

1 **AirSurf-Lettuce: an aerial image analysis platform for ultra-scale**
2 **field phenotyping and precision agriculture using computer vision**
3 **and deep learning**

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5 **Authors**

6 Alan Bauer^{1,2,3,+}, Alan.Bauer@earlham.ac.uk, orcid: 0000-0002-7443-1511

7 Aaron George Bostrom^{1,+}, aaron.bostrom@earlham.ac.uk, 0000-0002-7300-6038

8 Joshua Ball¹, Joshua.Ball@earlham.ac.uk, orcid: 0000-0003-4840-3768

9 Christopher Applegate¹, Christopher.Applegate@earlham.ac.uk

10 Tao Cheng⁴, tcheng@njau.edu.cn, orcid: 0000-0002-4184-0730

11 Stephen Laycock³, S.Laycock@uea.ac.uk, orcid: 0000-0002-7724-1369

12 Sergio Moreno Rojas⁵, sergio.morenorojas@GS-Growers.com

13 Jacob Kirwan^{5,*}, Jacob.Kirwan@GS-Growers.com

14 Ji Zhou^{1,2,3,*}, Ji.Zhou@njau.edu.cn, Ji.Zhou@earlham.ac.uk, orcid: 0000-0002-5752-5524

15

16 ¹Earlham Institute, Norwich Research Park, Norwich, NR4 7UZ, UK

17 ²Plant Phenomics Research Center, China-UK Plant Phenomics Research Centre, Nanjing
18 Agricultural University, Nanjing 210095, Jiangsu, China

19 ³School of Computing Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4
20 7TJ

21 ⁴National Engineering and Technology Center for Information Agriculture, MARA Key Laboratory
22 for Crop System Analysis and Decision Making, Jiangsu Key Laboratory for Information Agriculture,
23 Nanjing Agricultural University, Nanjing 210095, Jiangsu, China

24 ⁵G's Growers Limited, Ely, Cambridgeshire, CB7 5TZ

25

26 ⁺Joint first author ^{*}Corresponding author

27

28 **Corresponding authors**

29 Ji.Zhou@njau.edu.cn and Ji.Zhou@earlham.ac.uk; Jacob.Kirwan@GS-Fresh.com

30

31 **Abstract**

32 Aerial imagery is regularly used by farmers and growers to monitor crops during the growing season.
33 To extract meaningful phenotypic information from large-scale aerial images collected regularly from
34 the field, high-throughput analytic solutions are required, which not only produce high-quality
35 measures of key crop traits, but also support agricultural practitioners to make reliable management
36 decisions of their crops. Here, we report *AirSurf-Lettuce*, an automated and open-source aerial image
37 analysis platform that combines modern computer vision, up-to-date machine learning, and modular
38 software engineering to measure yield-related phenotypes of millions of lettuces across the field.
39 Utilising ultra-large normalized difference vegetation index (NDVI) images acquired by fixed-wing
40 light aircrafts together with a deep-learning classifier trained with over 100,000 labelled lettuce
41 signals, the platform is capable of scoring and categorising iceberg lettuces with high accuracy
42 (>98%). Furthermore, novel analysis functions have been developed to map lettuce size distribution in
43 the field, based on which global positioning system (GPS) tagged harvest regions can be derived to
44 enable growers and farmers' precise harvest strategies and marketability estimates before the harvest.

45

46 **Keywords**

47 AirSurf; lettuce; ultra-scale field phenotyping; deep learning; image analysis; precision agriculture

48

49 **Introduction**

50 As an important source of vitamins, minerals, and trace elements, leaf vegetables play crucial roles
51 in human nutrition¹. Lettuce (*Lactuca sativa* L., a plant of the Asteraceae family), one of the most
52 common staple vegetable foods, has a wide range of tastes, textures, and shapes that satisfy diverse
53 customer needs^{2,3}. Recent research indicates that lettuce consumption has positive effects on the
54 reduction of cardiovascular disease and chronic conditions, because it provides nutrients such as
55 vitamin A, *Beta-carotene*, folate, and iron content to support human growth and health^{4,5}. While
56 lettuce is an important and nutritional crop, fluctuating environments can increase the fragility of its
57 production⁶. For example, the bad weather in Spain in early 2017 led to retail prices for lettuce
58 products to nearly triple in UK supermarkets⁷. Severe weather not only can cause supply shortage, but
59 also affects the crop quality. According to studies on lettuce growth and development⁸⁻¹⁰, at newly
60 planted phase (i.e. from cotyledons unfolded to three true leaves stage), young crops require cool and
61 damp weather to develop into high-quality products after transplanting from greenhouse to the fields;
62 whereas lettuce leaves can rapidly become bitter and inedible if the plant growth is accelerated by
63 ambient temperature at the head maturity phase (i.e. before flowering). Because of the dynamic nature
64 of lettuce production, the actual yield of lettuces in commercial operations is around 70-80% of the
65 planted quantity^{11,12}. To ensure consistency of supply and quality, it is important for growers and
66 farmers to closely monitor lettuce growth and development, so that prompt and reliable agricultural
67 practices can be arranged under today's fluctuating agricultural conditions¹³.

68 Commercially, lettuce production offers an attractive economic profitability in comparison to many
69 other Agri-Food businesses^{14,15}. To date, lettuce-related businesses are worth billions of dollars and
70 employ hundreds of thousands of permanent and seasonal workers globally. According to the Food
71 and Agriculture Organisation of the United Nations¹⁶, European vegetable growers alone produced
72 2.95 million tonnes of lettuce (and chicory) in 2016, a total annual value of €2.5 billion. Spain, the
73 largest lettuce producer in Europe, is exporting approximately €420 million worth of lettuce products
74 every year; Germany, France and the UK are the three largest markets for lettuce consumption in
75 Europe, with a combined import of €350 million annually¹⁷. To serve diverse consumer tastes as well

76 as improve the actual yield of lettuces, lettuce breeders are constantly introducing new varieties to the
77 market, from the dense head (i.e. iceberg type) to the notched or frilly leaf varieties^{18,19}.

78 Further down the fresh produce supply chain, the planning and efficiency of many essential Agri-
79 Food activities are largely dependent on the maturity date and marketability of different sizes and
80 quality of crops²⁰. Logistics, trading, and marketing need to be organised several weeks before the
81 harvest²¹; moreover, the booking and reservation of lettuce distribution, agricultural equipment, and
82 commercial plans with retails are often determined between H1 (soft and spongy head) and H3
83 (mature and compact head) stages. So, crop can be harvested at the right time, with maximised
84 yield^{22,23}. To reliably measure and estimate potential yield (e.g. the number of lettuce heads) and
85 associated crop quality (e.g. lettuce size categories) for better marketing and supply chain
86 management, growers and farmers are continuously seeking new technologies to assist them with
87 better and more precise crop management decisions^{24,25}.

88 As a relative newcomer to life sciences, machine learning (ML) related techniques use statistics and
89 sparse representations to progressively build computational procedures to accomplish specific tasks
90 such as data classification, feature selection, clustering, and predictive modelling^{26,27}. Although the
91 unfamiliarity with ML often prevents plant researchers from effectively employing ML and its related
92 technology in biological studies²⁸⁻³⁰, many cases indicate that ML is the key to success in addressing a
93 variety of data-driven challenges in life sciences, if appropriately labelled training data³¹, suitable
94 learning algorithms³², and well-defined missions can be arranged. Some of the cases are: (1) the
95 analysis of big genomic data for annotation, assembly, and gene regulatory networks³¹, (2) the
96 classification of DNA and protein sequences for genetic and genomic studies³³, and (3) the prediction
97 of genome and phenome patterns based on high-dimensional feature datasets³⁴.

98 In this article, we present a cross-disciplinary approach that develops a new analytic software tool to
99 perform automated ultra-scale field phenotyping of iceberg lettuce. Our research and development
100 (R&D) activities integrates ultra-scale normalized difference vegetation index (NDVI) aerial imagery,
101 modern computer vision, state-of-the-art deep learning (i.e. convolutional neural networks, CNNs),
102 supervised machine learning, modular software engineering, and commercial lettuce production into
103 an open-source image analysis platform called *AirSurf-Lettuce* (AirSurf-L). The platform is capable

104 of performing phenotypic analysis of millions of lettuces across the field. A CNN model trained with
105 over 100,000 labelled lettuce signals has been embedded in AirSurf-L to quantify lettuce heads and
106 their plantation layout using ultra-large NDVI images collected by a fixed-wing light aircraft.
107 Unsupervised ML algorithms were used to classify lettuce heads into three size categories (i.e. small,
108 medium and large). To connect analysis results with marketability and crop management decisions, a
109 novel function has been developed to connect global positioning system (GPS) information with the
110 lettuce size distribution map, based on which a GPS-tagged harvest map was produced to enable
111 efficient and precise harvesting strategies for growers and farmers to increase marketable yield. The
112 analysis results generated by AirSurf-L show a strong correlation between machine counting and
113 specialist scoring. We are therefore confident that our work is promising in assisting vegetable
114 growers and farmers with their precision agriculture management activities. Together with recent
115 advances in unmanned aerial vehicles (UAV) technologies, ground-based remote sensors, and ML-
116 based modelling, AirSurf-L could have great significance to improve the fresh vegetable crop
117 production, distributing and logistics activities before the harvest. Furthermore, with additional
118 training data, necessary testing and validation, we believe that the analysis platform can be expanded
119 relatively easily to incorporate other crop species such as wheat and rice for ultra-scale aerial crop
120 phenotyping.

