interest¹. While traditional machine learning requires a user predefined feature extraction, the neural network
35 itself can learn the most suitable representation from the dataset and can therefore exert its power on high
36 content data. In ImageNet Large Scale Visual Recognition Challenge of 2012², a convolutional neural
37 network (CNN)-based architecture, namely AlexNet, outperformed the human image classification accuracy
38 to classify 1000 categories³. Since then, deep learning has gathered wide attraction in both the scientific and
39 industrial communities. Initially, deep learning was actively applied to image classification, however in
40 recent years, it has been further developed to process various tasks in computer vision, such as semantic
41 segmentation^{4,5}, object detection^{6,7}, and instance segmentation⁸.

42 Such deep-learning-based image analysis has also been influencing the field of agriculture. This
43 involves image-based phenotyping including weed detection⁹, crop disease diagnosis^{10,11}, fruit detection¹²,
44 and many other applications as listed in the recent review¹³. Meanwhile, not only features from images but
45 also with that of environmental variables, functionalized a neural network to predict plant water stress for
46 automated control of greenhouse tomato irrigation¹⁴. Utilizing the numerous and high context data generated
47 in the relevant field seems to have high affinity with deep learning.

48 However, one of the drawbacks of using deep learning is the need to prepare a large amount of
49 labeled data. The ImageNet dataset as of 2012 consists of 1.2 million and 150,000 manually classified images
50 in the training dataset and validation/test dataset, respectively². Meanwhile, the COCO 2014 Object Detection
51 Task constitutes of 328,000 images containing 2.5 million labeled object instances of 91 categories¹⁵. This
52 order of annotated dataset is generally difficult to prepare for an individual or a research group. In the
53 agricultural domain, it has been reported that sorghum head detection network can be trained with a dataset
54 consisting of 52 images with an average of 400 objects per image¹⁶, while a crop stem detection network was
55 trained starting from 822 images¹⁷. These case studies imply that the amount of data required in a specialized
56 task may be less compared to a relatively generalized task such as ImageNet classification and COCO
57 detection challenges. Nonetheless, the necessary and sufficient amount of annotation data to train a neural
58 network is generally unknown. The annotation process is highly stressful for researchers, as it is like running
59 a marathon without knowing the goal.

60 In such cases, domain adaptation (e.g. using ImageNet trained weights as initial network parameter
61 for the tasks in different domains; also known as transfer learning or fine-tuning) and image augmentation
62 (e.g. image flipping and rotating) have been the most commonly adopted techniques to compensate for the
63 lack of data. More recently, several reports have highlighted the challenges with incorporating active learning
64 or other approaches that loops the annotation and model training to minimize the labor cost¹⁸⁻²⁰.

65 On the lines of domain adaptation and data augmentation, learning from synthetic (e.g. CG-
66 generated) images has been highlighted, which is occasionally referred to as the *sim2real* transfer. One of the
67 important advantages of using synthetic dataset for training is that the ground truth annotations can be
68 automatically obtained without the need for human labor. A successful example can be found in person image
69 analysis method, that uses the image dataset with synthetic human models²¹ for various uses such as person

70 pose estimation²². One drawback of the sim2real approach are the gaps between the synthesized images and
71 the real scenes, e.g. non-realistic appearances. To counter this problem, many studies attempt to generate
72 realistic images from synthetic datasets, such as by domain adaptation techniques using generative
73 adversarial networks (GAN)²³. Although the methods for generating realistic images from synthetic images
74 were well studied in the CG community²⁴, GAN-based approaches are recently being paid attention for
75 generation of training dataset²⁵.

76 While the GAN-based approaches still require a large set of real images, another set of approaches
77 that are bridging the sim2real gap *domain randomization*, which trains the deep networks using large
78 variations of synthetic images with randomly sampled physical parameters. Although domain randomization
79 is somewhat related to data augmentation, synthetic environment enables representation of variations under
80 many conditions, which is generally difficult to attain by straightforward data augmentation techniques for
81 real images. An early attempt at domain randomization was made by generating the images using different
82 camera positions, object location, and lighting conditions; which is similar to the technique applied to control
83 robots²⁶. For object recognition tasks, Tremblay et al.²⁷ proposed a method to generate images with a
84 randomized texture on synthetic 3D models.

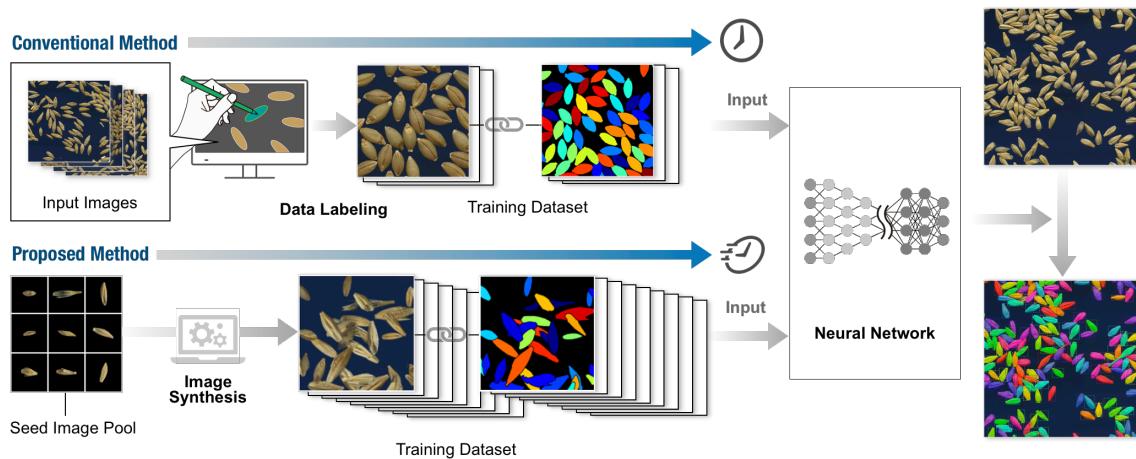
85 Such sim2real approaches have also been used for the preparation of training data for plant image
86 analysis. While Isokane et al²⁸ used the synthetic plant models for the estimation of branching pattern,
87 Giuffrida et al. used GAN-generated images to train a neural network for *Arabidopsis* leaf counting²⁹.
88 Similarly, Arsenovic et al. used StyleGAN³⁰ to create training images for the plant disease image
89 classification³¹. Meanwhile, Ward et al. generated artificial images of *Arabidopsis* rendered from 3D models
90 and utilized them for neural network training in leaf segmentation³². As far as difficulties in the collection
91 and annotation of training datasets is concerned, the use of synthetic images has a huge potential in the plant
92 phenotyping research field.

93 Among various crop phenotypes, seed morphology has been one of the most important traits. This
94 is because the seed shape directly influences the crop yield³³. Several studies report identification of genes
95 that enhance rice yield by utilizing Quantitative Trait Locus (QTL) involved in seed width^{34,35}. Moreover,
96 several studies utilized elliptic Fourier descriptors which enables to handle the seed shape as variables
97 representing a closed contour, successfully characterizing the characters of various species³⁶⁻³⁹. Focusing on
98 morphological parameters of seeds seems to be powerful metrics for both crop yield improvement and for
99 biological studies. However, including the said reports, many of the previous studies have evaluated the seed
100 shape either by qualitative metrics (e.g. whether the seeds are similar to the parental phenotype), by vernier
101 caliper, or by manual annotation using an image processing software. The phenotyping is generally labor-
102 intensive and cannot completely exclude the possibility of quantification errors that differ by the annotator.
103 To execute a precise and large-scale analysis, automation of the seed phenotyping step was preferred.

104 In recent years, several studies have been reported to systematically analyze the morphology of plant
105 seeds by image analysis. Ayoub et al. focused on barley seed characterization in terms of area, perimeter,
106 length, width, F-circle, and F-shape based on digital camera captured images⁴⁰. Herridge et al. utilized a
107 particle analysis function of ImageJ (<https://imagej.nih.gov/ij/>) to quantify and differentiate the seed size of

108 *Arabidopsis* mutants from the background population⁴¹. *SmartGrain* software has been developed to realize
109 the high throughput phenotyping of crop seeds, successfully identifying the QTL that is responsible for seed
110 length of rice⁴². Moreover, commercially available products such as *Germination Scanalyzer* (Lemnatec,
111 Germany) and *PT portable tablet tester* (Greenpheno, China) also aim or have the ability to quantify the
112 morphological shape of seeds. However, the aforementioned approaches require the seeds to be sparsely
113 oriented for efficient segmentation. When seeds are physically touching or overlapping each other, they are
114 often detected as a unified region, leading to an abnormal seed shape output. This requires the user to reorient
115 the seeds in a sparse manner, which is a potential bar to secure sufficient amount of biological replicate in
116 the course of high throughput analysis. In such situations, utilizing deep learning-based instance
117 segmentation can be used to overcome such a problem by segmenting the respective seed regions regardless
118 of their orientation. Nonetheless, the annotation process as described previously was thought to be the
119 potential limiting step.

120 In this paper, we show that utilizing a synthetic dataset that the combination and orientation of seeds
121 are artificially rendered, is sufficient to train an instance segmentation of deep neural network to process real-
122 world images. Moreover, applying our pipeline enables us to extract morphological parameters at a large
123 scale with precise characterization of barley natural variation at a multivariate perspective. The proposed
124 method can alleviate the labor-intensive annotation process to realize the rapid development of deep learning-
125 based image analysis pipeline in the agricultural domain as illustrated in Fig. 1. Our method is largely related
126 to the sim2real approaches with the domain randomization, where we generate a number of training images
127 by randomly locating the synthetic seeds with actual textures by changing its orientation and location.



