

GigaScience

Plant phenomics: an overview of image acquisition technologies and image data analysis algorithms --Manuscript Draft--

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Abstract:	The study of phenomes or phenomics has been a central part of biology. The field of automatic phenotype acquisition technologies based on images has seen an important advance in the last years. As other high throughput technologies, it bears from a common set of problems, including data acquisition and analysis. In this review, we give an overview of the main systems developed to acquire images. We give an in-depth analysis of image processing with its major issues, and the algorithms that are being used or emerging as useful to obtain data out of images in an automatic fashion.	
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Response to Reviewers:	<p>Dear Dr. Nogoy, thank you very much for the speedy review and the high quality of the referees you chose. We believe that their input has improved our manuscript and we have taken their and your advice into account.</p> <p>Please find enclosed a detailed response that can be also found in the manuscript. We have done a major rewriting to center it around two questions, image acquisition and data analysis. We have deleted a part we wrote about the different types of indexes used as it was informative but not really related to the major points.</p> <p>As a result, we believe that all the referees requests have been met. Some via direct correction and many by rewriting to clarify the points. In those cases where rewriting deleted a request, we have written it down on the letter found below.</p> <p>Please note that the new manuscript has all corrections and rewriting in red.</p> <p>We hope the referees will find this version suitable for publication.</p>	

1. Reviewer reports:

Reviewer #1: # Review giga science 20170317

The manuscript from Perez-Sanz et al is a review of image acquisition technologies and image data analysis algorithms used in plant sciences.

While the manuscript provides a nice overview of the available techniques and algorithms, I feel that it is, at least in this form, not suited for publication. The text needs to be clarified in numerous places (see comments below). Also, I think it is, too technical and would fit better in a more specialised journal such as Plant Methods.

General comments:

1- The English should be improved

We have used professional English edition to improve the language.

2- The scope in the text is varying a lot between sections, which makes the reading hard. Sometimes the text provide very precise information about a given experiment (e.g. line 59), while it stays very vague in other places. I think the whole text should be homogenised for easier understanding.

We have done a major rewriting to address this issue

3- The manuscript given the impression to be willing to make an overview of whole the existing sensors / algorithms used for plant image analysis. My feeling is that this tack is inherently huge, since each image acquisition / analysis problem will call for a specific solution. A thorough review would be enormous. This is not the case f this manuscript, which gives more of an overview.

We have addressed this problem by giving the major advantages/drawbacks and technical characteristics of image acquisition devices and image analysis procedures.

Some specific comments, to give an idea of the modifications that should be made (I haven't done the whole document):

4- Line 19 (first line of the abstract!): No, phenomics is not the field of atomic phenotype acquisition technologies. It is the field of phenome analysis and is not, strictly speaking, linked to any specific technology. Phenomics can be done by hand, with a ruler.

We agree with the referee and we have changed the formulation of the abstract.

5- Line 30: NDVI is not defined

We have defined NDVI and other abbreviations throughout the manuscript

6- line 41: the sentence discusses roots techniques, but cites shoot-related article

We have corrected this part by rewriting. The references in the previous manuscript(9- 12 now 11- 13 were correct and did refer to roots

7- line 41: what do you mean by "Analysis of direct imaging"?

We have changed the phrase as we refer to extraction of quantitative data from images (now line 42-43

8- line 44: I guess that author mean growing setups

We have corrected it (now line 46)

9- line 47-67: I am not sure to understand the aim of this paragraph. How does this fit with the rest of the text? I have the feeling it justifies to use of reporter lines, not the

use of imaging setups...

We have rewritten part of the paragraph to explain why. In principle reporter genes, specifically Green Fluorescent Protein and Luciferase fostered the use of artificial vision systems early on. (now 49-76)

10- line 67: why is drawback in crops?

We have clarified this point. Now line 72-76

11- Monovision: can't the infrared and fluorescence imaging setup be classified here? They would fit the definition given in line 101-103.

Although there are IR cameras acquiring a single wavelength most are RGB-IR so we have included this in the multispectral cameras section. (see lines 194-211)

12- 113: "developed to quantify QTL's" ->

We have corrected to "developed to identify QTL's" Now line 146

13- 114: "large POPULATION of RIL's"

This part was rewritten

14- 115: what do you mean by "elite lines"?