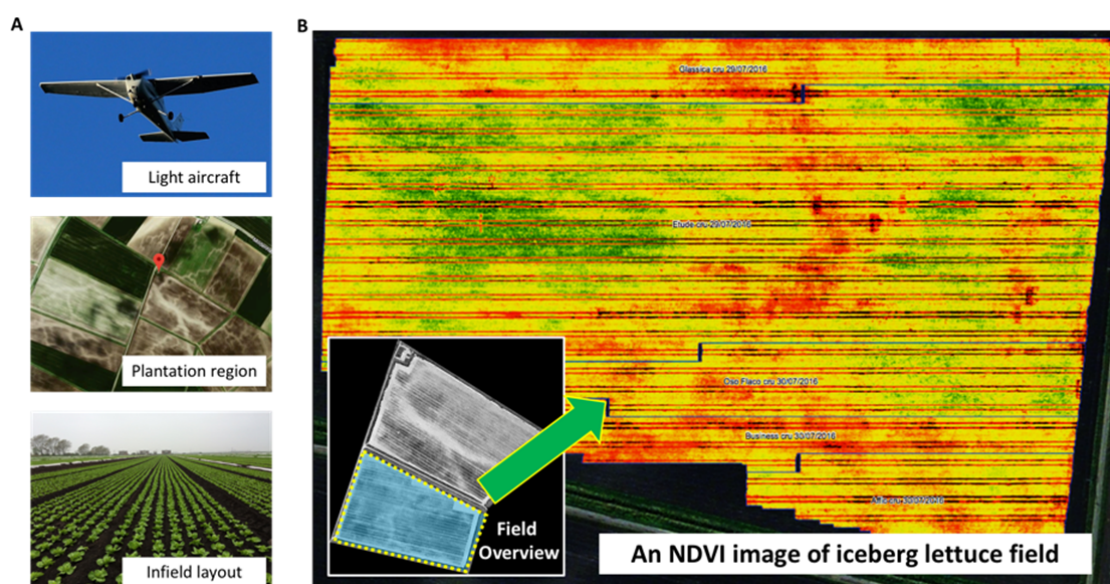
121

122 **Results**

123 *NDVI aerial imaging and data acquisition*

124 The ultra-large aerial NDVI imagery was acquired routinely (i.e. four-five times per season) using a
125 fixed-wing light aircraft operated by G's Growers, the second largest vegetable grower in the UK.
126 The flying route and the imaging protocol were designed to facilitate cross-site crop layout
127 assessment, yield prediction (based on vegetation indices), and disease monitoring (Fig. 1A), which
128 has been described previously³⁵. In this study, we used a series of collected ultra-large NDVI images
129 (1.5-2GB per image) at 3cm ground sample distance (GSD) spatial resolution, collecting iceberg
130 lettuce signals between H1 and H2 stages (i.e. moderate compact and crushable head), before lettuce

131 leaves were largely overlapped. Experimental fields were located in Cambridgeshire UK, ranging
132 from 10 to 20 hectares, with between 800,000 and 1.6 million lettuce heads in a single field. A field
133 planted with around 1 million lettuce heads (coloured light blue in Fig. 1B) was used in the following
134 sections to explain the analysis workflow of AirSurf-L. A high-level manual yield counting was
135 conducted by the grower's field specialists during the harvest, which was used to verify and improve
136 the AirSurf-L platform. Also, lettuces in subsections randomly selected from experimental fields were
137 scored manually by laboratory technicians, which were also used as training data for the CNN model.



138
139 **Figure 1: Ultra-large NDVI aerial imaging accomplished routinely through a fixed-wing light aircraft**
140 **operated by G's Growers.**

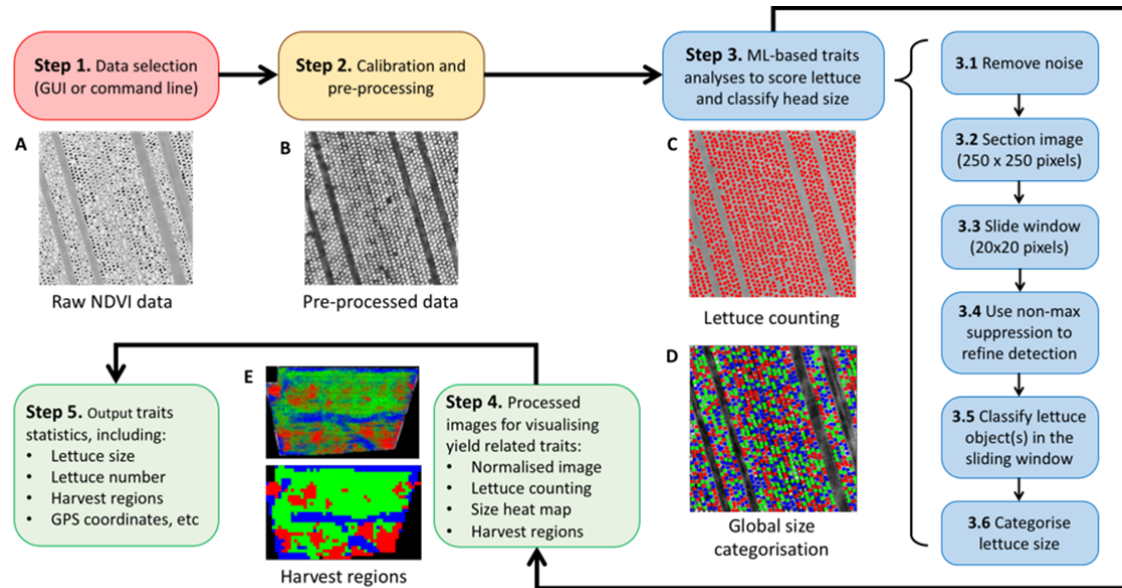
141 (A) The flying route and aerial imaging were designed to facilitate cross-site crop layout assessment and yield
142 prediction. (B) A series of ultra-large NDVI images at 3cm GSD spatial resolution were acquired to record 0.8-
143 1.6 million lettuce heads per field, at H1 and H2 stages.

144

145 *The analysis workflow of AirSurf-L*

146 The analysis of yield-related phenotypes was based on NDVI signals of iceberg lettuces across the
147 field. Figure 2 shows a high-level analysis workflow of AirSurf-L, which consists of five steps: data
148 input, image calibration and pre-processing, ML-based traits analyses, results visualisation, and
149 quantifications of yield-related phenotypes. *Step 1* accepts raw NDVI images as gray-level imagery

150 datasets. As pixels with extremely high NDVI signals usually have overflowed intensity values (i.e.
151 black pixels in Fig. 2A), a pre-processing step (*Step 2*) is designed to calibrate raw NDVI images, so
152 that intensity distribution can be normalised and overflowing pixels can be corrected. In this step, an
153 algorithm called contrast limited adaptive histogram equalization (CLAHE)³⁶ is applied to increase
154 the contrast between the foreground (i.e. lettuces) and background (i.e. soils) in a given NDVI image
155 (Fig. 2B). [Additional File 1](#) provides pseudo code and explanations of this step.



156

157 **Figure 2: A high-level analysis workflow of AirSurf-Lettuce.**

158 (A) Step 1 accepts raw NDVI images as input imagery data (pixels with extremely high NDVI signals are
159 overflowed). (B) Step 2 pre-processes the raw NDVI images to calibrate intensity distribution and correct
160 overflowing pixels. (C&D) Step 3 carries out ML-based traits analyses to quantify lettuce number and classify
161 head size across a given NDVI image. (E) Steps 4&5 visualise and export statistics of the traits analyses
162 detection, including yield-related phenotypes such as lettuce counting, size distribution, and harvest regions, and
163 associated GPS coordinates.

164

165 *Step 3* carries out ML-based traits analyses that quantify lettuce number (Fig. 2C) as well as classify
166 head size (Fig. 2D). It includes six steps: removing noise signals, partitioning a given image into
167 sections (250 x 250 pixels) for local analysis, producing a sliding window (20 x 20 pixels) to traverse
168 within a sectioned image, using non-max suppression to detect lettuces, and classifying recognised

169 lettuces into three size categorises (i.e. small, medium and large). The analysis result is visualised in
170 *Step 4*, where lettuce counting, size distribution map, and GPS-tagged harvest regions are saved as
171 processed images ([Fig. 2E](#)). At the final step (*Step 5*), statistics of yield-related traits are saved in a
172 comma-separated values (CSV) file, including lettuce counts per field, lettuce size distribution, lettuce
173 number and size measures within GPS-based grids, harvest regions, and their associated GPS
174 coordinates ([Additional File 2](#)). To enable users to carry out the above analysis workflow, a GUI-
175 based software application has been developed ([Supplementary Fig. 1](#)).

176

177 *Data construction for model training and testing*

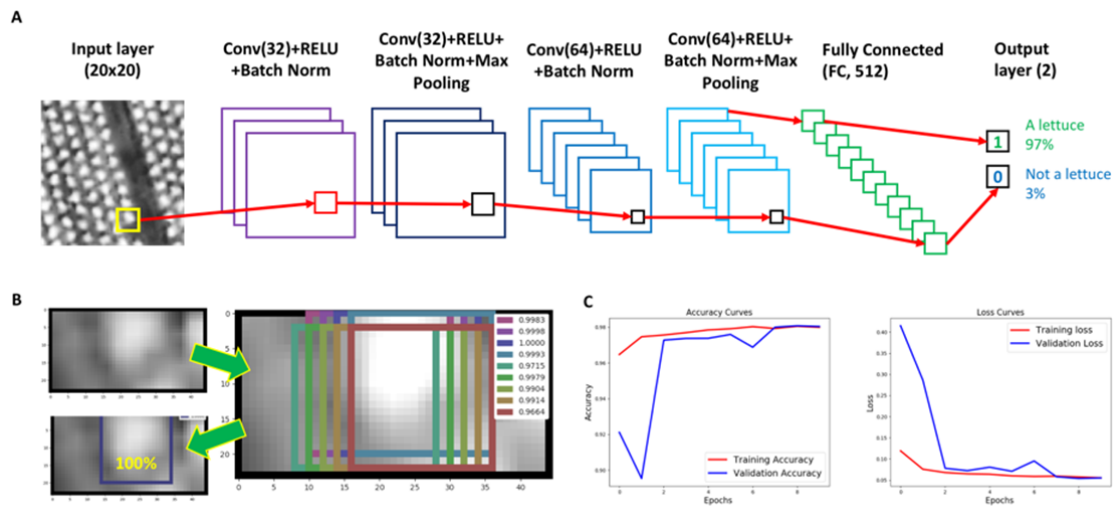
178 To generate a sound training and testing dataset for ML-based image analysis, we randomly selected
179 dozens of patches of a given field and manually labelled each lettuce in the patch with a red dot
180 ([Supplementary Fig. 2](#)). Then, each dot is identified by a bounding box, which is then used to build
181 the learning model. A training dataset with over 100,000 20x20-pixel labelled images has been
182 created, amongst which 50% are lettuces and the remaining are background signals such as soil, edges
183 of the field, and other non-lettuce objects. Following a standard CNN segmentation approach³⁷, we
184 designed a sliding window function to go through a given image to divide foreground and background
185 signals, splitting lettuce and non-lettuce objects. Training and testing datasets are equally balanced.
186 Validation sets are used alongside training sets to verify the performance of the model as well as to
187 prevent overfitting in model training, which is also an important step to allow us to fine-tune
188 hyperparameters of different learning layers³⁸.

189

190 *Neural network architecture*

191 Similar to AlexNet³⁹, a CNN-based learning architecture was established using the labelled datasets.
192 [Figure 3A](#) demonstrates the architecture of the CNN model, including (1) a convolutional (Conv2D)
193 layer with 32 filters and a 3x3 kernel, with a rectified linear unit (ReLU) as the activation function,
194 and batch normalisation to accelerate the learning process to enable higher learning rates⁴⁰; (2) the
195 same block is then repeated together with a max pooling layer to down-sample input using a 2x2

196 kernel based on the assumption that useful input features could be contained in sub-regions; (3) after
197 that, a second convolutional block is constructed, consisting of a Conv2D layer with 64 filters, a 3x3
198 kernel, a ReLU activation, and batch normalisation; (4) finally, this block is repeated, followed by
199 another max pooling layer (with a 2x2 kernel) to complete the learning procedure. After the
200 convolutional layers, layers are connected to a fully connected layer of size 512, which is followed by
201 a dropout layer with a 50% chance. To complete the learning architecture, a binary output generates
202 the probability of whether a given bounding box (20x20 pixels) contains a lettuce signal. If the
203 probability equals or is close to 1.0 (i.e. 100%), it indicates that it is highly likely that the bounding
204 box contains a complete lettuce head (Fig. 3B). The above architecture is commonly applied to
205 vision-based object detection problems⁴¹. The training and validation accuracy and loss curves are
206 reported in Figure 3C, showing that the model converges in only 10 epochs. To avoid overfitting, the
207 stopping criterion was designed to ensure that the validation accuracy is higher than the training
208 accuracy. By doing this, we can ensure the generalisation of the model. When training the CNN
209 model, the labelled data was also divided equally into train and validation sets.



