Initial results of the development of intelligent non-invasive phenotyping of raspberries using machine learning and 3D imaging

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Abstract

To distinguish candidate cultivars in fruit breeding, the characteristics of several thousand seedlings must be described and evaluated, which is done visually. Fruit counting and measuring in the field is currently the most used method. However, it is tedious and time-consuming and requires abundant manpower. In addition, visual evaluation is relatively subjective, and results may vary from different evaluators. The solution is to develop a machine vision-based method to automate and to intensify the fruit breeding process. In Latvia, the fruit breeding programme focuses on four fruit crops, including raspberries. Inclusion of raspberries (*Rubus idaeus*) in the breeding programme is related to the fact that there are no cultivars suitable for Latvian agroclimatic conditions. Breeding experience shows that the selections have insufficient ecological phenotype plasticity, which is a risk for cultivation of these cultivars in Latvian climate. In raspberries, yield depends on the sum of yield components, and each component is evaluated separately. The most important yield components are: number of laterals per shoot, number of flowers/fruits per lateral shoot, and average fruit mass. With the evaluation of these traits, image-based approaches to raspberry phenotyping are gaining momentum and provide fertile ground for non-invasive raspberry detection and categorization. The objective of this research is to develop a methodology and tools for non-invasive phenotyping of raspberry yield components based on red, green, blue (RGB) image colour value and 3D images, as well as provide descriptive and inferential statistics of raspberry cultivars. We propose a manually annotated 2D raspberry data set with ground truth region of interest (ROI)classifying labelled into five classes: "Buds", "Flowers", "Unripe Berries", "Ripe Berries", and "Damaged Berries" to verify and evaluate the raspberry detection problem using real-time deep neural network Yolo5. We also present three algorithms for 3D computational image processing to create 3D bounding boxes for raspberry parametrizing.

Keywords: raspberry, germplasm, multivariate statistics, phenotypic characterization, machine learning, RGB and 3D imaging

INTRODUCTION

The experience of raspberry growing in Latvia shows that the biggest part of most popular cultivars do not have a sufficiently high ecological phenotype plasticity, which poses a risk for the cultivation of these cultivars in the Latvian climate. The Institute of Horticulture (LatHort) in Dobele, Latvia has more than 40 years of experience in breeding raspberries, including description and visual evaluation of their phenotypic traits. The yield of raspberries depends on the sum of yield components, each of which is evaluated individually. The most important components are: number of laterals per cane, number of flowers/fruits per lateral shoot, and average fruit mass. The latter depends on the number and mass of individual drupes they form. The quality of raspberries is determined not only by flavour, which can be assessed by sensory evaluation, but also by the fruit's resistance to bruising, its colour,

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glossiness or bloom, and the firmness of the drupes. Initially, all the parameters for raspberries are estimated visually on a rating scale of 1-9, where 1 is the lowest and 9 the highest. To focus the evaluation process on the quality characteristics and yield components of raspberries and to reduce the influence of the human factor, appropriate imaging and data analysis methods should be introduced. Similar studies exist with imaging techniques for citrus (López-García et al., 2010). After all these traits have been evaluated, the next task is to integrate the entire raspberry breeding process into a ML-based (machine learning-based), non-invasive phenotyping model that would allow more productive, accurate, and faster evaluation of raspberries.

Plant phenotyping has been an important area of research in plant breeding. Although molecular breeding strategies have increased the emphasis on selection based on genotypic information, the following phenotyping data are still needed, since: 1) phenotypes are used for selection and to shape a prediction model in genomic selection; 2) a single phenotyping cycle is used to identify markers for subsequent selection through generations within the marker-assisted recurrent selection; 3) phenotyping is necessary to identify promising events in transgenic studies (Jannink et al., 2010). Advances in phenotyping are essential to take advantage of developments in conventional, molecular, and transgenic breeding. To achieve this goal, phenotyping involves experts from biological sciences, computer science, mathematics, and engineering. Given the rapid development of plant genomic technologies, the lack of access to plant phenotyping capabilities limits the ability to dissect the genetics of quantitative traits that are influenced by the environment. Qualitative data are mainly used to diagnose highly heritable traits that are not affected by environmental variation. These traits are easy to evaluate, allow rapid discrimination between germplasm, and are generally regulated by a few major genes. Accurate quantitative phenotype data are essential in plant breeding programmes to evaluate genotype performance and make selection (He et al., 2017). Recent advances in ML and deep learning (DL) make these techniques specifically applicable to the development of a high-precision, high-accuracy phenotyping platforms. Visible and 3D imaging techniques can capture not only two-dimensional visible features but also the three-dimensional shape and texture of the fruit. These techniques are non-invasive and can be used while the fruit is still attached to the plant. They also have the potential to detect normally invisible conditions such as water content, frost damage, etc. These data collected from plants of interest can be enriched with environmental data from sensors such as air and soil temperature and moisture, light conditions, etc., and the data sets are prepared for training a deep neural network (DNN) model for phenotyping.