This part was rewritten (line 148). An elite line is a genetic line useful for further breeding. Usually they have pyramided QTLs and or dominant alleles conferring superior traits sought after.

15- 123: isn't it a "DEPTH map"?

The mistake is corrected. Deep map is replaced by depth map. (Line 168)

16- 125: ToF is not defined

We have added a complete description of ToF devices-(line 228-249)

17- 134: why are stereo vision low throughput? Not sure it is true. Many plant phenotyping platform have a stereo vision imaging inside the imaging cabinet for 3D reconstruction -> high throughput

We have eliminated that text and added a paragraph with merits and drawbacks of 3-D systems. (Line 182-190)

Figure 1: What do the two arrows mean?

We have remade Figure 1 that describes the process of image acquisition and analysis

Reviewer #2: Due to the diversity of plant phenotyping techniques and different goals of plant research, it is a challenge to review and summarize major works enrolled in plenty of imaging techniques, image analysis pipeline, and image processing algorithms. The authors attempt to review some efforts of images acquisition and image processing, which is encouraged. However, the structure of review is confused, and massive fundamental knowledge of images analysis (read like a textbook of digital image processing) exists in this main text, which also lacks the references and the authors' own opinions. In addition, more applications of plant phenotyping should be cited in this review. More discussions and more comparisons with different image analysis should be summarized and added combined with the authors' suggestions, which can guide and benefit the readers.

1.Line 3: I wonder this review whether focus on plant phenomics, if yes, please change the title to plant phenomics.

We have changed the title as suggested

2.Line 30: please use the full name of "NDVI" and other abbreviations for the first time in this article.

We have modified all abbreviations and introduced first the name.

We have rewritten the complete part to make it easier to read, and deleted the part on different indexes. We have kept Figure 2 and on Table 2 different indexes can be

found.

3.Line 33: please add reference for the "analyse plant growth and biomass".
We have added a reference (Myneni et al Nature 1997) (Line 34, reference [2].

4.Line 41: what dose "direct imaging" mean?

We have changed the phrase as we refer to extraction of quantitative data from images (Line 42-43)

5.Line 48-52: please add references for the "Historically, the first type of screenings was developed using the Luciferase reporter gene driven by a promoter" and "Upon mutagenesis of a parental line harbouring a regulatory region activated or repressed by a certain biological process or an environmental condition, new germplasm has been recovered".

We have increased this part and included more references

6.line 69-72: The authors paid plenty of words to introduce the development of screening techniques in the second paragraph of the Background. However, why the purpose of this review is lacking. Why review of image acquisition and image analysis is needed?

This is a very good point, we have made a statement about this, as most literature about image processing is found in books describing how to do them and not as reviews about what to use and why. (Line 78-88)

7.Line 81: TDI is a new sensor? Or it is a new imaging technique with CCD?

TDI is not a new sensor; it is a special imaging acquisition technology that can be implemented over CCD or CMOS imaging sensors to improve their features. Currently it is possible to find TDI cameras in the portfolios of the most important cameras manufactures. We have modified the paragraph and included new references (Lines 102-108).

8.Line 77-93: please add references and add author's own opinion, instead of some general knowledge.

We have added some perspective about trends in all the types of cameras we have described (see last paragraph of each of the devices.

9.Line 96: five groups?

We have increased them to 7. This is a good point as it gives a clearer picture.

10.Line 105: please use the full name of "SPICY" for the first time.

We have corrected this throughout the paper

11.Line 99: "mono vision" should be changed to "mono RGB vision".

We have done this correction (now line 130 and following parts)

12.Line 121-123: please add reference for the "Basically, and after locating a point in two mono vision systems, it is possible to compute the distance from the point to the system. Images produced are known as deep maps".

We have added references

13.Line 125: please use the full name of "ToF" for the first time

We have corrected this point.

14.Line 134: the drawback of stereo vision system is low throughput, however, the author cited a reference "high-throughput stereo-vision system" in line 130.

We have corrected this and clarified it (line 192-190)

15.Line 138-139: "usually between 2 and 10?" please add reference.

This range that classify the multispectral cameras is changing along the last decade as technology is improved. We have found different manufacturers with multispectral cameras between 3 until 25 bands. We have added a reference for a multispectral camera with 25 bands. But in months, new cameras will be in the market with increased capacities. (Line 194-202).