210

211 **Figure 3: A CNN-based learning architecture established for lettuce counting.**

212 (A) The architecture of the trained CNN model, which generates a binary output representing the probability of
213 whether a yellow bounding box contains a lettuce signal. (B) If the probability is close to 1.0, it indicates that it
214 is highly likely that the bounding box encloses a lettuce. (C) The training and validation accuracy and loss
215 curves of the model.

216

217 *Phenotypic analysis of lettuce heads*

218 After a CNN classifier was trained, we used it to recognise and classify lettuce signals in ultra-large
219 NDVI images. The six-step approach discussed before (Fig. 2) is followed. However, during the
220 testing and implementation, we found that the CNN classifier could wrongly score lettuces as a lettuce
221 head is extremely tiny in an orthomosaic image (e.g. 11,330x6,600 pixels for a 7-hectare field when
222 GSD is 3cm, which can contain over half million lettuce heads). To resolve this issue, we have
223 designed a two-step approach: (1) sectioning the whole image into many 250x250 pixels sub-images,
224 and (2) using a fix-sized bounding-box (20x20 pixels) as a sliding window (with a stepping parameter
225 of 5 pixels to reduce the sliding calculation) to prune the detected lettuce objects in each sub-image.

226 Another reason that caused the CNN classifier's wrong detection is overlapped lettuce objects. Even
227 in a sub-image, overlapped lettuces could be detected repeatedly by the classifier. Hence, we
228 employed a non-maximum suppression (NMS) algorithm⁴² to rectify the detection result. NMS uses
229 probabilities to order the detected lettuce objects in a sub-image. After the sliding window function is
230 performed and many small patches have been identified in a sub-image, the NMS algorithm computes
231 an overlap coefficient to determine how to retain these patches. As lettuces are relatively well-spaced,
232 bounding boxes surrounding a complete lettuce signal will be retained, whereas partially covered
233 signals will be removed. To select the best overlap parameter for the NMS, a gradient descent method
234 is formulated. [Additional File 3](#) explains the NMS algorithm and its implementation in AirSurf-L.

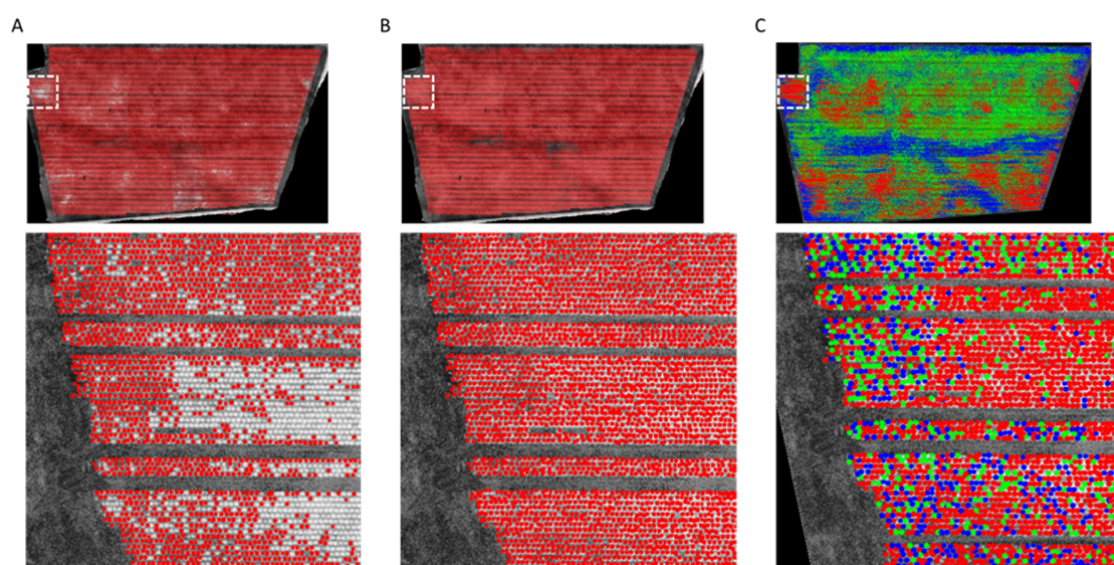
235

236 *Results improvement and size categorisation*

237 Initially, the training data selected was chosen randomly across the field. Using the data, AirSurf-L
238 can capture a broad range of sizes and orientations of lettuces with varying intensities. However,
239 when applying the initial CNN model, it failed to recognise lettuces in very bright regions and overly
240 count lettuces in very dark regions (e.g. approximately 50,000 lettuces were wrongly detected in the
241 one-million-head field, Fig. 4A). To resolve this issue, we enhanced the training datasets by manually
242 labelling an additional 500 lettuce signals within very bright or very dark regions. Then, newly

243 labelled data was inserted into the training datasets to retrain the model through the online-learning
244 approach⁴³. The improved model (available on our GitHub repository, see Availability of supporting
245 data) was tested on different experimental fields again and has dramatically enhanced the detection
246 result (Fig. 4B).

247 Identified lettuces are individually analysed to determine their associated size category. The size
248 classification is based on intensity and contrast values enclosed by the 20x20 bounding boxes, which
249 is computed using the dot product of the histogram of pixel intensities and a weighted vector towards
250 more pixel-based contrast values (see Methods). The assumption of this design is that higher NDVI
251 signals likely correlate with higher vegetation indices⁴⁴, which indicates bigger lettuce heads. The
252 categorisation result of all lettuce heads is clustered into three size groups. Each lettuce is then
253 coloured with a predefined colour code (i.e. small is blue, medium is green, and large is red, see Fig.
254 4C).



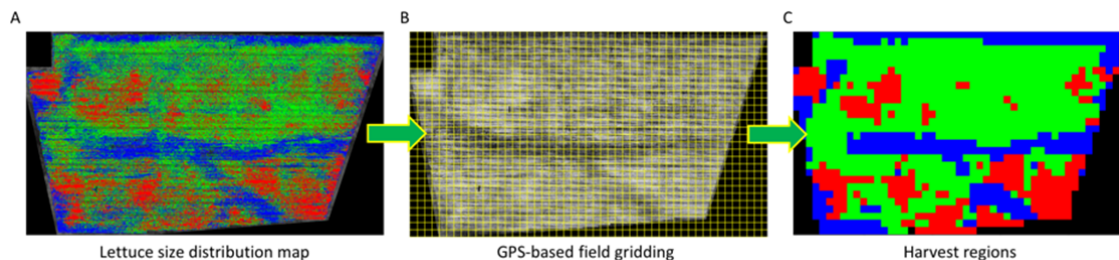
256 **Figure 4: The improved results of the CNN model and the size classification of lettuce heads.**

257 (A) Wrongly detected lettuces in very bright regions and overly counted lettuces in very dark regions, in a one-
258 million-head field. (B) Enhanced training datasets to retrain the model using the online-learning approach,
259 which led to much better detection results. (C) A predefined colour code (small is coloured blue, medium is
260 coloured green, and large is coloured red) is assigned to each recognised lettuce head across the field.

261

262 *GPS- tagged harvest regions*

263 The final phase of the phenotypic analysis is to define harvest regions based on colour-coded
264 lettuces. Using the size distribution map (Fig. 5A), the field is firstly segmented into many small grids
265 based on the optimal GPS resolution determined by the altitude of aerial imagery (3cm GSD, in our
266 case) as well as the size of the harvester machinery used by the grower. After dividing the field into
267 thousands of grids (Fig. 5B), GPS coordinates of each grid are recorded and each grid is coloured
268 with the most representative lettuce size category. By combining all coloured grids, a GPS-tagged
269 harvest map is produced, representing harvest regions of the field (Fig. 5C). The harvest map is then
270 used for designing harvesting strategies such as guiding a harvester to collect desired sized lettuces or
271 arranging logistics based on the lettuce number and size counting. To facilitate agricultural practices,
272 a result file (in .csv format, Additional File 2) is generated by AirSurf-L at the end of the analysis,
273 containing information of each harvest region, the associated GPS location, lettuce size, lettuce counts,
274 and the location in the field. To satisfy different needs for dissimilar requirements, the size of GPS-
275 based harvest grids can be modified manually in the AirSurf-L software.



276

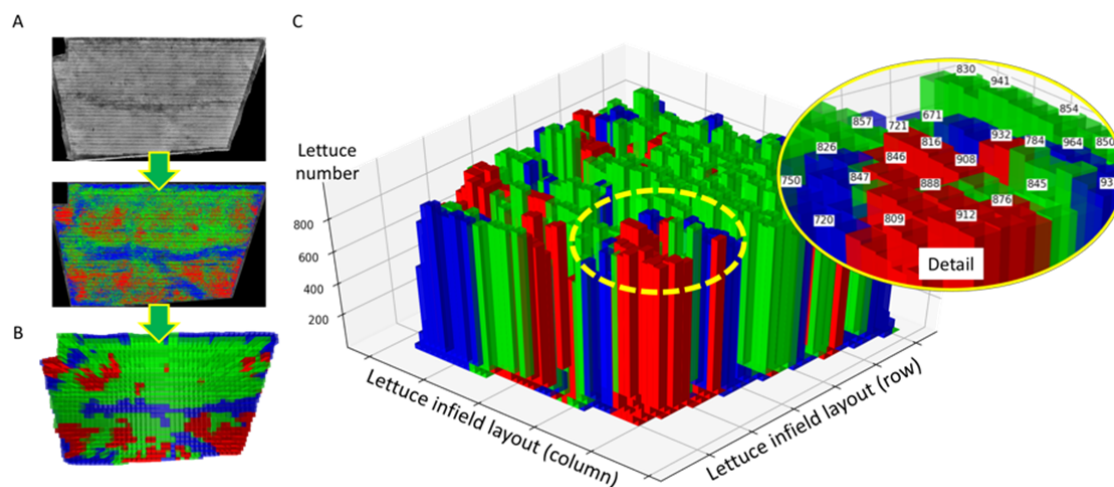
277 **Figure 5: A GPS-based harvest map based on lettuce size classification.**

278 (A) A colour-coded lettuce size distribution map. (B) The field is segmented into thousands of grids based on
279 the optimal GPS resolution and the size of the harvester machinery. (C) Grids are coloured with the most
280 representative lettuce size category across the image, representing harvest regions of the whole field.