The objective of this research was to develop a non-invasive ML based approach and tools for phenotyping the yield components of raspberries using RGB and 3D hyperspectral cameras. This will benefit raspberry breeding not only in Latvia, but also in other countries where fruit breeding programs are being developed.

MATERIAL AND METHODS

Plant material

The raspberry images were taken in an orchard of LatHort in Dobele, located in the southern part of Latvia (GPS: 56.6323154; 23.3425648). Phenotypic characterization was performed on a representative part (*Rubus* genotypes) of the LatHort collection. The cultivar evaluation plot was established in autumn 2018 with one-year-old plants, with spacing of 0.5 m in the row and 3.5 m between rows. The cultivars and selections were grown in a randomized complete block design with 1-4 replications of 10 plants each, with drip irrigation, in 3×0.5 m plots. The plants were grown with five fruiting cane per plant. Each spring 300 kg ha^{-1} of complex fertilizer YaraMila CROPCARE NPK 11-11-21 (Yara) was applied along the rows. The mineral content of the soil in the plantation in year 2018 was the following: P-99 mg kg^{-1}, K-136 mg kg^{-1}, C_{org}-2.0%; the acidity of the soil was 7.3 pH_{KCl}. Plants were grown following sustainable fruit growing practices (Directive 2009/128/EC).

Characterization and evaluation of raspberry fruit genotypes and elements

Fourteen floricane raspberry genotypes were evaluated for their yield components (number of flowers and fruit laterals per cane, number of fruits per fruit lateral) on 10 plants per genotype. Fruit parameters (fruit length, fruit width, fruit shape, number of drupes) were evaluated by measuring and/or counting. Shape index (relation between the fruit length and fruit width) was used for the characterization of fruit shape. The mean fruit weight (g) and yield cane^{-1} (g) were determined by weighing.

Fruit elements: number of flower buds, number of unripe and ripe berries were recorded with an RGB camera, but such fruit parameters as size and texture were determined from the 3D image data using Zivid One + 3D camera. All algorithms were developed in Python's data processing environment.

Statistical analysis

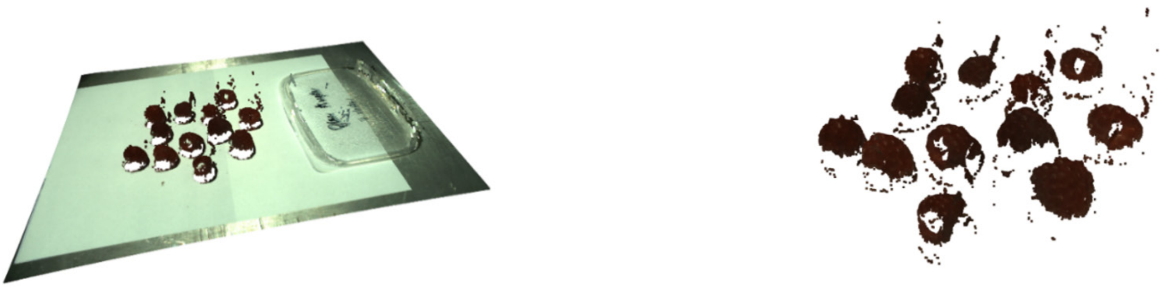
ANOVA was applied to evaluate the variability of traits: count fruit laterals per cane, berries per fruit lateral.