16.Line 156: the citing of "Figure 2" appeared earlier than Figure 1. Please check it carefully.

We have corrected this (line 222)

17.Line 160-163: please add references.
18.Line 167-169: please add references.
19.Line 173-177: please add references.

20.Line 178-181: More applications of plant phenotyping with LIDAR in recent years should be cited. Please discuss the disadvantage of the LIDAR.
21.Line 177: the end of the sentence lacks punctuation.
We have rewritten this whole part and included new references

22.Line 186: "14.000 nm" should be change to "14,000 nm". The image which obtained by thermographic camera should include a range of wavelength. Moreover, please add the reference.
We have added the reference. We have added fluorescence imaging with the corresponding ranges and references (line 297-322)

23.Line 196: "as a result of UV light excitation" is not rigorous, and please add the reference.
We have rewritten this part (see above line 397-322)

24.Line 75-203: more image acquisition techniques, such as x-ray CT, should be added. And the authors should summarize the merit and drawback of these imaging techniques.

25.Line 227-229: please add the reference of the "In fact, when information is measured as entropy, pre-processing causes a decrease in entropy". Or this is the author's own opinion.
We have rewritten this entire section

26.Line 235-265: please introduce the procedures of image correction and images enhancement more concisely, and please add the reference.
We have rewritten this entire section

27.Line 271-272: please add the references to the "Leaf Area Index (LAI), biomass, chlorophyll concentration, photosynthetic activity", respectively.
28.Line 287: please add the references to the "RDVI" and "MSR".
29.Line 294: what "NIR" and "VIS" represented?
30.Line 301: "EVI (enhanced vegetation index)" should be changed into "enhanced vegetation index (EVI)". Please check the similar mistake carefully in the main text.
31.Line 305: you should add the meaning of "RED" and "BLUE".
32.Line 267-312: the summarization of indexes in Table 1 is appreciated. But the "Vegetation indexes" part may not be appropriate for the "Image pre-processing" part, and this part is too redundant.
33.Line 320: 3D or 3-D.
34.Line 336-337: please add the references of the "1500-1590 nm" and "1390-1430 nm".
35.Line 355: Despite RGB and HSV colour space, other colour components such as ExG are also frequently used in plant detection. The authors should introduce more colour components.
36.Line 359-360: please add the references of the "hue can discriminate to detect chlorophyll".
37.Line 368: what is the meaning of "h(.)"?
38.Line 394: please add the references of the "Gaussian Mixture Model (GMM)". And what is the meaning of "l"?
39.Line 474: what are the meaning of "(892-934)" and "(281-245)"?
40.Line 476: 28 in SURF?
41.Line 487: please use the full name of "FAST" for the first time.
42.Line 442-517: The authors give too much detail about the features. Little was introduced about the application of these features in plant phenotyping.
43.Line 538-544: The authors should give some suggestion about when to select supervised/unsupervised techniques.
44.Line 545-547: I agree that the selection of ML algorithm require actual experimentation for optimal results. However, there are some general advices, the author should mention that and give some suggestions.

We have rewritten this part to make it more accessible. As a result, all the comments have been taken into account

	<p>45. According to the Figure 1, the author should review some popular algorithm or software of data analysis. And the structure of image analysis in the main text is confused, and the author should reorganize the review via the workflow of Figure 1.</p> <p>We have remade Figure 1 to make it clearer and matched the review with the Figure. We think that data analysis is a completely different topic. We have table 3 with popular software for image analysis.</p>
Additional Information:	
Question	Response
Are you submitting this manuscript to a special series or article collection?	No
<p>Experimental design and statistics</p> <p>Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends.</p> <p>Have you included all the information requested in your manuscript?</p>	No
<p>If not, please give reasons for any omissions below.</p> <p>as follow-up to "Experimental design and statistics</p> <p>Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends.</p> <p>Have you included all the information requested in your manuscript?</p> <p>"</p>	Not applicable
<p>Resources</p> <p>A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible.</p>	Yes

<p>Have you included the information requested as detailed in our Minimum Standards Reporting Checklist?</p>	
<p>Availability of data and materials</p> <p>All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript.</p> <p>Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?</p>	<p>Yes</p>

1 **Gigascience Review**

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3 **Plant phenomics: an overview of image acquisition technologies and**
4 **image data analysis algorithms**

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17 Abstract

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19 The study of phenomes or phenomics has been a central part of biology. The field of
20 automatic phenotype acquisition technologies based on images has seen an important
21 advance in the last years. As other high throughput technologies, it bears from a
22 common set of problems, including data acquisition and analysis. In this review, we
23 give an overview of the main systems developed to acquire images. We give an in-depth
24 analysis of image processing with its major issues, and the algorithms that are being
25 used or emerging as useful to obtain data out of images in an automatic fashion.