281

282 Figure 6 uses Python-based 3D Matplotlib library⁴⁵ to show the GPS-tagged harvest map. When
283 AirSurf-L reads an NDVI image, it first computes the number of lettuce heads and associated size
284 categories on the image (Fig. 6A). Then, by 3D visualising the relationship of GPS-based field grids,
285 the number of lettuces in the grid, and the representative size category (Fig. 6B), a dynamic 3D bar

286 chart is generated to present lettuce number using the z axis, infield layout (both columns and rows)
287 using both x and y axes, and the representative lettuce size using the predefined colours (Fig. 6C).
288 Through the 3D plot, users can zoom into one sub-region of the field to check detailed yield-related
289 traits within each infield grid and plan harvesting strategies accordingly.



290

291 **Figure 6: 3D visualisation of lettuce harvest regions.**

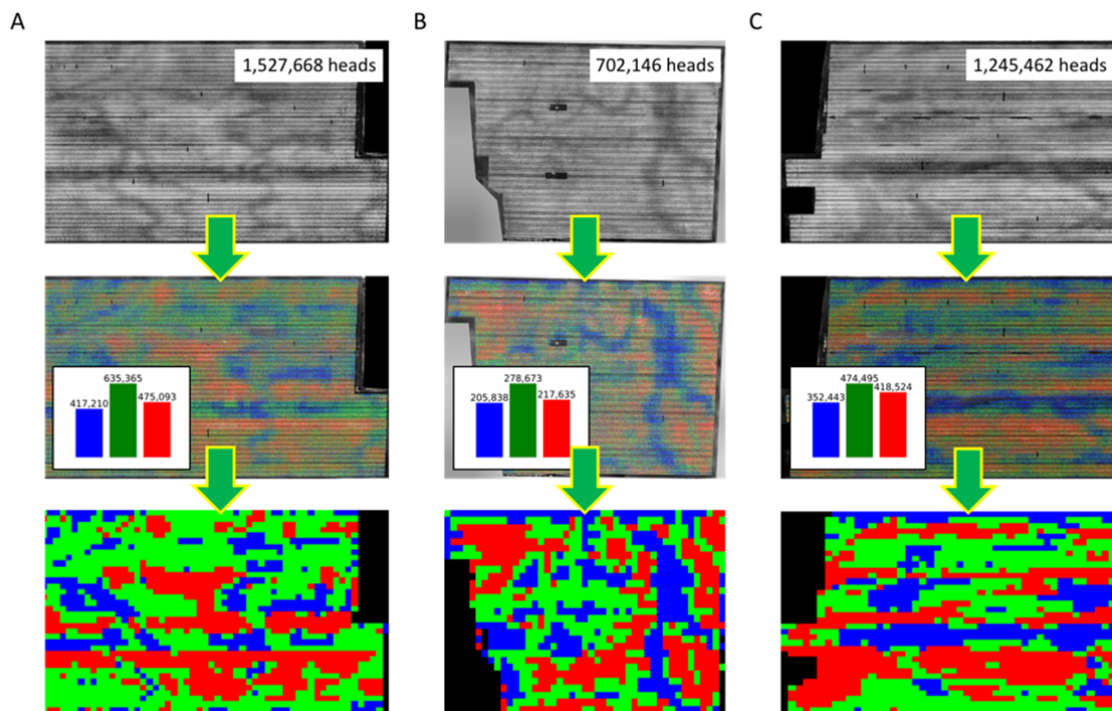
292 (A) AirSurf-L reads an NDVI image and exports a lettuce size distribution map. (B) 3D visualising GPS-based
293 field grids to represent the number of lettuces, representative size categories. (C) A dynamic 3D bar chart is
294 generated to present the relationship between lettuce number, infield layout, and the representative lettuce size.

295

296 *Validation of the platform*

297 To verify AirSurf-L and the generalisation of the learning model, we have applied the platform to
298 count and classify lettuce heads in three unseen experimental fields in Cambridgeshire, UK (Figs. 7A-
299 C). These fields contain around 700,000-1,500,000 lettuces and are located in different sites around
300 the county. Traits such as the number of lettuces per field and associated size categorisation quantified
301 by the platform were compared with industrial estimates, showing a highly correlated phenotypic
302 analysis (<5% difference). Besides the field-level comparison, we also randomly selected different
303 sizes of subsections in a given experiment field to evaluate AirSurf-L more precisely. We split these
304 subsections into two sets (i.e. 36 small regions and 21 large regions), where the small regions have
305 less than 400 lettuces and the large ones contain greater than 900 lettuces heads. After that, laboratory

306 technicians manually counted lettuce heads within these regions. The correlation between the manual
307 and AirSurf-L counting shows that, for the small regions, the difference between the human and
308 automatic counting is approximately 2%; for the large regions, the average difference is around 0.8%.
309 [Supplementary Figure 3](#) reports the strong correlations ($R^2 = 0.98$) between human and automatic
310 counting in both regions.



312 **Figure 7: Applying AirSurf-Lettuce to count and classify millions of lettuce heads in three plantation**
313 **fields across the Cambridgeshire, UK.**

314 (A-C) AirSurf-Lettuce is applied to count and classify millions of lettuce heads in three plantation fields in the
315 Cambridgeshire, UK.

316

317 **Discussion**

318 Traditionally, measuring infield crops on a large scale is very time-consuming and labour-intensive.
319 It often requires destructive techniques, potentially error-prone manual counting, or estimates of traits
320 that are key to yield production or crop quality⁴⁶. Recent advances in machine learning (including
321 deep learning) and computer vision (CV) based techniques have led to an explosion of phenotypic
322 analysis that is rapidly improving our abilities to mine phenotypic information from large and

323 complicated phenotyping datasets⁴⁷⁻⁴⁹. New data-driven analytic approaches are also changing plant
324 phenomics research – collecting big data (i.e. phenotyping) is no longer the bottleneck, instead how to
325 extract biologically relevant information (i.e. phenotypic analysis) from big data has become the
326 current challenge⁵⁰⁻⁵². Hence, along with the development of aerial imaging and remote sensing
327 technologies, it has become increasingly noticeable that the integration of scalable data collection,
328 high-throughput phenotypic analysis, and predictive modelling are key to crop research and precision
329 agriculture⁵²⁻⁵⁴.

330

331 *A combined research effort*

332 AirSurf-L addresses a specific challenge in ultra-scale field phenotyping and precision agricultural
333 practices through combining aerial NDVI imagery, CV, ML, and modular software engineering, with
334 commercial lettuce production. The software platform automates the measurement of millions of
335 lettuces in the field and our industrial partner has contributed key ideas of how to connect research-
336 based phenotypic analysis with real-world agriculture problems. As a cross-disciplinary project, our
337 project collaborators came from different backgrounds and hence many efforts were made on
338 understanding the requirements at the project initiation phase. Also, the academia-industry project
339 setting required a more agile R&D progress, because computational technologies and industrial
340 requirements were constantly changing while the project was still ongoing. From the project
341 development, one of the most valuable lessons we learned is that requirements and implementation
342 are unlikely to be clarified at the beginning and more efforts shall be made towards mutual
343 understanding. Similar to the case reported previously⁵⁵, all project parties need to be adaptive with
344 changeable requirements due to the dynamic nature of such a project; additionally, a successful
345 integration of project stakeholders requires all parties to manage expectation, mutual trust, and, more
346 importantly, clear communication channels.

347

348 *Machine learning in plant phenomics*

349 Another aim of this work is to further ML- and CV-based software solutions in plant phenomics.
350 High-throughput plant phenotyping is a fast-growing research domain, covering many disciplines,
351 from plant breeding, cultivation, remote sensing, to computing sciences^{56,57}. The modulated software
352 development allows us to apply different open-source learning architectures⁵⁸ (e.g. Scikit-Learn and
353 the TensorFlow frameworks) and CV algorithms^{59,60} (e.g. OpenCV and Scikit-Image libraries), when
354 implementing AirSurf-L. Notably, it is worth pointing out that ML is *not* a silver bullet for phenotypic
355 analysis, because: (1) learning algorithms could perform badly if training datasets are not well-
356 labelled; and, (2) although ML performs well in segmentation and classification if target objects are
357 well-defined, there is still a big gap between object recognition and traits analyses. Meaningful
358 phenotypic analysis not only requires sufficient biological understanding to define target traits in a
359 logical manner, but also needs bespoke algorithms to engineer features so traits can be soundly
360 extracted. Hence, biological questions, CV, data analysis, and software engineering shall be
361 considered collectively with ML techniques when resolving plant phenomics problems.

362

363 *Limitations of the platform*

364 Besides the high-accurate phenotypic analysis results presented in this article, there are still
365 limitations of the platform need to be considered: (1) AirSurf-L has been tested with top-view iceberg
366 lettuces mainly at H1 and H2 stages, which means that analysis error could increase if there are too
367 many overlaps between lettuce heads, e.g. from H3 stage onwards. (2) As AirSurf-L has only been
368 tested with NDVI imagery, it is therefore important to add new functions to the platform to
369 incorporate other vegetation indices measured through multi- and hyper-spectrum imaging sensors. (3)
370 As precision agriculture management decisions are normally based on imagery, soil and climate
371 conditions, AirSurf's results will be more reliable, if soil and climate data can be integrated in the
372 analysis. (4) The method was tested and validated in lettuce fields in a number of geographic
373 locations following a standard aerial imaging procedure, data collected from different sites via varied

374 aerial imaging strategies (e.g. different angles, altitudes and GSD) could improve the soundness and
375 compatibility of the platform.

376

377 *Prospects for crop research and precision agriculture*

378 The open-source software development opens up the potential for computational biologists to
379 include new learning models and analytic functions for other staple crops such as wheat and rice. For
380 example, the plant density of wheat is closely related to the yield due to its influences on the
381 allocation of water, light and fertilisers; however, it is not feasible to quantify the plant density solely
382 using ground-based RGB imagery⁶¹. Hence, utilising the ultra-scale NDVI aerial imagery and object
383 recognition methods embedded in AirSurf-L, it is likely possible to quantify wheat plants at the
384 emergence stage in different farming sites, which not only can benefit the assessment of sowing
385 performance, emergence rate, and plant distribution, but also will help breeders and cultivation
386 researchers make early predictions of the grain yield of different wheat genotypes in field experiments.

387 From a precision agriculture perspective, monitoring individual plant such as a lettuce head will
388 enable accurate monitoring of crops during key growth stages across a plantation site. It can provide
389 growers with the real number of crops in the field, based on which yield for harvest availability and
390 management plans can be quantified instead of estimated. The calculation of infield crops will also
391 lead to more accurate agricultural inputs, facilitating automated variable-rate application of fertiliser,
392 weed control, and pesticides through tractor software system with a precise crop distribution map⁶².
393 More interestingly, the close monitoring of key yield-related traits can also be used to guide farmers
394 and growers to reduce variability of agrichemical applications and irrigation in different fields,
395 leading to increased harvest yield and better operating profit margin⁶³. Finally, the AirSurf-L
396 approach fits in the cost-effective category in precision agriculture. The platform utilises existing
397 aerial imagery data routinely performed by the growers and farmers, which means that no extra data
398 collection cost is required by this approach and hence the adoptability of the technology, an important
399 factor for new Agri-Tech solutions to be accepted by the Agri-Food sector⁵³.