128
129 **Fig. 1 | Overview of the proposed training process of crop seed instance segmentation.**
130
131

132 **Contribution:** The contribution of this study is two folds. First, this is the first attempt to utilize a synthetic
133 dataset (i.e., a sim2real approach) with domain randomization for the crop seed phenotyping, which can
134 significantly decrease the manual labor for data creation (Fig. 1). Second, we propose a first method that can
135 be used against the densely sampled (i.e., touching or overlapping) seeds using instance segmentation.
136

137 Methods

138 Plant Materials

139 Barley seeds used in this research are 19 domesticated barley (*Hordeum vulgare*) accessions and one wild
140 barley (*H. spontaneum*) accession: B669, Suez (84); C319, Chichou; C346, Shanghai 1; C656, Tibet White
141 4; E245, Addis Ababa 40 (12-24-84); E612, Ethiopia 36 (CI 2225); I304, Rewari; I335, Ghazvin 1 (184);
142 I622, H.E.S. 4 (Type 12); I626, Katana 1 (182); J064, Hayakiso 2; J247, Haruna Nijo; J647, Akashinriki;
143 K692, Eumseong Covered 3; K735, Natsudaikon Mugi; N009, Tilman Camp 1 (1398); T567, Goenen (997);
144 U051, Archer; U353, Opal; and H602, wild barley. All the details of the said cultivars can be obtained at the
145 National BioResource Project (NBRP) (<https://nbrp.jp>). Meanwhile, seeds of rice (*Oryza Sativa*, cv.
146 Nipponbare), oat (*Avena sativa*, cv. Negusaredaiji), Lettuce (*Lactuca sativa*, cv. Great Lakes), and wheat
147 (*Triticum aestivum* cv. CS, Chinese Spring; N61, Norin 61; AL, Arina; and Syn01, a synthetic hexaploid
148 wheat line Ldn/KU-2076 which is generated by a cross between tetraploid wheat *Triticum turgidum* cv.
149 Langdon and *Aegilops tauchii* strain KU-2076)⁴³ were used in this report.

150 Image Acquisition

151 All the barley seeds were threshed using a commercial table-top threshing system (BGA-RH1, OHYA
152 TANZO SEISAKUSHO & Co., Japan). The seed images were captured on an EPSON GT-X900 A4 scanner
153 with the supplied software without image enhancement. Seeds were spread uniformly on the glass, scanned
154 at 7019 x 5100 px at 600 dpi using a blue colored paper background. For the image acquisition of seeds of
155 rice, oat, lettuce, and wheat, an overhead scanner ScanSnap SV600 (Fujitsu, Japan) was used with the image
156 size of 3508 x 2479 at 300 or 600 dpi.

157 Synthetic Image Generation

158 A total of 20 single seed images per cultivar were isolated and saved as an individual image file. The
159 background regions were removed such that the pixel value other than the area of the seed will be (0,0,0) in
160 RGB color value. As a result, a total of 400 (20 seed images for 20 cultivars) background clean images were
161 prepared to constitute a “seed image pool”. For the background image, four images at the fixed size of 1024
162 x 1024 were cropped from the actual background used in the seed scanning process and were prepared as a
163 “background image pool”.

164 The synthetic image generation process is described as follows. First, an image was randomly
165 selected from the background image pool and pasted to the virtual canvas of size 1024 x 1024. Second,
166 another image was randomly selected from the seed image pool. Image rotation angle was randomly set upon
167 selection. After rotation, the x and y coordinate at which the image was to be pasted was randomly determined,
168 however, the coordinate value was restricted to a certain range so that the image does not exceed the canvas
169 size, which its values were dependent on the selected seed image size and its rotation angle. Third, the seed

170 image was pasted to the canvas according to the determined values described above. When pasting, alpha
171 masks were generated and utilized in alpha blending such that the area outside of the seed will be transparent
172 and does not affect the canvas image. Moreover, utilizing the alpha mask, the seed perimeter was gaussian
173 blurred to decrease the artifacts resulting from the background removal process of the seed image. Notably,
174 if the region where the image was to be pasted in the canvas already had a seed image, the overlapping
175 proportion of the area of the seeds was calculated. If the calculated value exceeded the ratio of 0.25, pasting
176 was canceled, and another coordinate was chosen again. A maximum of 70 pasting trials were performed to
177 generate a single image.

178 During the synthetic image generation, a mask that has the same image size as the synthetic image
179 was created by first creating a black canvas and coloring the seed region with unique colors based on the
180 coordinate of the placing object. The coloring was performed when the seed were randomly placed in the
181 synthetic image. If a seed to be placed were overlapping an existing seed, the colors in the corresponding
182 region in the mask image were replaced by the foreground color.

183 The above procedure generates an image size of 1024 x 1024 with seeds randomly oriented inside
184 the canvas region. While in real-world images, seeds that are adjacent to the border of the image are cut off.
185 To replicate such a situation, the borders of synthetic images were cropped to obtain the final image. The
186 generated synthetic dataset constitutes 1200 set of data pairs of synthetic and mask image, in which each
187 image has a size of 768 x 768, that were used for neural network training.

188 Model Training

189 We used a Mask R-CNN⁸ implementation on the Keras/Tensorflow backend
190 (https://github.com/matterport/Mask_RCNN). Configuration predefined by the repository was used
191 including the network architectures and losses. The residual network ResNet101⁴⁴ was used for the feature
192 extraction. From the initial weights of ResNet101 obtained by training using MS COCO dataset, we
193 performed fine-tuning using our synthetic seed image dataset for 40 epochs by stochastic gradient descent
194 optimization with a learning rate of 0.001 and batch size of 2. Within the 1200 images of the synthetic dataset,
195 989 were used for training, 11 for validation, and 200 for the test dataset. No image augmentation was
196 performed during training. The synthetic training data has a fixed image size of 768 x 768; however, the input
197 image size for the network was not exclusively defined such that variable sizes of the image can be fed upon
198 inference. The network outputs a set of bounding boxes and seed candidate mask regions with a probability
199 value. A threshold value of 0.5 was defined to isolate the final mask regions.

200 Test dataset for Model Evaluation

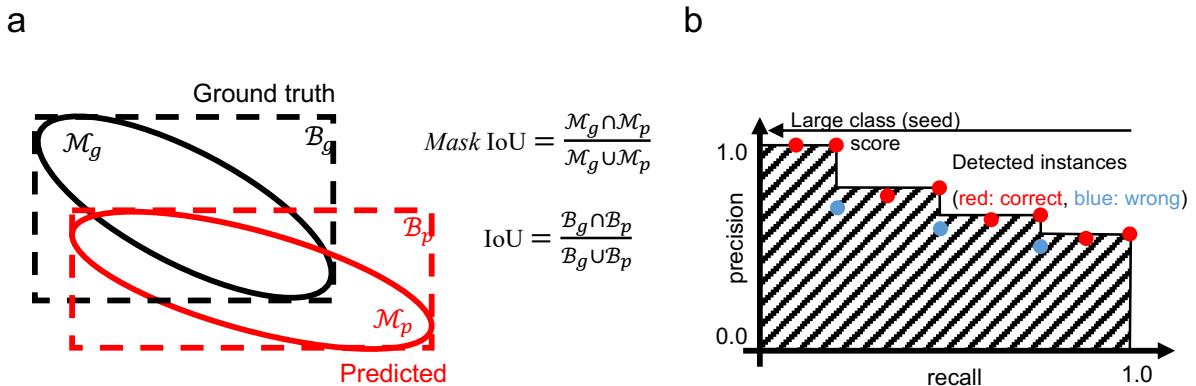
201 We prepared a test dataset consisting of 20 images and each image contained seeds derived from a
202 homogeneous population (Fig. 3a). Each image had a size of 2000 x 2000. AP₅₀, AP₇₅, and AP@[.5:.95] per
203 image (cultivar), as well as the mean AP of all images, was calculated. As the seeds to be detected per image
204 averages to approximately 100 objects per image and images itself were acquired under the same
205 experimental condition, we used one image per cultivar for model evaluation. For reference, we also prepared

206 200 synthetic images for testing (synthetic test dataset), which were not used for the model training or
 207 validation.

208 Metrics for Model Evaluation

209 To assess the accuracy of object detection using Mask R-CNN, we evaluated using two metrics, which were
 210 also used in the evaluation of the original report⁸. While they are commonly-used measures in object
 211 recognition and instance segmentation, such as in MS COCO¹⁵ and Pascal VOC⁴⁵ dataset, we briefly recap
 212 our evaluation metrics for clarity. During the experiment, the evaluation metrics were calculated using the
 213 Mask R-CNN distribution.

214



215
 216 **Fig. 2 | Evaluation metrics for object detection accuracy.** (a) The intersection-over-union (IoU) definitions
 217 for bounding boxes and masks. (b) The average precision (AP) defined as the area under the curves (AUC);
 218 shown as the area marked with slanted lines.

219

220

221 **Recall.** We first measured the recall, which evaluates how well the objects (i.e., seeds) are detected, which
 222 can be obtained by the ratio of true positive matches over the total number of ground-truth objects. To
 223 calculate the recall values, we determined the correct detection when the detection threshold of the
 224 intersection-over-union (IoU) between the ground-truth and predicted bounding boxes is over 0.5 (Fig. 2a).
 225 In other words, for each ground-truth bounding box, if a detected bounding box overlaps over 50%, it was
 226 counted as the true positive. Hereafter, we denote the recall measures as Recall₅₀.