26

27 **Keywords:** algorithms; artificial vision; deep learning; hyperspectral cameras; machine
28 learning; segmentation

29 Background

30

31 The development of systems to monitor large fields using Normalized Difference
32 Vegetation Index (NDVI), started a long successful career over 25 years ago when
33 NDVI was used in the so-called remote sensing field [1]. It was an important milestone
34 in the advance of automatic methods for analysing plant growth and biomass [2]. Ever
35 since, new technologies have increased our capacity to obtain data from biological
36 systems. The ability to measure chlorophyll status from satellite images allowed plant
37 health to be measured in large fields and predict crops and productivity in very large
38 areas such as the Canadian prairies, Burkina Faso or the Indian Basin in Pakistan [3–6].
39 Thus, the field of remote sensing is an important basis where knowledge about data
40 acquisition and analysis started. The development of phenotyping devices using local
41 cameras for crops took off using an array of technologies including Infrared
42 thermography to measure stomatal opening or osmotic stress [7–9]. Extraction of
43 quantitative data from images has been developed to study root development [10–12],
44 and has found a niche to identify germplasm resistant to abiotic stresses in plants such
45 as cereals [13], Arabidopsis [14] and for large-scale field phenotyping [15]. There are
46 several recent reviews addressing the different types of growing setups [16–22], and we
47 will not cover them in the current review.

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The development of reporter genes allows the external visualization of gene expression, sometimes in a non-invasive way. This led to the development of high throughput image acquisition devices to study gene expression. The use of high-throughput screening systems based on imaging techniques had a major impact in the identification of mutants involved in different processes [23]. Historically, the first type of screenings were developed using the Luciferase reporter gene driven by an endogenous promoter [24]. Upon mutagenesis of a parental line harbouring a regulatory region activated or repressed by a certain biological process or an environmental condition, new germplasm has been recovered [25]. This allowed the identification of a large number of mutants affecting complex traits such as response to abiotic stress [26] or circadian clock [27]. A second type of analysis based on measuring growth helped identify genes involved in chloroplast function [28]. Further studies using promoters driving a reporter gene have been used in Bryophytes such as *Physcomitrella patens*, or the unicellular green Algae *Chlamydomonas reinhardtii* to study circadian regulation [29,30]. Complex screens have been set up for instance to identify the formation of Cajal bodies in nuclei using alternatively spliced Green Fluorescent Protein (GFP) protein variants [31]. Once promoter driven lines are established they can be reused for further studies. A screen of 720 chemical compounds performed in Arabidopsis plants with a GIGANTEA promoter driving luciferase identified compounds that affect circadian clock and cause actin stabilization, an otherwise difficult parameter to measure [32]. Altogether, these screens have proven the importance of unbiased image acquisition systems, demonstrating the universal power of this approach for in-depth research in plants. Those studies based on transgenic material have been extensively used model systems such as Arabidopsis, *Physcomitrella* or *Chlamydomonas*. However transgenic-based studies present a major drawback for most crops, as the size of the plants makes them difficult to use for high throughput studies using reporter genes. Finally, image acquisition from large plants is challenging as growth chambers and setups need to be built for this purpose.

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The two aforementioned situations i.e. field and growth chamber setups have in common the large number of images produced when using automatic image acquisition technologies. Two main aspects to consider are the type of image acquired and how to process it. There are a number of recent reviews on phenomics and high-throughput image data acquisition [15,33–36]. In contrast, the majority of the literature concerning

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83 image processing and analysis is found in books where methods are described in detail
84 [37–41]. There are some very good reviews on aspects of data acquisition and analysis
85 i.e. imaging techniques [42], Machine Learning (ML) for high throughput phenotyping
86 [43] or software for image analysis [44], but a detailed review on different type of data
87 analysis is lacking. In this review, we cover the current and emerging methods of image
88 acquisition and processing allowing image-based phenomics (Figure 1).
89