400

401 **Conclusions**

402 *AirSurf-Lettuce* automatically measures infield iceberg lettuces using ultra-scale NDVI aerial images,
403 with a focus on yield-related traits such as lettuce number, size categories, field size distribution, and
404 GPS-tagged harvest regions. The analysis results are close to the manual and industrial counting and
405 can be used to improve existing crop measures and estimates. By monitoring millions of lettuces in
406 the field, we demonstrate the significant value of *AirSurf-L* in ultra-scale field phenotyping, lettuce
407 size distribution mapping, precise harvest strategies, and marketability estimates before harvesting.
408 We believe that our algorithm design, software implementation, lessons learned from applying ML-
409 and CV-based algorithms, and the academic-industrial R&D activities will be highly valuable for
410 future plant phenomics research projects that are destined to be dynamic and cross-disciplinary.
411 Finally, with continuous development work, we are confident that the analytic platform is likely to
412 support the Agri-Food sector with a smarter and more precise crop surveillance approach of vegetable
413 crops and therefore lead to better precision agriculture management decisions.

414

415 **Methods**

416 *Ultra-large field NDVI imagery and experimental fields*

417 The NDVI imaging sensor used is an industrial standard camera described previously³⁵. The aerial
418 imaging was carried out by a 'Sky Arrow' light aircraft, the lightest weight class (Very Light Aircraft,
419 VLA) of any commercial aircraft that is allowed for commercial work. The VLA let the pilot to fly
420 with very little fuel, less than an average farm vehicle while driving around the crops. Using VLA at
421 1000 feet (around 305 metres) in the sky, vast areas can be covered at a flight speed 180-200 km/hour.
422 The NDVI sensor can gather ultra-large crop imagery datasets at very low operating costs, as the
423 VLA can carry 45 kilograms of payload to cover four or five fields in a single flight. This aerial
424 imaging approach can also be used to understand the spectral changes associated with key disease
425 conditions. The NDVI lettuce signals used in this study were captured at H1 or H2 growth stage
426 (moderately compact and crushable head, when lettuce leaves are not largely overlapping with
427 neighbours). The experimental fields are operated by G's Growers near Ely UK, ranging from 10 to

428 20 hectares with at least 0.5 million lettuce heads in a single field. A rough manual yield estimate was
429 produced by specialists during the harvest, which were used for testing and improving AirSurf-L.

430

431 *Dataset preparation*

432 To generate a sound dataset for machine learning-based image analysis, we randomly selected 60
433 patches of the field of varying sizes, each containing between 300 and 1,000 individual lettuce heads,
434 and manually labelled each lettuce in the selected patches. Each labelled lettuce is extracted as a
435 20x20 pixel image representing a single lettuce head. We then used these images, along with images
436 that did not correspond to a lettuce head, to train a CNN classifier to recognize and separate potential
437 lettuces from the ultra-large field images. The pixels contained within the potential lettuces were used
438 for further phenotypic analysis of lettuce size. To format the manually labelled dataset for building the
439 model, we created another training dataset with over 100,000 20x20 pixel images, among which 50%
440 are lettuces and 50% are background signals. The background images were selected using regions
441 other than the labelled lettuces across the field together with a non-overlapping sliding window
442 function to extract background patches. These images are then split into two equally balanced training
443 and validation sets.

444

445 *Construction of deep neural network architecture*

446 We built our deep neural network based on the architecture of AlexNet. We used a shallower
447 architecture as opposed to AlexNet and other modern deeper architectures for several reasons: (1) the
448 size of our dataset is relatively small for deep learning studies, where larger and deeper networks tend
449 to require bigger training datasets; (2) additionally, ours is only a binary classification problem as
450 opposed to the ImageNet classification task; (3) larger neural networks often require more time to
451 train, which can be slower to execute and not feasible to train the model without specialised hardware
452 such as GPUs. In our case, we wanted a relatively simple, but powerful model that could execute in a
453 broad range of environments and in a timely manner.

454 Like AlexNet, we used rectified linear units (ReLU) as our activation function, which is now a
455 common standard in CNNs. This is because ReLU reduces the vanishing gradient problem¹⁹. After
456 each convolutional layer, we also perform batch normalisation. This reduces the covariance shift,
457 which helps ensure that the model generalises well and the network converges faster. Finally, we
458 included two max pooling layers to reduce the problem into smaller samples. Other architectures
459 might use more max pooling layers, but our input images were segmented and hence quite small. In
460 order to avoid too much information loss from the training procedure, we trained the CNN on our
461 datasets until the validation accuracy was greater than the training accuracy. The training and
462 validation accuracy and loss are reported in [Figure 3](#), where it is shown that the model converged in
463 only 10 epochs. More importantly, to avoid over fitting, the stopping criterion was set for when the
464 validation accuracy is higher than the train accuracy.

465

466 *Size categorisation*

467 After AirSurf-L identifies a list of square pixel patches containing single lettuces, it is important to
468 perform automatic unsupervised size categorisation. Lettuce sizing in this work is split into three size
469 categories: small, medium and large; however, the method can be easily changed to classify more size
470 categories. The pixel regions are extracted from the image and then NDVI values are put into bins
471 with similar pixel values. Originally, the histogram included 10 bins that are evenly spread across the
472 value range, i.e. 0-255. However, treating all pixel values equally performed poorly in practice. We
473 therefore included two important aspects in the size categorisation. Firstly, the lower NDVI
474 surrounding value does not determine the actual size of the lettuce; secondly, the higher NDVI values
475 are much more important in indicating size. As such, we created a geometric pattern of cut-off values
476 for each bin. These were: 64, 128, 160, 192, 208, 224, 232, 240, 244, 248, 250, 252, 253, and 254.
477 With these cut-off values, most of the background pixels were captured in the first two bins, with
478 increasing granularity as the values approached the maximum of 255.

479 Having transformed the pixel regions into a series of histogram count vectors, we were able to
480 compare regions and cluster the patches into groups. The count vectors are grouped into three distinct

481 sizes by using k-means clustering with k set to three. They are sorted into size order through
482 calculating the dot product between the weight vector and the cluster centres count vector. These
483 sorted values then determine which cluster corresponds to which size, and subsequently, applies to
484 each lettuce. Three colours are used to indicate size categories: blue for small, green for medium, and
485 red for large.

486

487 *Common Pitfalls*

488 The CNN trained on a set of approximately 100,000 images. Despite the reported training and
489 validation accuracies being quite high, in practice the network performed poorly because it could not
490 distinguish lettuces in patches where most lettuces appear particularly bright. As the initial training
491 datasets were chosen randomly, not enough representative samples from extreme regions were
492 selected during the training. Without sufficient training data, the network was undercounting by 5% in
493 large fields. To solve this problem, we manually labelled further 500 lettuces and added them to the
494 training dataset. The neural network was retrained and converged faster than the previous iteration.
495 The algorithm was updated with the new model with improved results. The above training issue could
496 be a common pitfall for many deep-learning analytic solutions, because key features were constructed
497 by learning algorithms instead of engineered. Many learning models were vulnerable when facing up
498 to totally undefined datasets.

499

500 **Availability and requirements**

501 Project name: AirSurf-Lettuce with G's Growers

502 Project home page: <https://github.com/Crop-Phenomics-Group/Airsurf-Lettuce>

503 Operating system(s): platform independent

504 Programming language: Python 3

505 Requirements: Packaged for both Mac and Windows

506 License: BSD-3-Clause available at <https://opensource.org/licenses/BSD-3-Clause>

507

508 **Abbreviations**

509 Comma-separated values (CSV), computer vision (CV), convolutional neural networks (CNNs), deep
510 learning (DL), global positioning system (GPS), ground sample distance (GSD), machine learning
511 (ML), non-maximum suppression algorithm (NMS), normalized difference vegetation index (NDVI),
512 rectified linear units (ReLU), the United Kingdom (UK), and Unmanned Aerial Vehicles (UAVs).

513

514 **Availability of supporting data**

515 The datasets supporting the results presented here is available at [https://github.com/Crop-Phenomics-](https://github.com/Crop-Phenomics-Group/Airsurf-Lettuce/releases)
516 [Group/Airsurf-Lettuce/releases](https://github.com/Crop-Phenomics-Group/Airsurf-Lettuce/releases). Source code and other supporting data are also openly available in
517 the GitHub repository.

518

519 **Author contributions**

520 J.Z., A.B., A.G.B. and J.K. wrote the manuscript, S.M.R. performed the NDVI imaging. J.K. provided
521 harvest information and biological expertise. J.Z., A.G.B., and C.A. designed the analysis algorithms.
522 A.B., A.G.B., C.A. and J.Z. developed and implemented the core algorithms. A.B. and A.G.B. built
523 the deep learning models for AirSurf-L. J.B. packaged the GUI executables. J.Z., A.B., A.G.B., C.A.,
524 S.L. and J.B. tested the software. J.Z., A.G.B and A.B. performed the data analysis. All authors read
525 and approved the final manuscript.

526

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540

541 **Competing interests**

542 The authors declare no competing financial interests.

543

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676

677 **Figures**

678 **Figure 1: Ultra-large NDVI aerial imaging accomplished routinely through a fixed-wing light**
679 **aircraft operated by G’s Growers.**

680 **(A)** The flying route and aerial imaging were designed to facilitate cross-site crop layout assessment
681 and yield prediction. **(B)** A series of ultra-large NDVI images at 3cm GSD spatial resolution were
682 acquired to record 0.8-1.6 million lettuce heads per field, at H1 and H2 stages.

683

684 **Figure 2: A high-level analysis workflow of AirSurf-Lettuce.**

685 (A) Step 1 accepts raw NDVI images as input imagery data (pixels with extremely high NDVI signals
686 are overflowed). (B) Step 2 pre-processes the raw NDVI images to calibrate intensity distribution and
687 correct overflowing pixels. (C&D) Step 3 carries out ML-based traits analyses to quantify lettuce
688 number and classify head size across a given NDVI image. (E) Steps 4&5 visualise and export
689 statistics of the traits analyses detection, including yield-related phenotypes such as lettuce counting,
690 size distribution, and harvest regions, and associated GPS coordinates.

691

692 **Figure 3: A CNN-based learning architecture established for lettuce counting.**

693 (A) The architecture of the trained CNN model, which generates a binary output representing the
694 probability of whether a yellow bounding box contains a lettuce signal. (B) If the probability is close
695 to 1.0, it indicates that it is highly likely that the bounding box encloses a lettuce. (C) The training and
696 validation accuracy and loss curves of the model.

697

698 **Figure 4: The improved results of the CNN model and the size classification of lettuce heads.**

699 (A) Wrongly detected lettuces in very bright regions and overly counted lettuces in very dark regions,
700 in a one-million-head field. (B) Enhanced training datasets to retrain the model using the online-
701 learning approach, which led to much better detection results. (C) A predefined colour code (small is
702 coloured blue, medium is coloured green, and large is coloured red) is assigned to each recognised
703 lettuce head across the field.

704

705 **Figure 5: A GPS-based harvest map based on lettuce size classification.**

706 (A) A colour-coded lettuce size distribution map. (B) The field is segmented into thousands of grids
707 based on the optimal GPS resolution and the size of the harvester machinery. (C) Grids are coloured
708 with the most representative lettuce size category across the image, representing harvest regions of the
709 whole field.