227

228 **Average precision (AP) using mask IoUs.** The drawbacks of the recall measure include penalizing the false
 229 positive detections and evaluating using the overlaps of bounding boxes that are poor approximation of the
 230 object shape. We, therefore, calculated the average precision (AP) using *mask* IoUs, which can be a measure
 231 of the detection accuracy (in terms of both recall and precision) as well as providing a rough measure of mask
 232 generation accuracy. During the computation of APs, we first compute the IoU between the instance masks
 233 (*mask* IoU), as shown in Fig. 2a. AP can be obtained based on the number of correct (i.e., true positive) and
 234 wrong (i.e., false positive) detection determined using a certain threshold of *mask* IoUs. Fig. 2b summarizes

235 the computation of the AP. We sort the detected instances using the class score (i.e., the confidence that the
236 detected object is a seed, in our case) in the descending order. For the n-th instance, the precision and recall,
237 based on the mask IoU threshold, are calculated for the subset of instances from 1st to n-th detections. By
238 repeating the process for each of the instances, we obtain a receiver operating characteristics (ROC) curve
239 shown in Fig. 2b. The AP is defined as the ratio of the rectangle approximations of the area under the curve
240 (AUC), which is shown as the area marked by slanted lines in the figure. APs thus takes the value from 0.0
241 to 1.0 (i.e., 100%). We evaluated APs using multiple mask IoU thresholds. AP₅₀ and AP₇₅ are computed using
242 the mask IoU threshold of 0.5 and 0.75, respectively. AP₇₅ becomes a stricter measure than AP₅₀, because
243 AP₇₅ requires the correct matches with more accurate instance masks. Similar to MS COCO evaluation, we
244 also measured AP@ [.5:.95], which is the average value of APs with IoU thresholds from 0.5 to 0.95 with
245 the interval of 0.05.

246 Quantification of Seed Morphology

247 The main application of the seed instance segmentation is to quantify phenotypes of seeds for analyzing and
248 comparing morphological traits. In the mask image, morphological variables of seed shape such as area,
249 width, and height were calculated using the *measure.regionprops* module of the scikit-image library,
250 respectively. To analyze the characteristics of seeds across different cultivars, principal component analysis
251 (PCA) was applied to the variables. In the result section, we briefly present the analysis using different types
252 of descriptors, computed by elliptic Fourier descriptors (EFD) and variational autoencoder (VAE) both of
253 which are described below.

254

255 **Post-processing: Selection of isolated seeds.** The instance segmentation network outputs a set of bounding
256 boxes and seed area candidates as mask images, where some seeds overlap with each other. To analyze the
257 seed morphology (or use for further phenotyping applications), it is required to select the seeds that are
258 isolated (i.e., not partly hidden) from neighboring seed instances. To select such seeds, post-processing step
259 was introduced. First, the bounding box coordinates were checked whether it resides inside the 5 px margin
260 of the image. The bounding boxes that protrude the margin were removed. Second, using the solidity (ratio
261 of the region of interest area against its convex hull area) of the respective mask as a metric, the 25% lower
262 quantile threshold was determined and used to remove the outliers. Similarly, further outliers were removed
263 by a 5% lower and 95% higher quantile threshold of length-to-width ratio. The threshold was empirically
264 determined during the analysis.

265

266 **Elliptic Fourier descriptors (EFD).** EFD⁴⁶ has been used to quantify the contour shape of seeds³⁶, which
267 approximate the contour shape as the set of different ellipses. During the computation of EFD, segmented
268 seed images were first converted to binary mask image where the background pixel value was 0 and the seed
269 area is 1. Next, the contour of the seed was detected by the *find_contours* module of the scikit-image library.
270 The detected contours were converted to EFD coefficients using the *elliptic_fourier_descriptors* module of
271 *pyefd* library (<https://github.com/hbldh/pyefd>) under the condition of harmonics 20 and with normalization

272 so as to be rotation and size-invariant. The output was flattened, which converted the shape of the array from
273 4 x 20 to 80. As the first three coefficients are always or nearly equal to 1, 0, 0 due to the normalization
274 process, they were discarded upon further analysis. A total of 77 variables were used as descriptors for
275 principal component analysis (PCA).

276

277 **Variational autoencoder (VAE).** Autoencoder (AE) is a type of neural network with an encoder-decoder
278 architecture that embeds a high-dimensional input data (e.g., images) to a low-dimensional latent vector, to
279 correctly decode the input data from the low-dimensional vector. Variational autoencoder (VAE)⁴⁷ is a variant
280 of AE, where the distribution in the latent space is generated to fit a prior distribution (e.g., Gaussian
281 distribution, $N(0,1)$). In a generative model, the low-dimensional parameters in the latent space is often used
282 as the nonlinear approximation (i.e., dimensional reduction) of the dataset. Similar to other approximation
283 methods like PCA, the parameters in the latent space estimated by VAE can be used for interpolation for the
284 data distribution; the input data with different characteristics (e.g., different species) is often well separated
285 in the space⁴⁸ compared to the conventional methods (e.g., PCA), without using the ground-truth labels for
286 the classes during the training. We used a VAE with a CNN-based encoder-decoder network to visualize the
287 latent space. In brief, the network receives an RGB image which has a shape of 256 x 256 x 3. For the encoder,
288 input data were first passed through 4 layers of convolution with a filter number of 32, 64, 128, 256,
289 respectively. Since we fit the latent space to the Gaussian distribution, the log variance and the mean of the
290 latent space are computed after full-connection layers. For the decoder, the output of the encoder was passed
291 through 4 layers of deconvolution with filter number of 256, 128, 64, 32, respectively. Finally, the convolution
292 layer with 3 filters was added to convert the data back to an RGB image with its shape identical to the input
293 image. In our analysis, we utilized the two-dimensional latent space (i.e., the final output of the encoder of
294 VAE) to visualize the compressed features of the input image.

295 Software Libraries and Hardware

296 Computational analysis in this study was performed using Python 3.6. Keras (ver.2.2.4) with Tensorflow (ver.
297 1.14.0) backend for deep learning related processes. OpenCV3 (ver. 3.4.2) and scikit-image (ver. 0.15.0) was
298 used for operations in morphological calculations of the seed candidate regions as well as basic image
299 processing. A single GPU was used for network training and inference. R (ver. 3.5.1) was used for ANOVA
300 and Tukey post hoc HSD test analysis.

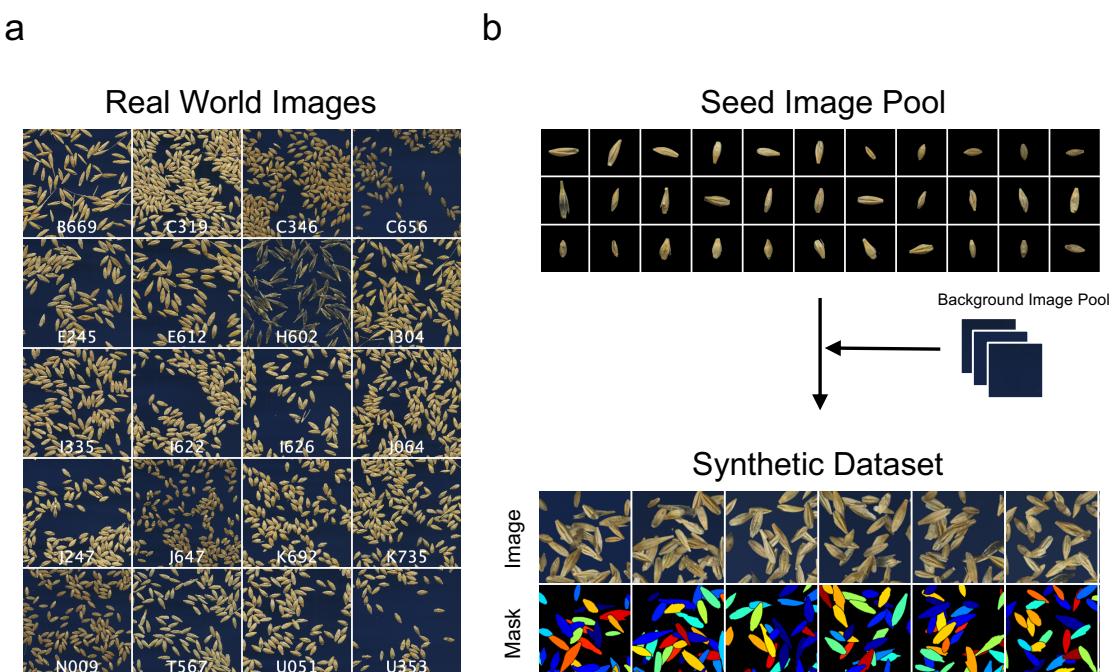
301

302 **Results**

303 **Preparation of Barley Seed Synthetic Dataset**

304 Examples of seed images captured by the scanner are shown in Fig. 3a. The morphology of barley seeds is
 305 highly variable between cultivars, in terms of size, shape, color, and texture. Moreover, the seeds randomly
 306 come in contact with or partially overlap each other. Determination of the optimal threshold for binarization
 307 may enable isolation of the seed region from the background; however, conventional segmentation methods
 308 such as watershed fail to segment the seed area for morphological quantification (see the results shown in
 309 Fig. S1), indicating that employing a sophisticated segmentation method (in our case, instance segmentation
 310 using Mask R-CNN⁸) is indeed required for successful separation of the individual seeds. However, Mask R-
 311 CNN requires annotations of bounding boxes—which circumscribes the seed—and mask images that
 312 necessarily and sufficiently cover the seed area (Fig. S2). Given that the numbers of seeds per image are
 313 abundant (Fig. 3a), the annotation process has been predicted to be labor-intensive.

314



315
 316 **Fig. 3 | Data prepared in this study.** (a) Images of barley seeds scanned from 20 cultivars. Cultivar names
 317 are described in white text in each image. These images were also used as a real-world test dataset shown in
 318 Table 1. (b) Scheme of generating synthetic images. Images are generated by combining real images of
 319 scanned seeds with the background images on to the virtual canvas. Simultaneously generated ground truth
 320 label (Mask) is shown at the bottom, wherein each seed area is marked with a unique color.

321

322

323 Fig. 3b shows the seed image pool and synthesized dataset obtained using the proposed method (see
 324 Methods for details). Instead of labeling real-world images for use as a training dataset, Mask R-CNN was

325 trained using the synthetic dataset (examples shown at the bottom of Fig. 3b), which is generated from the
326 seed image pool and background image pool (Fig. 3b top) using a domain randomization technique.