90 Review

91 Image acquisition

92
93 Image acquisition is the process through which we obtain a digital representation of a
94 scene. This representation is known as image and its elements are called pixels (picture
95 elements). The electronic device used to capture a scene is known as imaging sensor.
96 CCD (charge-coupled device) and CMOS (complementary metal oxide semiconductor)
97 are the most broadly used technologies in image sensors. A light wavelength is captured
98 by small analogic sensors, which will acquire major or minor charge depending on the
99 amount of incident light. These signals are amplified, filtered, transported and enhanced
100 by means of specific hardware. A suitable output interface and a lens in the same
101 housing is all that it is needed to perform image acquisition. The elements enumerated
102 above conform the main element of computer vision systems, the camera. Time delay
103 and integration (TDI) is an imaging acquisition mode that can be implemented over
104 CCD [45] or CMOS [46]. It improves the features of the image acquisition system
105 considerably. TDI is used in applications that require the ability to operate in extreme
106 lighting conditions, requiring both high speed and high sensitivity, for example: inline
107 monitoring, inspection, sorting, and remote sensing (for weather o vegetation
108 observation) [46].

109 The aforementioned technologies, CCD, CMOS and TDI confer unique characteristics,
110 which define the type of data a camera can provide with a degree of robustness. There
111 are fundamental differences in the type of performance the different sensors offer. In the
112 last years CMOS technology, has outperformed CCDs in most visible imaging
113 applications. When selecting an imaging sensor (a camera), CCD technology causes less
114 noise and produces higher quality images, mainly in scenes with bad illumination. They
115 have a better depth of colour due to their higher dynamic range. On the other hand, the

116 CMOS sensors are faster at processing images. Due to the hardware architecture for
117 pixel extraction, they need less electrical power to operate, they allow a Region of
118 Interest (ROI) to be processed on the device and are cheaper than CCDs. Furthermore,
119 TDI mode with CCD or CMOS imaging sensors is used for high speed and low light
120 level applications [47]. The latest technological developments in cameras show that the
121 trend of the manufacturers such as IMEC, world-leader in nanoelectronics, is to fuse
122 TDI technology with the CCD and CMOS characteristics in the same device [48]. TDI
123 technology is expected to be applied to high throughput phenotyping processes in the
124 nearby future.

125
126 The field of image acquisition is extremely developed with considerable literature but
127 image acquisition systems can be classified into seven groups that are suitable for
128 phenotyping.

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130 1. Mono-**RGB** vision

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132 **Mono-**RGB**** vision systems are composed of a set comprising a lens, imaging sensor,
133 specific hardware and IO interface. Depending if they use a line or matrix of pixels,
134 they are classified in line cameras (or scanners) and matrix cameras. Most computer
135 vision phenotyping devices are based on **mono-**RGB**** vision systems. Examples of
136 mono-**RGB** vision devices include “**Smart tools for Prediction and Improvement of**
137 **Crop Yield (SPICY)**”, an automated phenotyping prototype of large pepper plants in the
138 greenhouse. The system uses multiple **RGB** cameras to extract two types of features:
139 features from a 3D reconstruction of the plant canopy and statistical features derived
140 directly from **RGB** images [49]. A different approach has been used with two cameras
141 inside a growth chamber to measure **circadian growth features** of *Petunia*, *Antirrhinum*
142 and *Opuntia* [50]. **Two cameras with low and high magnifications were used to carry-**
143 **out phenotype studies of *Arabidopsis thaliana* seeds. The system is mounted on a three-**
144 **axis gantry and the rotation of the samples allow the gravitropic bending response to be**
145 **determined in the roots and its posterior quantification [51].** Recently a high-throughput
146 **RGB** system has been developed to **identify Quantitative Trait Loci (QTL)** involved in
147 yield in large recombinant inbred lines in maize [52], demonstrating the increasing
148 impact of this approach in **phenomics**.