Characterization of raspberry fruits using 3D image data

Another focus of raspberry fruit characterization was the use of a 3D fruit phenotyping approach. To achieve this, the idea of object detection algorithm in 3D point clouds was developed. The main task was to detect and identify raspberries as an object. In this project, we used 3D bounding boxes for the result. The evaluation was done using three algorithms: *k*-nearest neighbours (KNN) algorithm (Cover and Hart, 1967), "imaginary square" algorithm and object recognition by using object projection approach (Guo et al., 2013). The 3D point clouds were created using the Zivid One + 3D camera.

KNN algorithm

KNN was used to detect colours. First, the KNN algorithm must be trained – one or more colour samples of the object must be presented as training data. Then the presented colours are classified in a 3D graph. At least one object class is created. Based on the training colours, the KNN algorithm tries to find the same or similar colours in the test point cloud. The simplest case is when the object has a monotone colour. If the object has many different colours describing this object, then all descriptive colours must be assigned to the object class. The main task of the KNN algorithm in this project was to remove the background and unwanted noise. As a result, only the object, in this case the raspberries, remains (Figure 1).



(a) (b)

Figure 1. Object detection with KNN algorithm, (a) before using KNN, (b) after KNN.

"Imaginary square" algorithm

In general, there can be more than one object pattern in a point cloud. All patterns must be detected separately. The "imaginary square" algorithm is developed to separate one object pattern from other patterns. The "imaginary square" algorithm is used after the KNN algorithm. The analyses of the "imaginary square" algorithm start at the maximum 'y' value (at the edge of the point cloud). The square is created at the maximum 'y' point. As the square gets larger and larger, more and more points are needed. After each step, the square gets larger and the number of points that are in that square also gets larger. If the number of points in the square in step ‘n+1’ is greater than in step ‘n’, then the square will continue to grow. Conversely, if the number of points in step ‘n+1’ remains the same, it means that a possible object has been detected. The next step is to analyse whether it is an object or not.

If the two objects are located close, to each other than square treats two objects as one. In this case, it makes sense to define the largest possible object. If the size of the square is larger than the largest possible object, there is no point in increasing the size of the square further. When the maximum size of the square is reached, there might be one or more object patterns in the square. In this case, this area can be analysed separately with another algorithm (Figure 2).

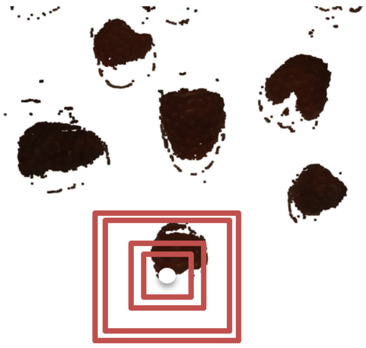


Figure 2. “Imaginary square” algorithm.

Object projection analysing

The third algorithm is the most precise algorithm, but it takes more time to detect an object than the “imaginary square” algorithm. The third algorithm uses the object projection on the base to analyse the possible object. To obtain the base projection of the object, the KNN algorithm is used a second time, but this time in reverse order. This time the objects are deleted, and the background is used. Using the background, it is possible to obtain outlier points of the base. The 3D camera that captures the patterns of the raspberries cannot capture what is behind them. When the berries are deleted from the point cloud, there is empty space under the berries – holes in the base. In this case the holes can be interpreted as projections of the object onto the base. Outlier points are the outlines of the object projection (Figure 3).

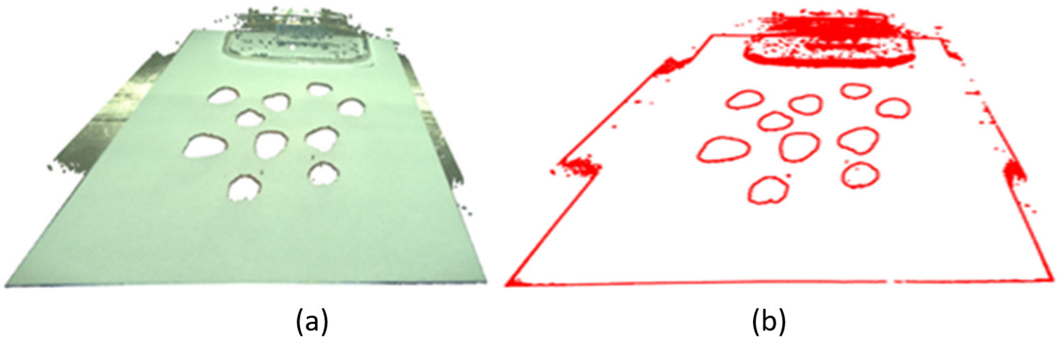
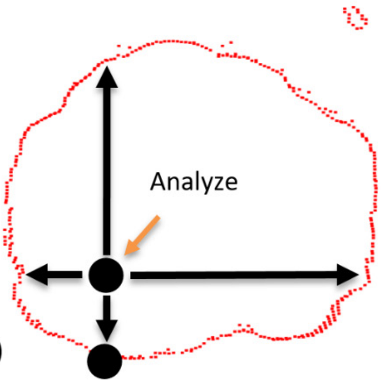