327 Model Evaluation

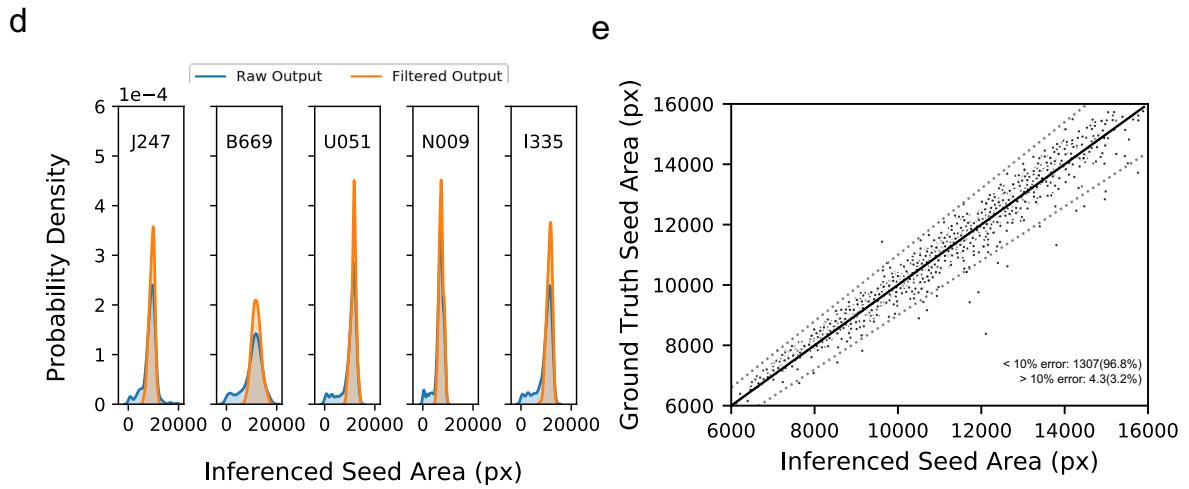
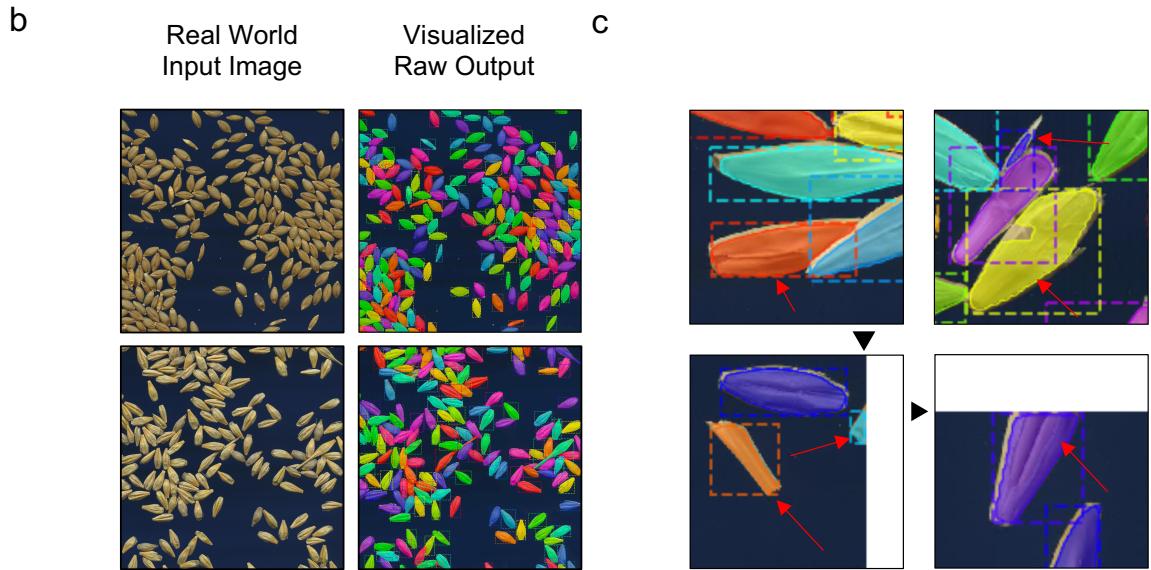
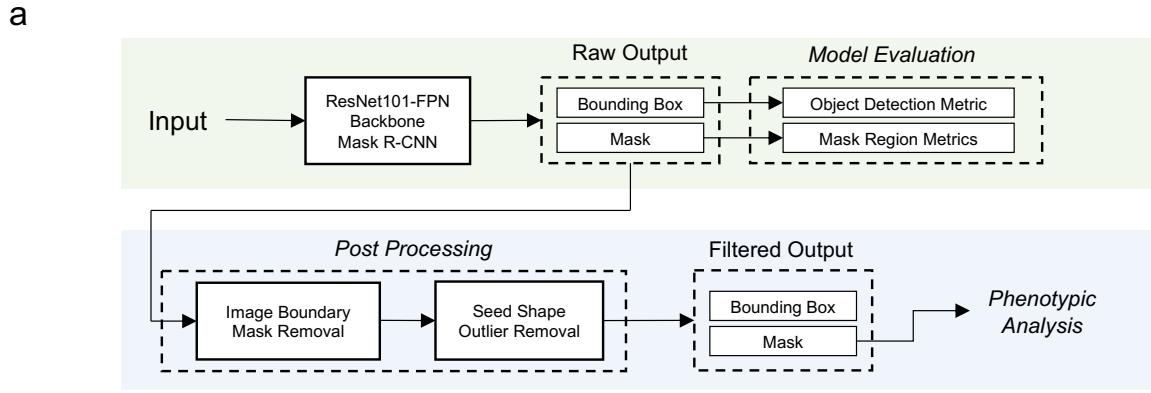
328 We show herein the visual results and a quantitative evaluation of object detection and instance segmentation
329 by Mask R-CNN. The trained Mask R-CNN model outputs a set of bounding box coordinates and masks
330 images of seed regions (Raw Output) (Fig. 4a top row). Examples of visualized raw output obtained from the
331 real-world images show that the network can accurately locate and segment the seeds regardless of their
332 orientation (Fig. 4b and Fig. S3). Table 1 summarizes the quantitative evaluation using the recall and AP
333 measures (see Method section for details). The efficacy of seed detection was evaluated using the recall
334 values computed for bounding box coordinates at 50% Intersection of Union (IoU) threshold (Recall₅₀). The
335 model achieved an average of 95% and 96% on the synthetic and real-world test datasets, respectively. This
336 indicates that the trained model can locate the position of seeds with very low false negative rate. From the
337 Average Precision (AP) values, which were computed based on mask regions at varying mask IoU thresholds,
338 comparable AP₅₀ were achieved between the synthetic (96%) and real-world (95%) datasets. For higher IoU
339 threshold (AP@ [.5:.95] and AP₇₅), the values of the synthetic test dataset (73%) exceeded that of the real-
340 world test dataset (59%). These results suggest that the model's ability to segment the seed region is better in
341 the case of the synthetic than the real-world images; however, considering the visual output interpretation
342 (Fig. 4B) and the values of AP₅₀ (95%), we judged that seed morphology can be sufficiently determined from
343 real-world images. The relatively low AP in high IoU in the real-world test dataset is possibly derived from
344 the subtle variation in the manual annotation of seed mask regions.

345

	Object Detection Metric	Mask Region Metrics		
		Recall ₅₀	AP@[.5:.95]	AP ₅₀
Synthetic Test Dataset	0.95	0.73	0.96	0.93
Real World Test Dataset	B669	0.92	0.56	0.92
	C319	0.95	0.62	0.91
	C346	0.98	0.64	0.97
	C656	0.96	0.61	0.95
	E245	0.95	0.63	0.94
	E612	0.96	0.66	0.98
	H602	0.87	0.42	0.78
	I304	0.99	0.64	0.98
	I335	0.97	0.67	0.93
	I622	0.93	0.62	0.93
	I626	0.96	0.65	0.95
	J064	0.93	0.65	0.97
	J247	0.94	0.65	0.97
	J647	0.98	0.62	0.98
	K692	0.98	0.69	0.98
	K735	0.95	0.62	0.92
	N009	0.99	0.63	0.99
	T567	0.98	0.63	0.98
	U051	0.96	0.65	0.96
	U353	1.00	0.65	0.98
	Average	0.96	0.59	0.95
				0.86

346

347 **Table 1 | Model Evaluation.** Table describing the evaluation result of the trained Mask R-CNN raw output.
 348 Recall values at the IoU threshold of 50% (Recall₅₀) and Average Precision (AP) at the IoU 50% (AP₅₀),
 349 75% (AP₇₅), and the mean value from IoU 50% to 95% with the step size of 5% (AP@[.5:.95]) are shown.



350

351 **Fig. 4 | Image Analysis pipeline.** (a) Summary of the image analysis pipeline. (b) Examples of graphical
 352 output of the trained Mask R-CNN on real-world images. Different colors indicate an individual segmented
 353 seed region. Note that even though the seeds overlap or touch each other, the network can still distinguish
 354 them as independent objects. (c) Examples of detected candidate regions to be filtered in the post-processing
 355 step, indicated using red arrows. Black arrowheads indicate the input image boundary. (e) Probability density
 356 of the seed areas of the raw output and filtered output. (f) Scatterplot describing the correlation of the seed

357 area that was measured by the pipeline (Inferred Seed Area) and by manual annotation (Ground Truth Seed
358 Area). Each dot represents the value of a single seed. Black and gray lines indicate the identity line and the
359 10% error threshold line, respectively. The proportion of the seeds exhibiting error lower or higher than the
360 10% mark is also displayed.