149 These devices have excellent spatial and temporal resolution, i.e. they can produce a
150 very large number of images in very short periods and at a very low cost. They are
151 portable and there are many software tools to perform image processing (Table 1).
152 Systems based on mono-RGB vision allow a quantification of the plant canopy [53], as
153 well as sufficient computation of vegetation indices, for most purposes. The main
154 disadvantages are caused by the overlap of plant organs during growth and nutation
155 phases and the relative position of the organs with respect to the device that makes the
156 precise quantification difficult. In addition, these devices are affected by variations in
157 illumination when used outdoors. The trend in outdoor plant phenotyping is to combine
158 mono-RGB systems with other systems such as Light Detection and Ranging LIDAR
159 devices (see below), thermal imaging or adding new bands or filters to the camera that
160 allow the segmenting of specific regions of the spectrum [54,55].

161 162 2. Stereo vision 163

164 Stereo vision systems try to correct a drawback of mono-RGB vision systems for
165 distance measurement. The architecture of stereo vision systems emulates the behaviour
166 of human vision using two mono vision systems. Basically, and after locating a point in
167 two mono vision systems, it is possible to compute the distance from the point to the
168 system. Images produced are known as depth maps [56]. A stereo vision system has
169 been used by Biskup and colleagues [57] to obtain structural features of plant canopies.
170 The 3D reconstruction has been successfully employed to obtain 3-D models of plants,
171 thus demonstrating the power of this approach [58]. Simple depth reconstructions
172 helped to define stems, leaves and grapes showing the potential of this technology [59].
173 A RGB camera mounted on a mobile robot is used as an automated 3D phenotyping of
174 vineyards under field conditions. Sequentially, the system captures a set of images,
175 which are used to reconstruct a textured 3D point cloud of the whole grapevine row
176 [60]. A stereo vision has been developed to perform high throughput analysis of
177 rapeseed leaf traits. The system uses two identical RGB cameras to obtain stereo images
178 for canopy and 3-D reconstruction [61]. Developing a 3D-mesh segmentation has
179 allowed cotton growth to be analysed [62], showing the further possibilities of 3D
180 imaging.

182 The main advantage of 3-D systems is their simplicity, two cameras are enough to
183 obtain depth maps. The stereo vision has evolved in multi-view stereo (MSV) and has
184 found a place in plant phenotyping [63]. Furthermore, the MSV is a low cost 3D image
185 acquisition system compared with other technologies such as LIDAR or tomography
186 imaging [64]. Stereo vision systems have important weaknesses. They are affected by
187 changes of the scene illumination, they need a high performance computational system
188 to carry out stereo matching algorithms, and they have a poor depth resolution [65].
189 These limitations are increased in outdoor environments, as image segmentation
190 becomes more challenging.

191 192 3. Multi and hyper spectral cameras

193
194 The multispectral and hyperspectral cameras have been used in numerous fields of
195 science and in industrial applications [66–71]. The spectral resolution is the main factor
196 that distinguishes multispectral imagery from hyperspectral imagery [72]. Multispectral
197 cameras are devices able to capture images from a number of discrete spectral bands.
198 The number of bands has increased in the last decade as technology has improved.
199 Currently, the main camera manufacturers offer multispectral cameras acquiring
200 between three and twenty five bands, including the visible RGB channels, Near Infra-
201 Red (NIR) or a set of custom bands, with a tendency to provide increasing number of
202 bands [73]. The spectral bands may not be continuous, thus for one pixel we obtain a
203 vector of information comprising the number of elements corresponding to the number
204 of bands registered. Hyperspectral systems may reach resolutions of a few nanometers
205 in wavelength, obtaining for each pixel a digital signature that may contain several
206 hundreds of continuous bands within a specific range of wavelengths [74].
207 Traditionally, both multispectral and hyperspectral imaging have been used for remote
208 sensing and have an increased number of applications in phenomics. A multispectral
209 system has been developed to improve the original colour of images for fruit
210 recognition [75]. The authors fused the original colour image with an infrared image
211 using the nonlinear Daubechies wavelet transform (DWT). Thus, the additional
212 information from a second image allows the original one to be improved.

213
214 The use of hyperspectral cameras is increasing in phenotyping experiments as they
215 allow the identification of physiological responses, pathologies or pests in a non-

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216 invasive way. Using hyperspectral images, a system has been developed to identify
217 pathogens in barley leaves using probabilistic topic models [76]. A hyperspectral
218 microscope was used to determine spectral changes on the leaf and cellular level of
219 barley (*Hordeum vulgare*) during resistance reactions against powdery mildew
220 (*Blumeria graminis f.sp. hordei, isolate K1*) [77]. A detailed description of the different
221 wavelengths and combinations used in multispectral and hyperspectral cameras can be
222 seen in Figure 2, and their uses in Table 2. We expect to see an increase in phenomic
223 setups using multispectral and hyperspectral cameras in the future. An emerging issue
224 will be the data analysis as the number of pictures doubles with each additional
225 spectrum used for analysis (see below).