710

711 **Figure 6: 3D visualisation of lettuce harvest regions.**

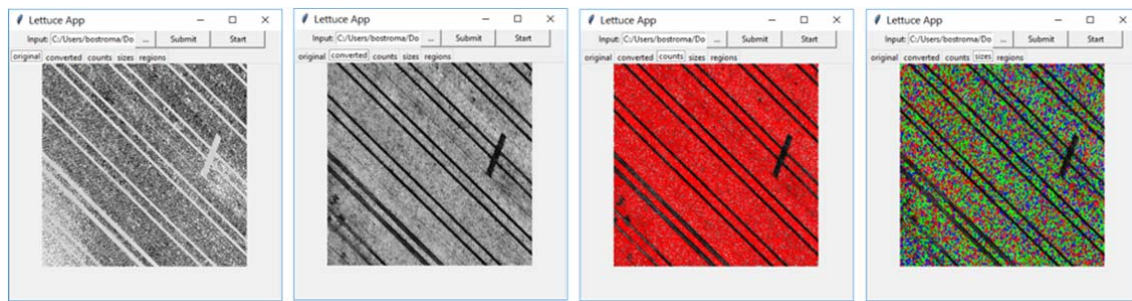
712 (A) AirSurf-L reads an NDVI image and exports a lettuce size distribution map. (B) 3D visualising
713 GPS-based field grids to represent the number of lettuces, representative size categories. (C) A
714 dynamic 3D bar chart is generated to present the relationship between lettuce number, infield layout,
715 and the representative lettuce size.

716

717 **Figure 7: Applying AirSurf-Lettuce to count and classify millions of lettuce heads in three**
718 **plantation fields across the Cambridgeshire, UK.**

719 (A-C) AirSurf-Lettuce is applied to count and classify millions of lettuce heads in three plantation
720 fields in the Cambridgeshire, UK.

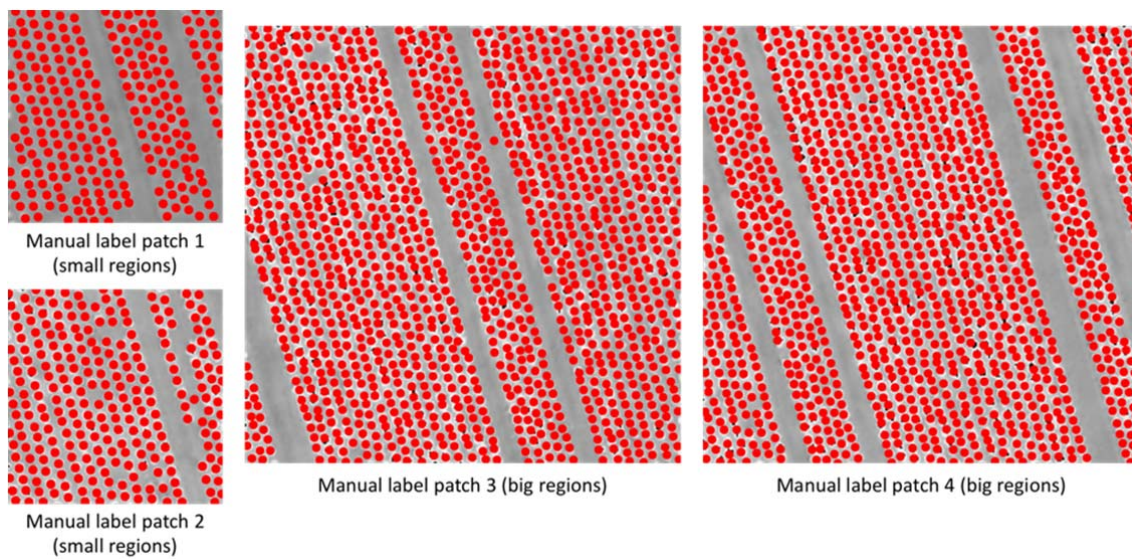
721



722

723 **Supplementary Figure 1: The GUI interface of AirSurf-Lettuce and the analysis workflow.**

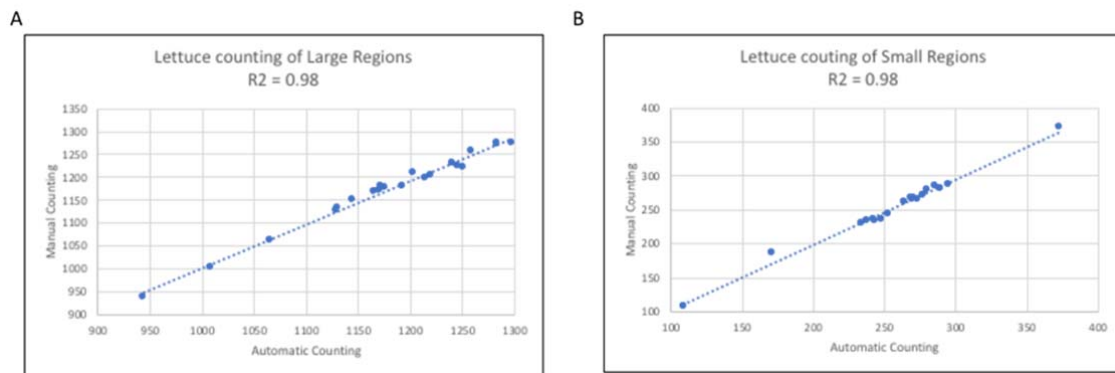
724



725

726 **Supplementary Figure 2: Manually labelled lettuces in randomly selected patches using red dots.**

727



728

729 **Supplementary Figure 3: The correlation between human counting and AirSurf-L scoring ($R^2 =$**

730 **0.98).**

731

A



B

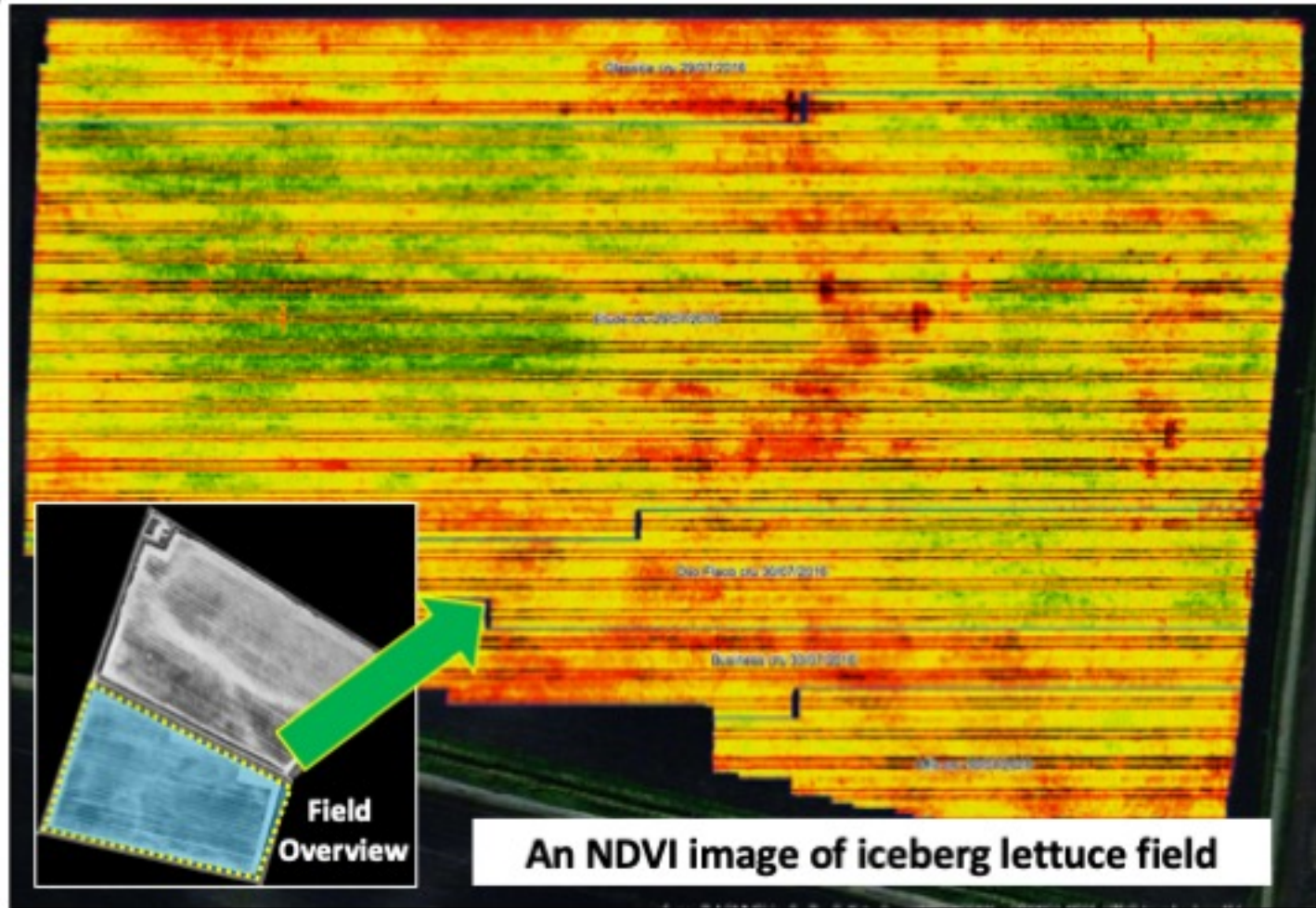
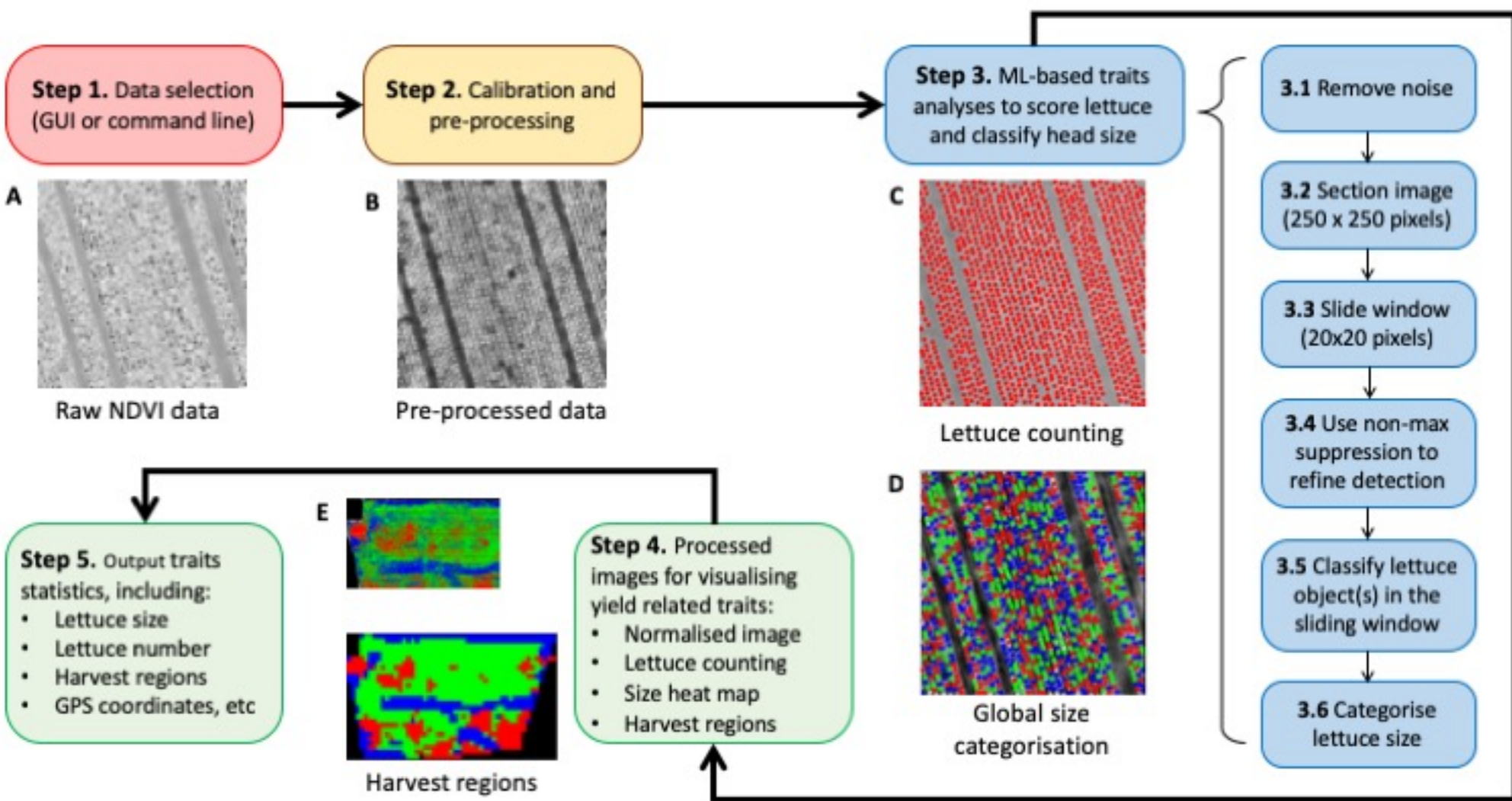
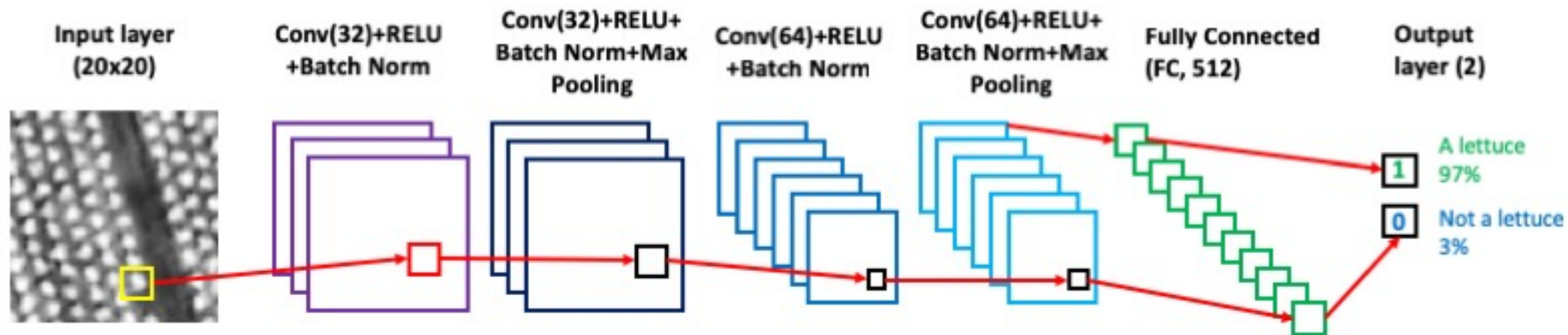