361

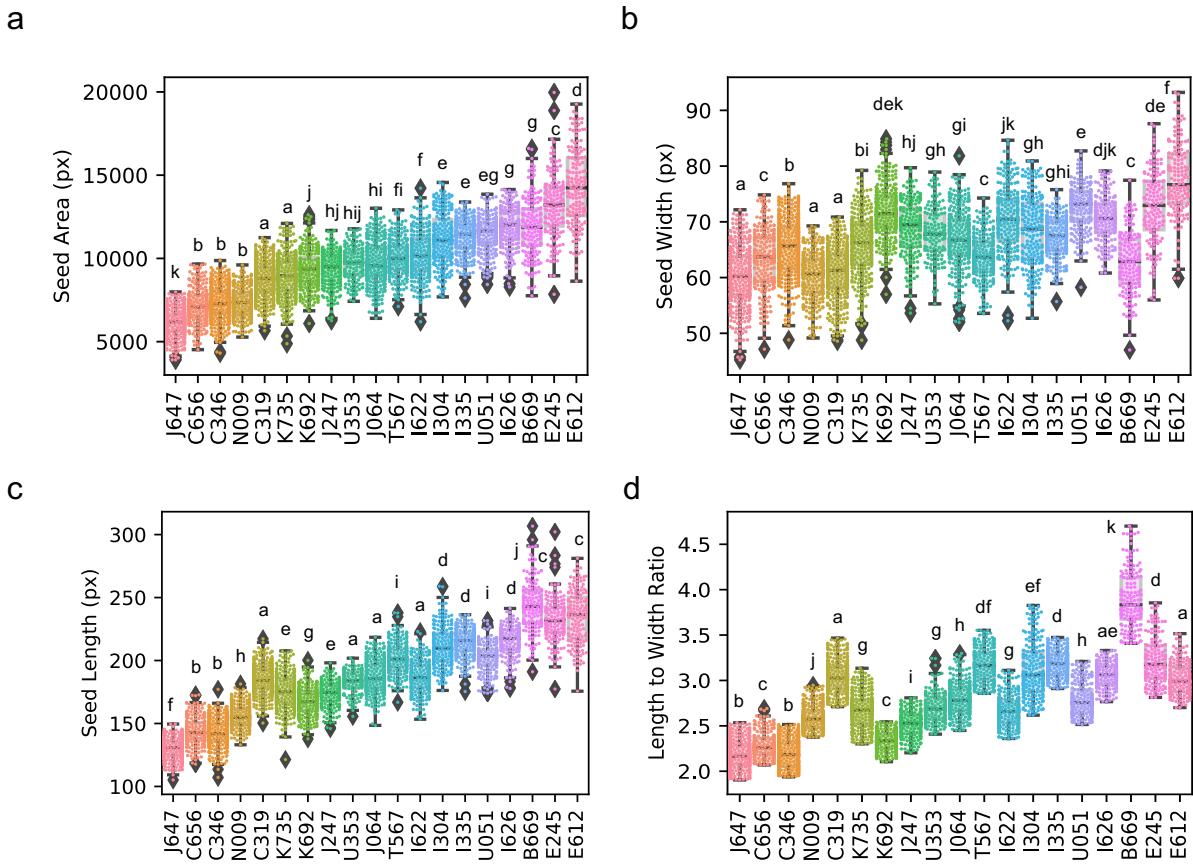
362 Post-Processing

363 As described in the Methods section, we introduced a post-processing step to the raw output to eliminate
364 detections that are not suitable for further analysis. This process removes seed occlusion due to physical
365 overlap, incomplete segmentation by the neural network, non-seed objects such as dirt or awn debris, or the
366 seeds which were partly hidden due to the location being outside the scanned area (Fig. 4c). Fig. 4d and 4e
367 show the distribution of the seed area before and after post processing. Even though the seed area itself was
368 not used as a filtering criterion, the area values in the respective cultivars shift from a long-tailed to a normal
369 distribution, which well reflects the characteristics of a homogenous population (Fig. 4d). A comparison of
370 the filtered output (Inferred Seed Area) and hand-measured (Ground Truth Area) values displays a strong
371 correlation, where the Pearson correlation value is 0.97 (Fig. 4e). These results suggest that the filtered output
372 values obtained from our pipeline are reliable for further phenotypic analyses.

373 Morphological Characterization of Barley Natural Variation

374 Our pipeline learns from synthetic images, which eases the training dataset preparation process. This pipeline
375 enables large-scale analysis across multiple cultivars or species. To highlight the important advantages of the
376 proposed pipeline, we herein demonstrate an array of analyses to morphologically characterize the natural
377 variation of barley seeds, which highlights the crucial biological features that will provide guidance for
378 further investigation. We selected 19 out of 20 cultivars which were used to train the neural network; however,
379 we have acquired a new image that was not used for training or testing in further analysis. One accession,
380 H602, was excluded from the analysis because the rachis could hardly be removed by husk threshing;
381 therefore, the detected area did not reflect the true seed shape. From the pipeline, we obtained 4,464
382 segmented seed images in total (average of 235 seeds per cultivar).

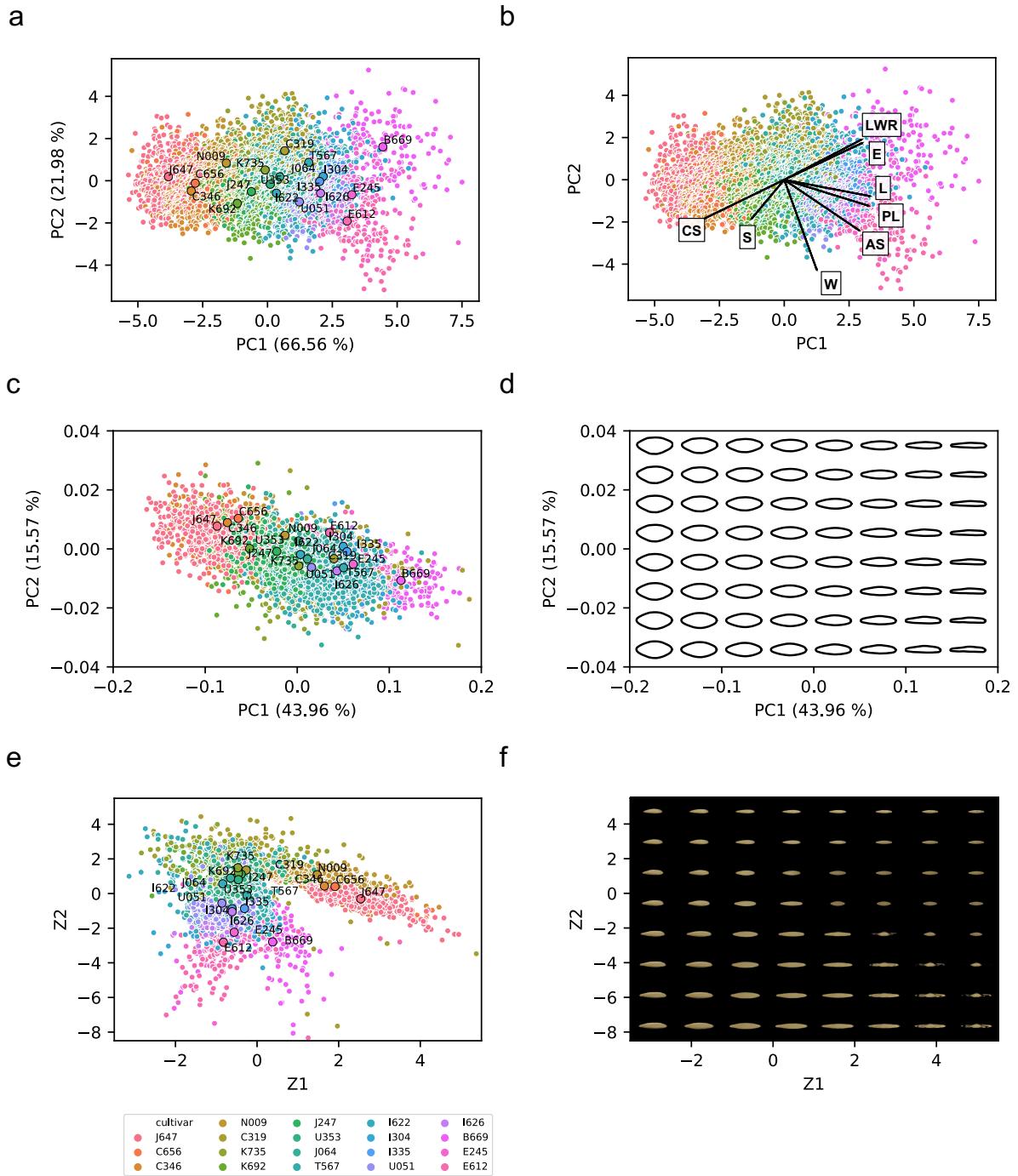
383 As simple and commonly used morphological features, the seed area, width, length, and length-to-
384 width ratio per cultivar were extracted from the respective images and are summarized in Fig. 5a-d. With a
385 sufficient number of biological replicates, we can not only compare the inter-cultivar difference (e.g. median
386 or average) but also consider the intra-cultivar variance. We applied the Analysis of variance (ANOVA) with
387 Tukey's post-hoc test to calculate the statistical difference between cultivars. Interestingly, many cultivars
388 that visually display similar distribution patterns or medians are grouped into statistically different clusters
389 (e.g., K735 and K692 in Fig. 5a). The categorization into numbers of clusters in the respective morphological
390 features suggest that they are regulated by multiple quantitative trait loci. To gain further insight into the
391 morphology of barley cultivars characterized by various descriptors, we performed a multivariate analysis.



392

393 **Fig. 5 | Analysis of natural variation of barley seed morphology.** Whiskerplot overlaid with a swarmplot
 394 (colored dot) grouped by barley cultivars. (a) Seed area, (b) seed width, (c) length, and (d) length-to-width
 395 ratio. Diamonds represent outliers. Statistical differences were determined by one-way ANOVA followed by
 396 Tukey post-hoc analysis. Different letters indicate significant differences ($p < 0.05$).
 397
 398

399 First, we show the results of a principal component analysis (PCA) using eight predefined descriptors
 400 (area, width, length, length-to-width ratio, eccentricity, solidity, perimeter length, and circularity). The first
 401 two principal components (PC) could explain 88.5% of the total variation (Fig. 6a, b). Although there were
 402 no discrete boundaries, the data points tended to form a cluster unique to the cultivar in the latent space,
 403 indicating that cultivars can be classified to a certain extent according to the said descriptors. (Fig. 6a).
 404 Variables such as seed length (L) and perimeter length (PL) mainly constituted the first PC, with seed
 405 circularity (CS) oriented towards the opposite direction, while seed width (W) and length-to-width ratio had
 406 a major influence in PC2 (Fig. 6b). This is exemplified by the distribution of the slenderest B669 and the
 407 circular-shaped J647 at the far-right and far-left orientation in the latent space. Notably, while width (W)
 408 mainly constituted PC2, the direction of its eigenvector differs from that of length (L). Along with the
 409 moderate value of Pearson's correlation between length and width (0.5, $p < 0.01$) (Fig. S4), it is implied that
 410 genes that control both or either of size and length may coexist in the determination of barley seed shape, as
 411 reported in rice⁴⁹.