Figure 3. Object projection analysing: (a) base, (b) the outlier points of the base.

The analysis of the projection starts with the region in the square of the algorithm “imaginary square”. If there are two objects in this square, there are also two object projections. This algorithm analyses them one after the other. The point with the highest ‘y’ value is determined and defined as a point on the projection edge (perimeter). Then a point with a small delay is created inside the projection. This point is called analysis point (Figure 4a).



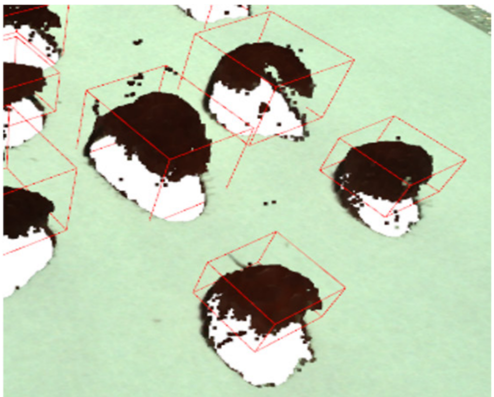


Figure 4. (a) object projection analysing method, (b) 3D bounding boxes.

From the analysis point, four vectors are created to locate the four nearest edge points of the object projection. Using these four points, some information about the object projection can be obtained. To get more precise information about the projection perimeter, you can move the analysis point a little and use four vectors again. When the object projection is located, the object is located above the projection and the 3D bounding boxes for each raspberry has been created (Figure 4b).

RESULTS AND DISCUSSION

Descriptive yield components

The study in 2021 was carried out for 14 floricane raspberry cultivars and hybrids. The largest number of fruit laterals per cane was found for hybrid S 2-6-13 and the cultivars 'Ruvi' and 'Sulamifa'. The largest number of fruits per fruit lateral was for the cultivar 'Lubetovskaja' and hybrids S2-6-8, S11-25a-4 and S2-6-13 (Table 1) compared to cultivar 'Glen Ample'. Significant differences between evaluated genotypes ($p<0.001$) were found related to fruit laterals per cane, berries per fruit lateral and yield per cane. The diversity of genotypes with regard to yield components is important for the development of smart non-invasive methods for phenotyping these traits.

Table 1. Characterization of yield and yield components in year 2021.

Cultivars	Fruit laterals per cane (count)	Berries per fruit lateral (count)	Average berry weight (g)	Yield (g cane^{-1})
S2-6-13	21.5	11.7	2.0	503.1
S2-6-8	18.2	14.4	1.8	471.7
S11-25a-4	15.1	12.4	2.5	468.1
Patricija	15.6	8.7	2.3	312.2
Lubetovskaja	13.2	10.1	2.1	280.0
Līna	11.7	8.5	2.7	268.5
S1-12-13	15.4	9.1	1.8	252.3
Ruvi	18.3	9.8	1.4	251.1
Kapriz Bogov	13.9	7.8	2.1	227.7
Bozhestvennaja	10.5	7.2	2.7	204.1
Sulamifa	18.6	7.8	1.3	188.6
Octavia	8.7	8.8	2.2	168.4
Shahrizada	9.7	6.0	2.3	133.9
Glen Ample	6.9	7.3	2.2	110.8
Probability levels of significance by ANOVA	<0.001	<0.001	n.s.	<0.001

Characterization of raspberry fruit parameters

The largest fruits with elongated form and highest account of drupelets were for cultivars 'Bozhestvennaja' and 'Patricija' (Table 2). These cultivars have the highest shape index. The diversity of fruit parameters of different genotypes is important for the development of smart non-invasive methods for phenotyping using 3D images.