Fig. 1

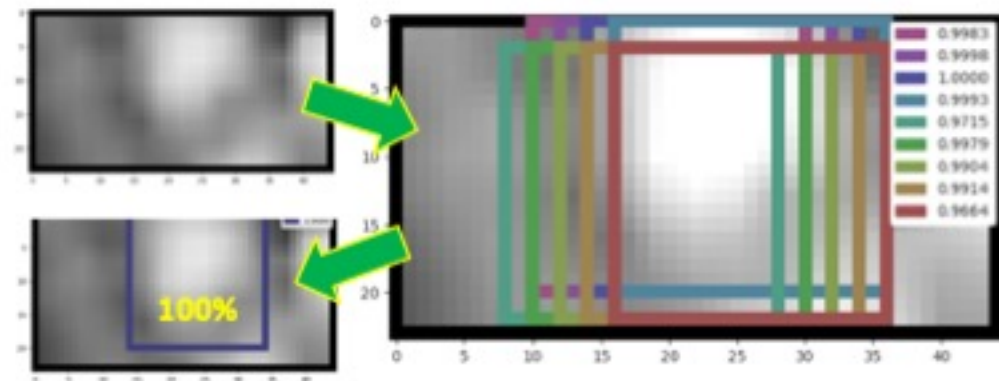
Fig. 2



A



B



C

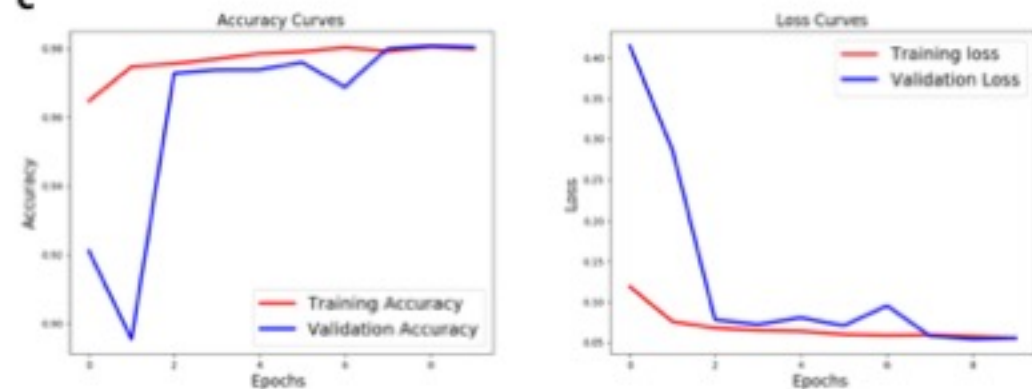
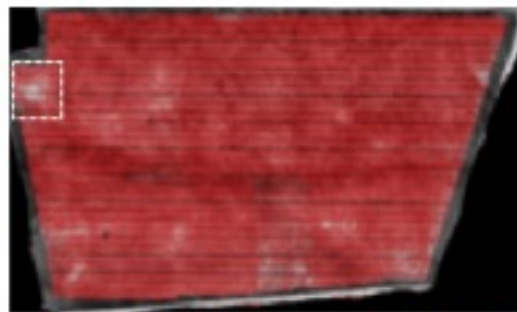
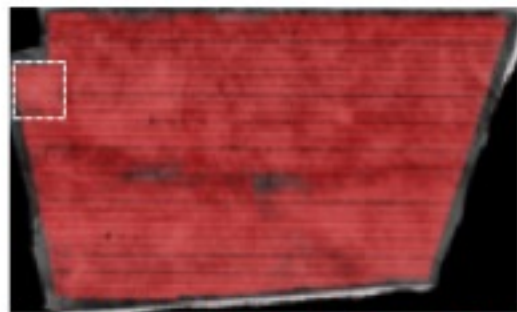
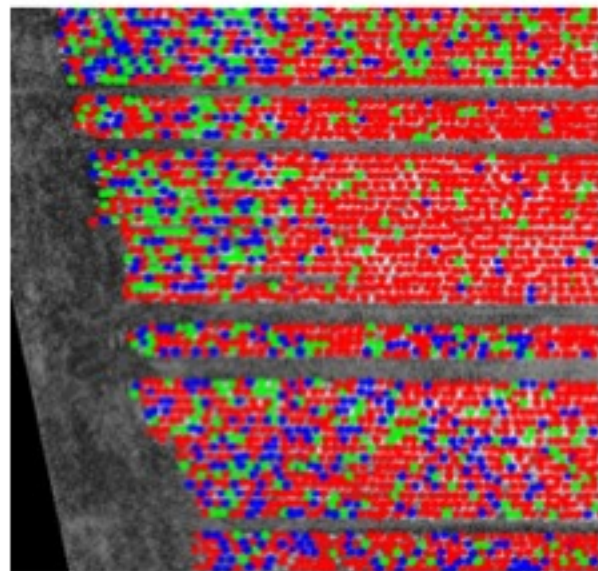
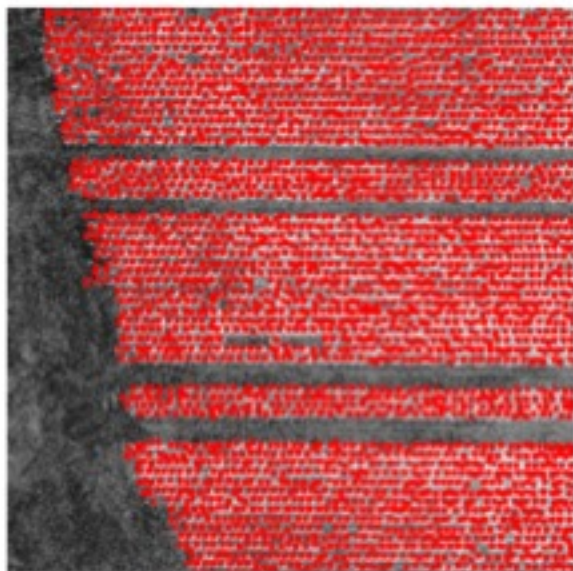
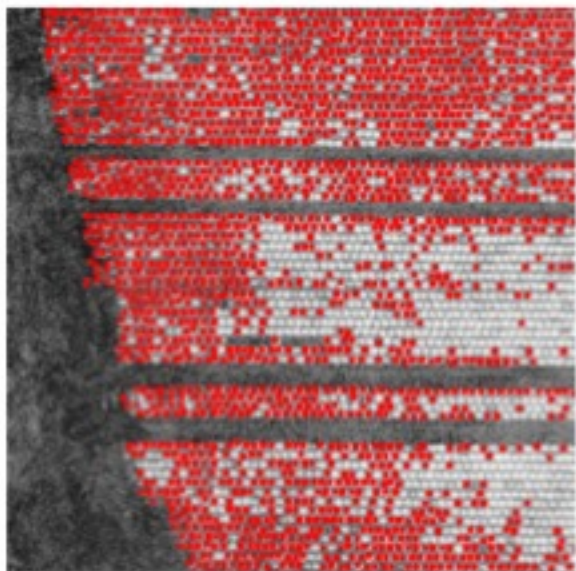
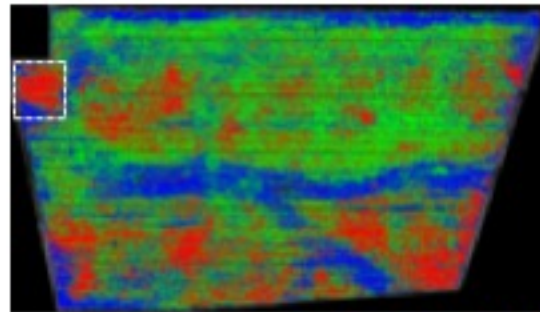
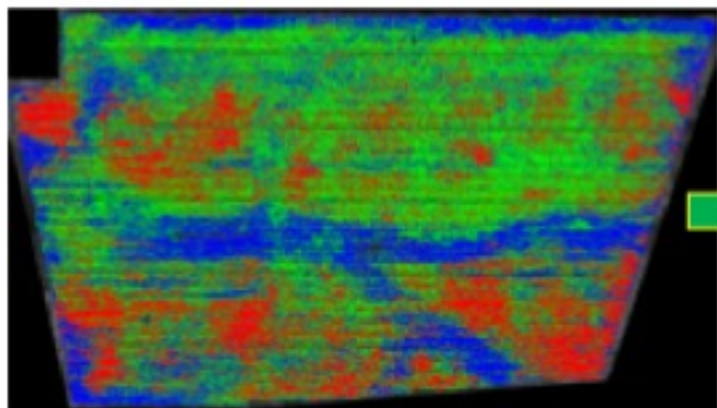