412

413 **Fig. 6 | Multivariate analysis of barley seed morphology.** (a,b) Principal Component analysis (PCA) with
 414 morphological parameters of barley seeds. Each point represents the data point of the respective seed. The
 415 colors correspond to those defined in the color legend displayed below (e). Mean PC1 and PC2 values of
 416 each cultivar are plotted as large circles with text annotations in (a). Eigenvectors of each descriptor are
 417 drawn as arrows in (b). LWR, length-to-width ratio; E, eccentricity; L, seed length; PL, seed perimeter length;
 418 AS, seed area; W, seed width; S, solidity; CS, seed circularity. (c,d) PCA with elliptic Fourier descriptors
 419 (EFD). The colors and point annotated in (c) follow those of (a). Interpolation of the latent space followed
 420 by reconstruction of the contours are displayed in (d). (e,f) Latent space visualization of Variational
 421 Autoencoders (VAE). The colors and point annotated in (e) follows those of (a). Interpolation of the latent

422 space followed by image generation using the generator of VAE are displayed in (f).

423

424

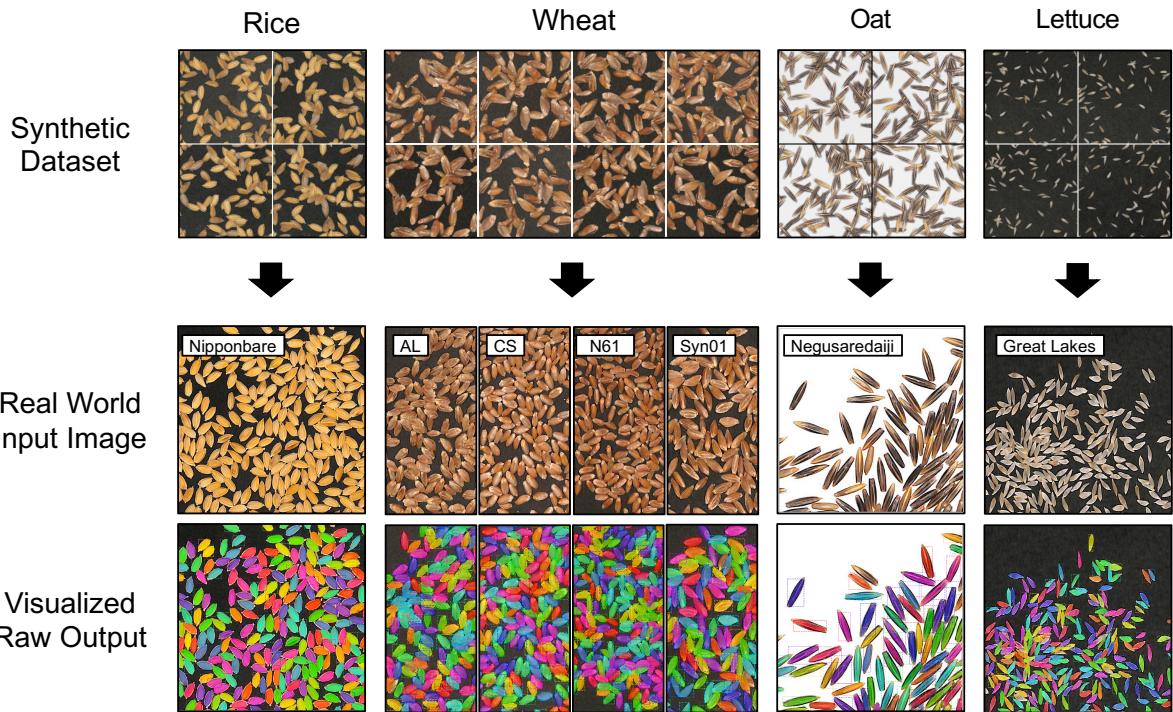
425 Next, we extracted the contour shapes of seeds using elliptic Fourier descriptors (EFDs) followed
426 by PCA (Fig. 6b,c), which is also used in other studies for seed morphological analysis^{36,37}. Compared to the
427 PCA based on the eight morphological descriptors in Fig. 6a, the distributions of respective seeds were
428 relatively condensed, while the clusters by cultivars were intermixed (Fig. 6c), possibly because the size
429 information is lost upon normalization; therefore, EFD can utilize only the contour shape. Interpolating the
430 latent space in the PC1 axis direction clearly highlights the difference in slenderness of the seed (Fig. 6d and
431 Fig. S4a, left). PC2 did not show an obvious change in shape when compared to PC1 (Fig. 6d); however, it
432 seemed to be involved in the sharpness of the edge shape in the longitudinal direction (Fig. S5a, right).
433 Although further verification is required, rendering the average contours which represent the shapes of the
434 respective cultivars implies the difference in such metrics (Fig. S5b).

435 Finally, we trained a variational autoencoder (VAE) for latent space visualization⁴⁷. Unlike other
436 methods using the shape descriptors (i.e., eight simple features or EFDs), the VAE inputs the segmented seed
437 images, which can thus obtain a representation that well describes the dataset without feature predefinition
438 (see Methods for details). The learned representation can be visualized into a two-dimensional scatterplot
439 similar to a PCA (Fig. 6e). Compared to the PCA-based methods, VAE seems to cluster the cultivar in the
440 latent space more explicitly. While the predefined morphological descriptors extract a limited amount of
441 information from an image, VAE can handle an entire image itself; hence, the latter theoretically can learn
442 more complex biological features. Overall, Z1 tend to be involved in the seed color (i.e. brightness) and size,
443 while Z2 is in seed length (Fig. 6f). Generally, unsupervised learning, utilizing deep neural networks such as
444 VAE, requires a sufficient amount of data to fully exert its power to learn the representation of the dataset.
445 The large-scale analysis across various cultivars provides researchers with a novel option to execute such
446 analyses as demonstrated.

447 Application in various crop seeds

448 We further extended our method to verify the efficacy of our approach for other crop seeds. In this report, we
449 newly trained our model to analyze the seed morphology of wheat, rice, oat, and lettuce, with the respectively
450 generated synthetic datasets (Fig. 7, top row). Processing the real-world images resulted in a clear
451 segmentation of each species, regardless of seed size, shape, texture, or color, and background (Fig. 7 middle
452 and bottom rows). In conclusion, these results strongly suggest the high generalization ability of our presented
453 method.

454



455
456
457
458
459
460

Fig. 7 | Application of our proposed pipeline to seeds of various species. Synthetic data of respective species were generated (top row) and the neural networks were independently trained. The inference result against the real-world input images (middle row) were visualized (bottom row). The name of the cultivar per species is overlaid, respectively.

461 Discussions

462 In this research, we showed that utilizing a synthetic dataset can successfully train the instance segmentation
463 neural network to analyze the real-world images of barley seeds. The values obtained from the image analysis
464 pipeline was comparable to that of manual annotation (Fig. 4e), thus achieving high throughput quantification
465 of seed morphology in various analysis. Moreover, our pipeline requires a limited number of synthesized
466 images to be added to the pool for creating a synthetic dataset. This is labor cost-efficient and practical
467 compared to labeling numerous amounts of images required for deep learning.

468 To completely understand the use of synthetic data for deep learning, we must have a precise
469 understanding of “*what type of features are critical to represent the real world dataset*”. In the case of seed
470 instance segmentation, we presumed that the network must learn the representation that is important for
471 segregating physically touching or overlapping seeds into an individual object. Therefore, in the course of
472 designing synthetic images, we prioritized the dataset to contain numerous patterns of seed orientation, rather
473 than to contain massive patterns of seed textures. Based on the result that the model showed sufficient result
474 against the test dataset (Fig. 4B, S3, and Table 1), it is suggested that our presumption was legitimate to a
475 certain extent. However, because the neural network itself is a *black box*, we cannot discuss more than *ex*
476 *post facto* reasoning. Recently, there have been challenges to understand the representation of biological

477 context by various interpretation techniques, exemplified in plant disease classification^{11,50}. Extending such
478 techniques applicable to an instance segmentation neural network as used in our study will help verify the
479 authenticity of both the synthesized dataset and the trained neural network in future studies.

480 We introduced post-processing to exclude nonintegral mask regions prior to phenotypic analysis (Fig.
481 4a, bottom row and 4c,d). Theoretically, if we can add a category label to the synthetic dataset to determine
482 whether the respective regions are suitable for analysis, the neural network may acquire the classification
483 ability to discriminate such integrity. However, the complexity of synthetic data generation increases, and
484 miss-detected or incomplete mask regions cannot be excluded. We presume heuristic-based post-processing
485 is a simple yet powerful approach. Nonetheless, our outlier removal process is based on the assumption that
486 the seed population is homogeneous. It is important to verify if such filtering is valid against the
487 heterogeneous population. Notably, *SmartGrain* also introduces a post-processing step, involving a repetitive
488 binary dilation and erosion. Those processes were reported to be effective in analyzing the progenies of two
489 cultivars in rice upon QTL analysis⁴². As the post-processing is independent of the neural network in our
490 pipeline, designing and verifying various methods are important for expanding the functionality of the
491 analysis pipeline.