226

227 4. ToF cameras

228

229 The Time of Flight cameras or ToF cameras have been one of the last imaging devices
230 to be incorporated into automatic plant phenotyping [78]. ToF has as a general principle
231 the measurement of the distance between the objective of the camera and each pixel.
232 This is achieved measuring the time it takes for a signal emitted in NIR to come back,
233 reflected by the object. This allows a precision 3D reconstruction. Stereo vision coupled
234 with ToF images have been implemented to increase the performance of methods of
235 image segmentation to obtain leaf areas [79]. Beyond the tedious hand work required
236 for manual analysis sampling is done in a non-destructive way. Depth maps obtained by
237 a ToF camera together with colour images are used to carry out the 3D modelling of
238 leaves. The system is mounted on a robotic arm which allows image acquisition to be
239 automated [80]. A ToF has been successfully used to identify QTL regulating shoot
240 architectures of *Sorghum* by mean of 3D reconstruction [81].

241 Microsoft Kinect is a low cost image acquisition system designed for video gaming
242 which can be used for characterization and for tracking of phenological parameters [82].
243 The device is composed of an infrared projector and camera that generates a grid from
244 which the location of a nearby object in 3 dimensions can be ascertained [83]. Kinect
245 has been used to measure plant structure and size for two species growing in California
246 grassland [84]. The quantitative 3D measurements of the architecture of the shoot and
247 structure of the leaves can be performed when proper segmentation algorithms are
248 developed suggesting some potential for ToF systems [85].

249

1 250 The main disadvantages of this acquisition system are the low resolution, a reduced
2 251 distance range of a few meters and the high dependence on the reflecting surface for
3 252 imaging. As a result, they cannot operate under strong sunlight and are more appropriate
4 253 for indoor conditions. Its reduced cost and the possibility of obtaining 3D structures of
5 254 entire plants, as well as of individual organs make these devices very attractive for
6 255 indoor phenotyping.

7 256

8 257 5. LIDAR technology

9 258

10 259 Light Detection and Ranging (LIDAR) is a remote sensing technology developed at the
11 260 beginning of the 70s to monitor the Earth's Surface [86]. LIDAR uses a laser pulse light
12 261 to measure the distance between the light source and the object by calculating the time
13 262 of emission and time of reflected light detection. It allows the creation of a cloud of
14 263 points that reconstruct the 3D structure of an object [87,88]. LIDAR has been used in
15 264 image acquisition from distances of thousands of kilometres to centimetres,
16 265 demonstrating the great potential of these type of devices. Satellite-based LIDAR
17 266 systems are used for the measurements of vegetation canopy height, area, volume or
18 267 biomass, etc. [89–91]. Recent development using both manned and unmanned flights
19 268 have allowed the estimation of biomass dynamics of a coniferous forest using Landsat
20 269 satellite images together with ground and airborne LIDAR measurements
21 270 [92]. Terrestrial LIDAR sensors are applied to detect and discriminate maize plants and
22 271 weeds from soil surface [93]. Short range LIDAR can be deployed for high-throughput
23 272 phenotyping (HTP) systems for cotton plant phenotyping in the field [94] or tomato leaf
24 273 area by 3-D laser reconstruction [95]. Fully automated crop monitoring is feasible using
25 274 centimetre ranges from robotized or gantry systems [53]. An autonomous robotic
26 275 system has allowed 3D mapping of plant structures to be performed with millimetric
27 276 precision [96]. A LASER SCAN mounted on a XYZ gantry system was used to
28 277 estimate the growth measures and structural information of plants through laser
29 278 triangulation techniques [97]. Thus, using different devices LIDAR has an impressive
30 279 range of possibilities for plant phenomics.