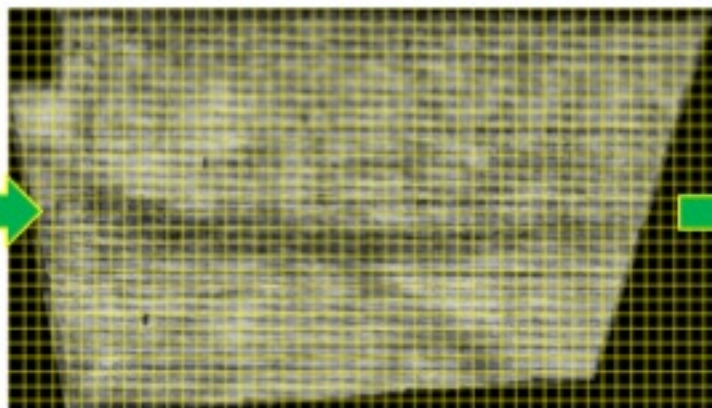
Fig.4**A****B****C**

A



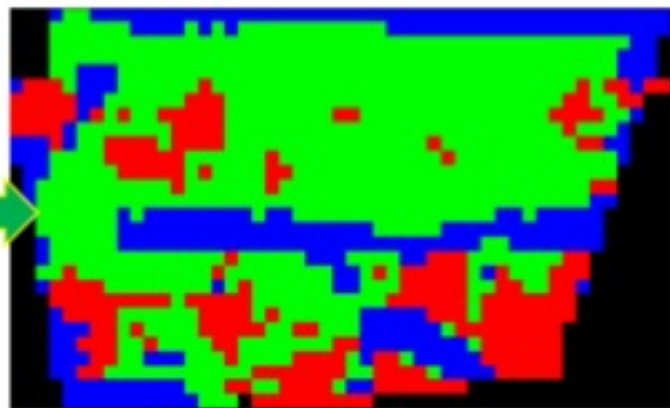
Lettuce size distribution map

B



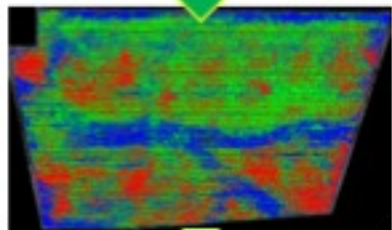
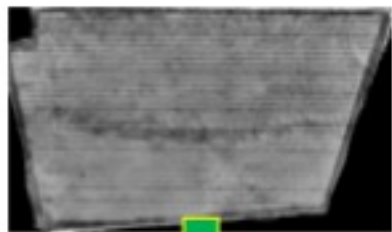
GPS-based field gridding

C

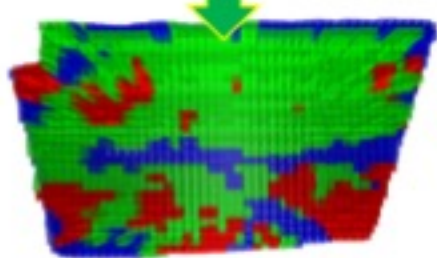


Harvest regions

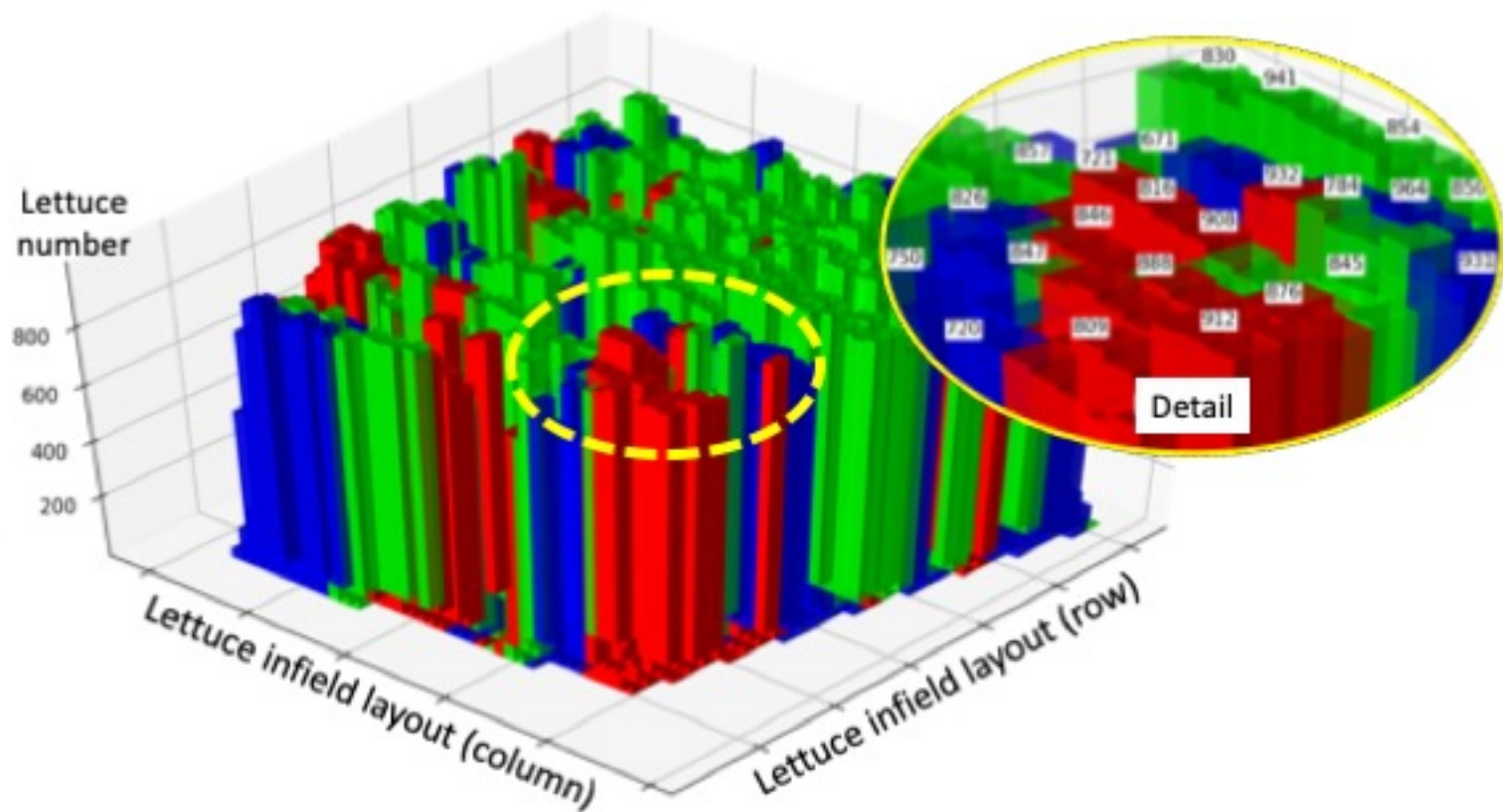
A



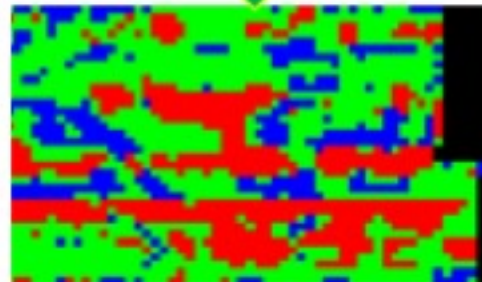
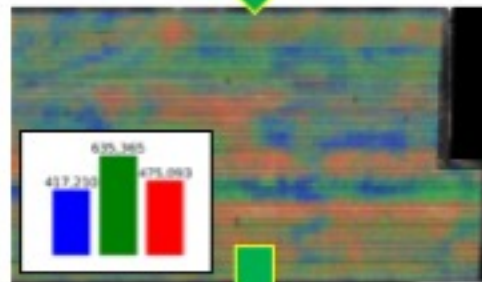
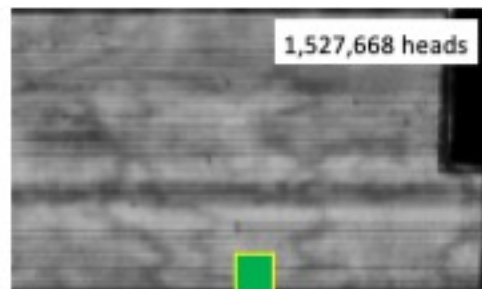
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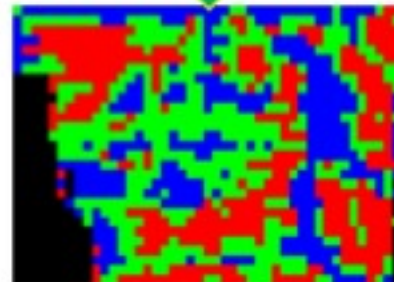
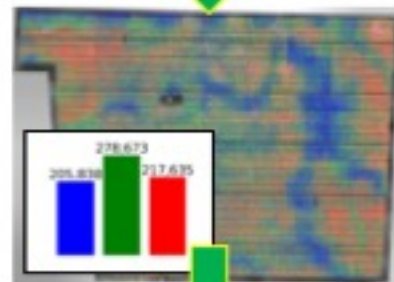
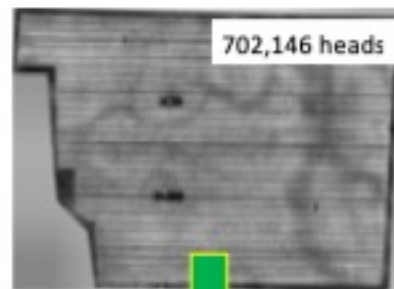
C



A



B



C