492 The shape and size of seeds (grains) are important agronomic traits that determine the quality and
493 the yield of crops³³. In recent years, a number of genes have been identified and characterized through genetic
494 approach, accompanied by laborious phenotyping. In previous studies, researchers manually measured the
495 shape and size of seeds, which is time-consuming and erroneous; it restricts the number of seeds that the
496 researcher can analyze. The researchers used to manually select several seeds that seemed to represent the
497 population in a subjective manner and for this reason, small phenotypic differences between genotypes could
498 not be detected. Our pipeline can phenotype a large number of seeds without the need to consider the seed
499 orientation to be sparse in image acquisition and thereby can obtain large amount of data in a short period of
500 time. This allows easy and sensitive detection of both obvious and subtle phenotypic differences between
501 cultivars supported by statistical verification (Fig. 5). This will be a breakthrough in identifying
502 agronomically important genes, especially for molecular genetic research such as genome-wide association
503 study (GWAS), quantitative trait locus (QTL) analysis, and mutant screening. Thus, will open a new path to
504 identify genes that were difficult to isolate by conventional approaches.

505 Moreover, the application of our pipeline is not restricted to barley but can be extended to various
506 crops such as seeds of wheat, rice, oats, and lettuce (Fig. 7). Our results strongly suggest that our approach
507 is applicable to any varieties or species in principle, thus is expected to accelerate research in various fields
508 with similar laborious issues. One example can be an application in characterization of and gene isolation
509 from seeds of wild species. Cultivated lines possess limited genetic diversity due to bottlenecks in the process
510 of domestication and breeding, therefore many researchers face challenges to identify agronomically
511 important genes from wild relatives as a source of genes for improving agronomic traits. As the appearance
512 of the seeds of wild species is generally more diverse than that of cultivated varieties, development of a
513 universal method to measure both traits were difficult. Another example is in understanding the development
514 of seed morphology of wheat. Although the shapes of small florets can be manually quantified from the image

515 of a scanned spikelet, the automated quantification has not been realized owing to excess non-seed objects
516 (e.g., glume, awn, and rachis) in the image. Applying another domain of randomization for synthesizing a
517 training dataset can be utilized to functionalize a neural network to quantify seed phenotype from such images.

518 Collectively, we have shown the efficacy of utilizing the synthetic data, based on the concept of
519 *domain randomization* to train the neural network for real-world tasks. Recent technical advances in the
520 computer vision domain have enabled us to generate a realistic image, or even a realistic “virtual reality”
521 environment, thus will provide more possibilities to give solutions to current image analysis involved
522 challenges in the agricultural domain. We envision that a collaboration with plant and computer scientists
523 will open a new point of view for generating a workflow that is valuable for plant phenotyping, leading to a
524 further understanding of the biology of plants through the complete use of machine learning/deep learning
525 methods.

526 Data Availability

527 Synthetically generated datasets and real-world test datasets can be obtained from the following Github
528 repository (https://github.com/totti0223/crop_seed_instance_segmentation). Code to reproduce the
529 deployment of the trained Mask R-CNN and multivariate analysis is formatted as IPython notebooks and can
530 also be obtained from the same repository. Other data and information regarding the manuscript are available
531 upon reasonable request.

532 Conflicts of Interest

533 The authors declare that there are no conflicts of interest regarding the publication of this article.

534 Author Contribution Statement

535 YT directed and designed the study, wrote the program codes, generated the synthetic test dataset, performed
536 the experiments with assistance from FO, HT, DS, and KT. HT and DS collected and scanned the barley seed
537 images and JI collected wheat images. YT annotated the test dataset. YT, HT, and DS was involved in the
538 conceptualization of this research. YT, FO, and HT wrote the manuscript with assistance from DS, KT, JI,
539 and SO, furthermore with verification of scientific validity from all the coauthors.

540 Acknowledgments

541 We thank Ms. Yoko Tomita at Nagoya University for assistance in the labor-intensive annotation to generate
542 a ground truth test dataset. We also thank Dr. Miya Mizutani for a comprehensive discussion and critical
543 reading of the manuscript. The graphical abstract was rendered by Dr. Issey Takahashi who is a member of
544 the Research Promotion Division in ITbM of Nagoya University. Dr. Shunsaku Nishiuchi provided
545 Nipponbare rice seeds used in this study. Dr. Toshiaki Tameshige amplified and provided wheat seeds. Dr.

546 Kentaro Shimizu amplified and provided wheat Arina seeds and Drs. Shigeo Takumi and Yoshihiro Matsuoka
547 established, amplified and provided synthetic wheat Ldn/KU-2076 (Syn01) seeds. This work was supported
548 by Japan Science and Technology Agency (JST) PRESTO [Grants nos. JPMJPR17O5 (YT) and
549 JPMJPR17O3 (FO)], JST CREST [Grant Number JPMJCR16O4 (HT, DS, SO)], MEXT KAKENHI
550 [Numbers 16H06466 and 16H06464 (HT), 16KT0148 (DS), and 19K05975 (JI)], and JST ALCA [Number
551 JPMJAL1011 (TK)]. All the barley materials are provided by the National BioResource Project (NBRP:
552 Barley).

553

554

555 **Figure Legends**

556

557 **Fig. 1 | Overview of the proposed training process of crop seed instance segmentation.**

558 **Fig. 2 | Evaluation metrics for object detection accuracy.** (a) The intersection-over-union (IoU) definitions
559 for bounding boxes and masks. (b) The average precision (AP) defined as the area under the curves (AUC);
560 shown as the area marked with slanted lines.

561 **Fig. 3 | Data prepared in this study.** (a) Images of barley seeds scanned from 20 cultivars. Cultivar names
562 are described in white text in each image. These images were also used as a real-world test dataset in Table
563 1. (b) Scheme of generating synthetic images. Images are generated by combining actual scanned seed images
564 over the background images on to the virtual canvas. Simultaneously generated ground truth label (Mask) is
565 shown at the bottom in which each seed area is marked with a unique color.

566 **Fig. 4 | Image Analysis pipeline.** (a) Summary of the image analysis pipeline. (b) Examples of graphical
567 output of the trained Mask R-CNN on real-world images. Different colors indicate an individual segmented
568 seed region. Note that even if the seeds are overlapping or touching each other, the network can discriminate
569 them as an independent object. (c) Examples of detected candidate regions to be filtered in the post-
570 processing step indicated in red arrows. Black arrowheads indicate the input image boundary. (e) Probability
571 density of the seed areas of the raw output and filtered output. (f) Scatterplot describing the correlation of the
572 seed area that was measured by the pipeline (Inferred Seed Area) and by manual annotation (Ground Truth
573 Seed Area). Each dot represents the value by a single seed. Black and gray lines indicate the identity line and
574 the 10% error threshold line, respectively. The proportion of the seeds that have lower or higher than the 10%
575 error is also displayed.

576 **Fig. 5 | Analysis of natural variation of barley seed morphology.** Whiskerplot overlaid with a swarmplot
577 (colored dot) grouped by barley cultivars. (a) Seed area, (b) seed width, (c) length, and (d) length to width
578 ratio. Diamonds represent outliers. Statistical differences were determined by one-way ANOVA followed by
579 Tukey post hoc analysis. Different letters indicate significant differences ($p < 0.05$).

580 **Fig. 6 | Multivariate analysis of barley seed morphology.** (a,b) Principal Component analysis (PCA) with
581 morphological parameters of barley seeds. Each point represents the data point of respective seed. The colors
582 correspond to that defined in the color legend displayed below (e). Mean PC1 and PC2 values of each cultivar
583 are plotted as large circle with text annotations in (a). Eigenvectors of each descriptor are drawn as arrows in
584 (b). LWR, length to width ratio; E, eccentricity; L, seed length; PL, seed perimeter length; AS, seed area; W,
585 seed width; S, solidity; CS, seed circularity. (c,d) PCA with elliptic Fourier descriptors (EFD). The colors
586 and point annotated of (c) follows that of (a). Interpolation of the latent space followed by reconstruction of
587 the contours are displayed in (d). (e,f) Latent space visualization of Variational Autoencoders (VAE). The
588 colors and point annotated of (e) follows that of (a). Interpolation of the latent space followed by image
589 generation using the generator of VAE are displayed in (f).

590 **Fig. 7 | Application of our proposed pipeline to seeds of various species.** Synthetic data of respective
591 species were generated (top row) and the neural networks were independently trained. The inference result
592 against the real-world input images (middle row) were visualized (bottom row). The name of the cultivar per

593 species is overlaid, respectively.

594 **Table 1 | Model Evaluation.** Table describing the evaluation result of the trained Mask R-CNN raw output.
595 Recall values at the IoU threshold of 50% (Recall_{50}) and Average Precision (AP) at the IoU 50% (AP_{50}), 75%
596 (AP_{75}), and the mean value from IoU 50% to 95% with the step size of 5% ($\text{AP}@[.5:.95]$) are shown.

597

598 References

599

600 1. Goodfellow, I., Bengio, Y. & Courville, A. *Deep Learning*. (The MIT Press, 2016).

601 2. Russakovsky, O. *et al.* ImageNet large scale visual recognition challenge. *Int. J. Comput. Vis.* **115**, 211–252 (2015).

602

603 3. Krizhevsky, A., Sutskever, I. & Hinton, G. E. ImageNet classification with deep convolutional neural networks. *Commun. ACM* **60**, 84–90 (2017).

604

605 4. Ronneberger, O., Fischer, P. & Brox, T. U-Net: Convolutional networks for biomedical image segmentation. in *Proc. Medical Image Computing and Computer-Assisted Intervention (MICCAI)* 234–241 (Springer International Publishing, 2015).

606

607

608 5. Shelhamer, E., Long, J. & Darrell, T. Fully convolutional networks for semantic segmentation. *IEEE Trans. Pattern Anal. Mach. Intell.* **39**, 640–651 (2017).