Table 2. Characterisation of floricane raspberry fruits in year 2021.

Cultivars	Length (mm)	SD (mm)	Width (mm)	SD (mm)	Shape index	SD	Number of druplets	SD
S2-6-13	17.0	1.7	15.1	1.3	1.1	0.1	94.8	24.6
S2-6-8	19.0	1.7	18.2	1.4	1.0	0.1	75.5	11.3
S11-25a-4	17.3	1.3	16.6	1.1	1.0	0.1	80.1	8.2
Patricija	25.7	4.2	18.1	1.1	1.4	0.2	112.3	22.5
Lubetovskaja	17.4	1.1	15.4	1.6	1.1	0.1	71.0	9.2
Bozhestvennaja	23.1	2.0	15.6	1.5	1.5	0.2	106.2	18.5
Glen Ample	17.7	1.4	18.2	1.5	1.0	0.1	64.5	6.6
Meteor	14.9	1.3	15.7	1.1	0.9	0.1	60.0	9.9

SD: standard deviation.

Raspberry fruit detection with Yolo5 and raspberry data set

Compared to manual measurements (e.g., weighing equipment or ruler), non-invasive image-based phenotyping of fruit plants aims to develop a comprehensive phenotyping model for fruit plants based on computer vision techniques and tools. To reach this goal throughout the season from flowering to harvest, computer vision, DL architectures and imaging algorithms using quantitative data from massive 2D, and 3D images of raspberry plants were considered. In this work, a representative raspberry data set (Raspberry data set) was created consisting of 2072 raspberry images before final data cleaning. The images were acquired at different times during the raspberry growth phases from different angles and distances. The pixels of healthy, symptomatic, or damaged raspberries were classified into five classes: "Buds", "Flowers", "Unripe Berries", "Ripe Berries", and "Damaged Berries". The images size was 1773×1773 pixels. See Figure 5 for the parameters and average proportion of the Raspberry data set.

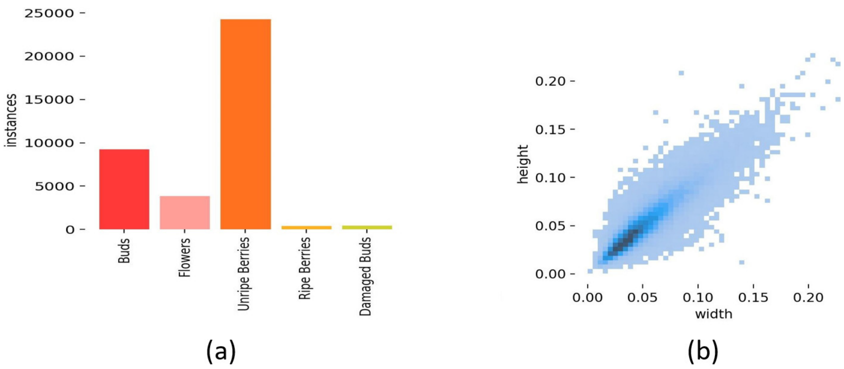
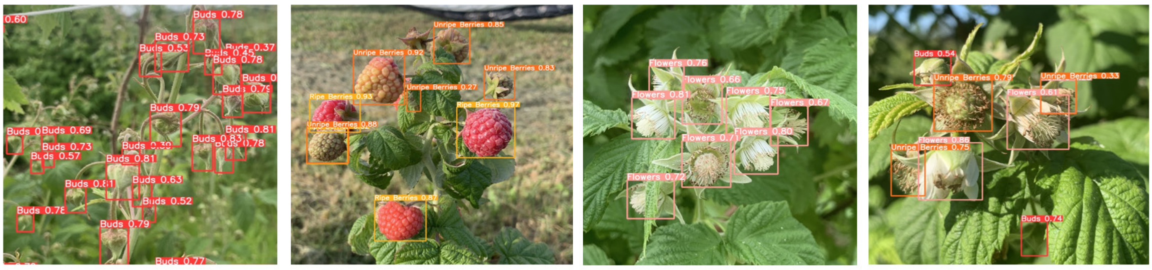


Figure 5. Parameters of the Raspberry data set, (a) the number of instances for each class, (b) the average proportions of the height and width of the instances.