609

610 6. Girshick, R., Donahue, J., Darrell, T. & Malik, J. Rich feature hierarchies for accurate object detection and semantic segmentation. in *Proc. IEEE Conference on Computer Vision and Pattern Recognition* 580–587 (IEEE, 2014). doi:10.1109/CVPR.2014.81

611

612

613 7. Girshick, R. Fast R-CNN. in *Proc. IEEE International Conference on Computer Vision (ICCV)* 1440–1448 (IEEE, 2015). doi:10.1109/ICCV.2015.169

614

615 8. He, K., Gkioxari, G., Dollar, P. & Girshick, R. Mask R-CNN. in *Proc. IEEE International Conference on Computer Vision (ICCV)* 2980–2988 (IEEE, 2017). doi:10.1109/ICCV.2017.322

616

617 9. Milioto, A., Lottes, P. & Stachniss, C. Real-time blob-wise sugar beets vs weeds classification for monitoring fields using convolutional neural networks. *ISPRS Ann. Photogramm. Remote Sens. Spatial Inf. Sci.* **IV-2/W3**, 41–48 (2017).

618

619

620 10. Mohanty, S. P., Hughes, D. P. & Salathé, M. Using deep learning for image-based plant disease detection. *Front. Plant Sci.* **7**, 1419 (2016).

621

622 11. Ghosal, S. *et al.* An explainable deep machine vision framework for plant stress phenotyping. *Proc. Natl. Acad. Sci. USA* **115**, 4613–4618 (2018).

623

624 12. Bresilla, K. *et al.* Single-shot convolution neural networks for real-time fruit detection within the tree. *Front. Plant Sci.* **10**, 611 (2019).

625

626 13. Kamilaris, A. & Prenafeta-Boldú, F. X. Deep learning in agriculture: A survey. *Comput. Electron. Agr.* **147**, 70–90 (2018).

627

628 14. Kaneda, Y., Shibata, S. & Mineno, H. Multi-modal sliding window-based support vector regression for predicting plant water stress. *Knowl-Based Syst.* (2017). doi:10.1016/j.knosys.2017.07.028

629

630 15. Lin, T.-Y. *et al.* Microsoft COCO: Common objects in context. in *Proc. European Conference on Computer Vision (ECCV)*, 740–755 (Springer International Publishing, 2014).

631

632 16. Guo, W. *et al.* Aerial imagery analysis - Quantifying appearance and number of sorghum heads for applications in breeding and agronomy. *Front. Plant Sci.* **9**, 1544 (2018).

633

634 17. Jin, X. *et al.* High-throughput measurements of stem characteristics to estimate ear density and above-ground biomass. *Plant Phenomics* **2019**, 1–10 (2019).

635

636 18. Ghosal, S. *et al.* A weakly supervised deep learning framework for sorghum head detection and counting. *Plant Phenomics* **2019**, 1–14 (2019).

637

638 19. Chandra, A. L., Desai, S. V., Balasubramanian, V. N., Ninomiya, S. & Guo, W. Active learning with weak
639 supervision for cost-effective panicle detection in cereal crops. *arXiv arXiv:1910.01789*, (2019).

640 20. Nath, T. *et al.* Using DeepLabCut for 3D markerless pose estimation across species and behaviors. *Nat.*
641 *Protoc.* **14**, 2152–2176 (2019).

642 21. Varol, G. *et al.* Learning from synthetic humans. in *Proc. IEEE Conference on Computer Vision and Pattern*
643 *Recognition (CVPR)* 4627–4635 (IEEE, 2017). doi:10.1109/CVPR.2017.492

644 22. Doersch, C. & Zisserman, A. Sim2real transfer learning for 3D pose estimation: Motion to the rescue. in
645 *Proc. Annual Conference on Neural Information Processing Systems (NeurIPS)* (2019).

646 23. Goodfellow, I. J. *et al.* Generative adversarial networks. in *Proc. Annual Conference on Neural Information*
647 *Processing Systems (NeurIPS)* (2014).

648 24. Johnson, M. K. *et al.* CG2Real: Improving the realism of computer generated images using a large collection
649 of photographs. *IEEE Trans. Vis. Comput. Graph.* **17**, 1273–1285 (2011).

650 25. Shrivastava, A. *et al.* Learning from simulated and unsupervised images through adversarial training. in *Proc.*
651 *IEEE Conference on Computer Vision and Pattern Recognition (CVPR)* 2242–2251 (IEEE, 2017).
652 doi:10.1109/CVPR.2017.241

653 26. Peng, X. B., Andrychowicz, M., Zaremba, W. & Abbeel, P. Sim-to-real transfer of robotic control with
654 dynamics randomization. in *Proc. IEEE International Conference on Robotics and Automation (ICRA)*
655 3803–3810 (IEEE, 2018). doi:10.1109/ICRA.2018.8460528

656 27. Tremblay, J. *et al.* Training deep networks with synthetic data: Bridging the reality gap by domain
657 randomization. in *Proc. IEEE/CVF Conference on Computer Vision and Pattern Recognition Workshops*
658 (*CVPRW*) 1082–10828 (IEEE, 2018). doi:10.1109/CVPRW.2018.00143

659 28. Isokane, T., Okura, F., Ide, A., Matsushita, Y. & Yagi, Y. Probabilistic plant modeling via multi-view image-
660 to-image translation. in *Proc. IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR)*
661 2906–2915 (IEEE, 2018). doi:10.1109/CVPR.2018.00307

662 29. Giuffrida, M. V., Scharr, H. & Tsaftaris, S. A. ARIGAN: Synthetic arabidopsis plants using generative
663 adversarial network. in *Proc. IEEE International Conference on Computer Vision Workshops (ICCVW)*
664 2064–2071 (IEEE, 2017).

665 30. Karras, T., Laine, S. & Aila, T. A style-based generator architecture for generative adversarial networks. in
666 *Proc. IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR)* (IEEE, 2019).

667 31. Arsenovic, M., Karanovic, M., Sladojevic, S., Anderla, A. & Stefanovic, D. Solving current limitations of
668 deep learning based approaches for plant disease detection. *Symmetry* **11**, 939 (2019).

669 32. Ward, D., Moghadam, P. & Hudson, N. Deep leaf segmentation using synthetic data. in *Proc. British*
670 *Machine Vision Conference Workshops (BMVCW)* (2018).

671 33. Shomura, A. *et al.* Deletion in a gene associated with grain size increased yields during rice domestication.
672 *Nat. Genet.* **40**, 1023–1028 (2008).

673 34. Song, X.-J., Huang, W., Shi, M., Zhu, M.-Z. & Lin, H.-X. A QTL for rice grain width and weight encodes a
674 previously unknown RING-type E3 ubiquitin ligase. *Nat. Genet.* **39**, 623–630 (2007).

675 35. Weng, J. *et al.* Isolation and initial characterization of GW5, a major QTL associated with rice grain width
676 and weight. *Cell Res.* **18**, 1199–1209 (2008).

677 36. Williams, K., Munkvold, J. & Sorrells, M. Comparison of digital image analysis using elliptic Fourier

678 descriptors and major dimensions to phenotype seed shape in hexaploid wheat (*Triticum aestivum* L.).
679 *Euphytica* **190**, 99–116 (2013).

680 37. Ohsawa, R., Tsutsumi, T., Uehara, H., Namai, H. & Ninomiya, S. Quantitative evaluation of common
681 buckwheat (*Fagopyrum esculentum* Moench) kernel shape by elliptic Fourier descriptor. *Euphytica* **101**,
682 175–183 (1998).

683 38. Iwata, H., Ebana, K., Uga, Y., Hayashi, T. & Jannink, J.-L. Genome-wide association study of grain shape
684 variation among *Oryza sativa* L. germplasms based on elliptic Fourier analysis. *Mol. Breeding* **25**, 203–215
685 (2010).

686 39. Eguchi, M. & Ninomiya, S. Evaluation of soybean seed shape by elliptic Fourier descriptors. in *Proc. World*
687 *Conference on Agricultural Information And IT* (2008).

688 40. Ayoub, M., Symons, J., Edney, J. & Mather, E. QTLs affecting kernel size and shape in a two-rowed by six-
689 rowed barley cross. *Theor. Appl. Genet.* **105**, 237–247 (2002).

690 41. Herridge, R. P., Day, R. C., Baldwin, S. & Macknight, R. C. Rapid analysis of seed size in *Arabidopsis* for
691 mutant and QTL discovery. *Plant Methods* **7**, 3 (2011).

692 42. Tanabata, T., Shibaya, T., Hori, K., Ebana, K. & Yano, M. SmartGrain: High-throughput phenotyping
693 software for measuring seed shape through image analysis. *Plant Physiol.* **160**, 1871–1880 (2012).

694 43. Takumi, S., Nishioka, E., Morihiro, H., Kawahara, T. & Matsuoka, Y. Natural variation of morphological
695 traits in wild wheat progenitor *Aegilops tauschii* Coss. *Breed. Sci.* **59**, 579–588 (2009).

696 44. He, K., Zhang, X., Ren, S. & Sun, J. Deep residual learning for image recognition. in *Proc. IEEE Conference*
697 *on Computer Vision and Pattern Recognition (CVPR)* 770–778 (IEEE, 2016). doi:10.1109/CVPR.2016.90

698 45. Everingham, M., Van Gool, L., Williams, C. K. I., Winn, J. & Zisserman, A. The Pascal Visual Object
699 Classes (VOC) challenge. *Int. J. Comput. Vis.* **88**, 303–338 (2010).

700 46. Kuhl, F. P. & Giardina, C. R. Elliptic Fourier features of a closed contour. *Computer Graphics and Image*
701 *Processing* **18**, 236–258 (1982).

702 47. Kingma, D. P. & Welling, M. Auto-encoding variational bayes. in *Proc. International Conference on*
703 *Learning Representations (ICLR)* (2014).

704 48. Doersch, C. Tutorial on variational autoencoders. *arXiv preprint arXiv:1606.05908*, (2016).

705 49. Li, N., Xu, R., Duan, P. & Li, Y. Control of grain size in rice. *Plant Reprod.* **31**, 237–251 (2018).

706 50. Toda, Y. & Okura, F. How convolutional neural networks diagnose plant disease. *Plant Phenomics Article*
707 **ID 9237136**, 14 pages (2019).

708