To detect the most important yield components (number of laterals per cane, number of flowers/fruits per lateral, and average mass of fruits), an object detector was developed. It is based on the Yolo5 DNN architecture and has been trained on a Raspberry data set (Figure 6) After training our detector, we made for predictions for the new and unseen raspberry images in our test set. It can be seen that the detector currently correctly with appropriate probability can detect labelled raspberry classes. However, it struggles to correctly differentiate between one or multi-class objects when the visibility of the objects to be classified is occluded by the same class or other class object.



(a) (b) (c) (d)

Figure 6. Detection results obtained with the trained Yolo5 detector on random images from the test set of the Raspberry data set. (a) "Buds", (b) "Unripe Berries" and "Ripe Berries", (c) "Flowers", (d) "Flowers", "Buds", and "Unripe Berries".

To evaluate the convergence performance three different types of loss shown in Figure 7 were taken into consideration: box loss, objectness loss and classification loss. The box loss represents how well the predicted bounding box covers an object and how well the algorithm can locate the centre of an object. Classification loss gives an idea how well the algorithm can predict the correct class of a given object. Objectness is a measure of the probability that a given object exists in a proposed region.

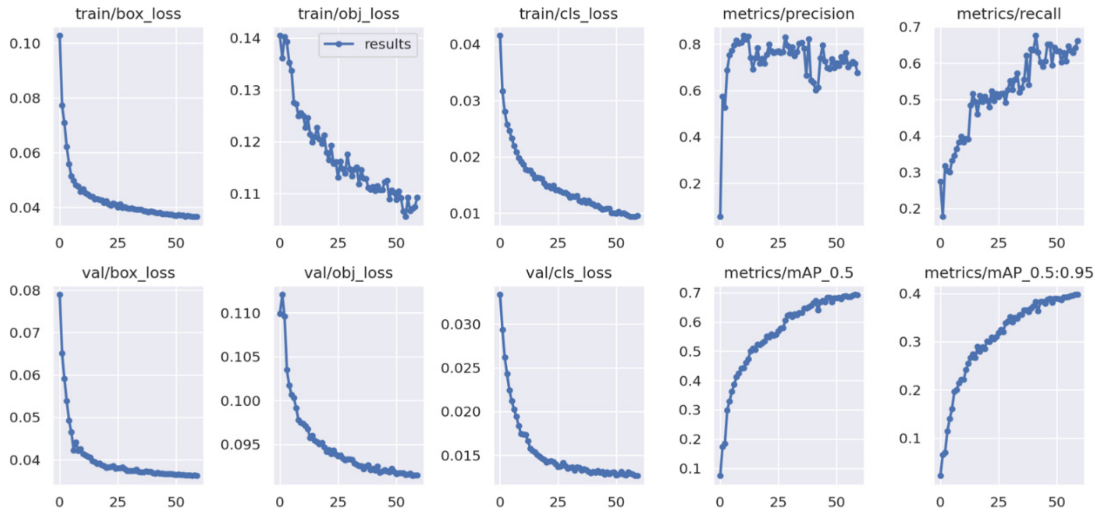


Figure 7. Convergence of Yolo5 object detector during DNN training, where X-axes indicate number of epochs, Y-axes indicate parameter value.

The raspberry object detector improved swiftly in terms of precision, mean average precision and recall before plateauing after about 50 epochs. The box, objectness and classification losses of the validation data also shown decline around epoch 50. To select the best weights for the model an early stopping was used. In using 3D image approach, the most important consideration was the localization of the object in point cloud. The next step will be to analyse the object parameters, such as length, width, depth, surface smoothness, ripeness of raspberries and so on.

CONCLUSIONS

The diversity of fruit parameters and yield components of different genotypes is important for the development smart non-invasive methods for phenotyping.

In the first part of the project, the DNN Yolo5 has been developed for the detection and localization of raspberries in RGB images. The DL model has been trained to estimate raspberry yield, and achieves after calculation of average precision for each raspberry class and taking a mean value over all classes, an average accuracy mAP of more than 70%. 3D image data processing is also considered to estimate raspberry parameters in 3 dimensions. Currently, the 3D model can separate raspberries by their height, width and depth in a 3D point cloud. Future research will include and focus on raspberry texture analysis which is supposed leading towards intelligent non-invasive phenotyping categorization.

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