31 280

32 281 Some shortcomings of LIDAR devices for plant phenotyping are the absence of colour
33 282 in the measurement, excessive time to compute the cloud points, low precision for
34 283 massive phenotyping, scanning noises caused by wind, rain, insects, small particles in

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the air, and the requirement of calibration. Recent advantages suggest that the use of LIDAR technologies could overcome some of challenges for the next-generation phenotyping technologies [98]. Developments in multispectral LIDAR instruments show novel systems which are capable of measuring multiple wavelengths and of obtaining vegetation indexes (see below) [99,100] or to measure arboreal parameters [101]. The massive adoption of LASER technologies by autonomous car manufactures has fostered the development of 3D High Definition LIDAR (HDL) with real time (RT) capacities. The new 3D HDLs are capable of generating 1.3 million points per second with precisions of 2 cm and distances of up to 120 meters [102]. These new devices open the door to the RT massive phenotyping in outdoor and indoor crops.

6. Thermography and Fluorescence Imaging

Thermography is a widely-used technology in remote sensing and plant phenotyping [103–106]. Thermographic cameras are able to acquire images at wavelengths ranging from 300 to 14,000nm [107], thus allowing the conversion of the irradiated energy into temperature values, once the environmental temperature is assessed. Plants open stomata in response to environmental cues and circadian clock depending on the type of photosynthetic metabolism they have [108,109]. The evapotranspiration can be assessed with thermography [110], and quantification can be made at different scales such as a leaf, a tree, a field or a complete region. Water stress and irrigation management are two fields of application of thermography imaging [111–114]. Thermography imaging can detect local changes of temperature produced due to pathogen infection or defence mechanisms [115]. *Oerke et al.* used a digital infrared thermography to correlate the maximum temperature difference (MTD) of apple leaves with all stages of scab development [116].

Fluorescence imaging has been used in a large number of experimental setups as UV light in the range of 340-360 nm is reflected by different plant components as discrete wavelengths [42]. The corresponding wavelengths emitted are cinnamic acids in the range of green-blue (440-520 nm). Early experiments using reflected fluorescence allowed the identification of phenylpropanoid synthesis mutants in *Arabidopsis* [117]. Chlorophyll fluorescence emits in red and far-red (690-740 nm). It is an important parameter that has been studied as a proxy for different biological processes such as

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318 circadian clock or plant health [8,118,119]. A system based on a UV light lamp and a
319 conventional camera provided of a UV-filter to avoid RGB and IR images has been
320 used to identify changes in UV absorbance related to pollination [120]. Multicolour
321 fluorescence detection uses the combination of chlorophyll and secondary metabolites
322 emitted fluorescence to determine plant health in leaf tissues [121].

323
324 Thermography imaging results in an estimable tool for monitoring of genotypes and
325 detection of plant diseases [122] where all the specimens are located under strict control
326 conditions: temperature, wind velocity, irradiance, leaf angle or canopy leaf structures
327 are potential issues for quality image acquisition. The next generation of thermography
328 imaging for phenotyping will have to resolve drawbacks related to temporal variations
329 of environment conditions, aspects relating to angles of view, distance, sensitivity and
330 reproducibility of the measurements [114]. Both thermographic and fluorescent images
331 capture a single component and images are in principle easy to analyse as segmentation
332 based on thresholds can be applied to the acquired images. Combining thermographic
333 and fluorescent imaging requires sophisticated data analysis methods based on neural
334 networks to obtain quality data but are an emerging solution [121].

335

336 7. Tomography imaging

337
338 Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique which uses
339 Radio Frequency (RF) magnetic fields to construct tomographic images [123].
340 Commonly MRI has been used to investigate the anatomy structure of the body
341 (especially the brain) in both health and disease [124]. In plant phenomics, MRI is used
342 to visualize internal structures and metabolites. This method poses a great potential to
343 monitor physiological processes occurring *in vivo* [125]. MRI has allowed the
344 development of root systems over time in bean to be mapped [126], moisture
345 distribution to be visualized during development in rice [127] and the water presence to
346 be analysed during maturity process of barley grains [128].

347 Positron Emission Tomography (PET) is a nuclear medicine imaging modality that
348 allows the assessment of biochemical processes *in vivo*, to diagnose and stage diseases
349 and monitor their treatment [129]. *Karve et al.* [130] presented a study about C-
350 allocation (Carbon allocation from CO₂ through photosynthesis) in large grasses such as
351 *Sorghum bicolor*. The study concluded that the commercial PET scanners can be used

1 352 reliably, not only to measure C-allocation in plants but also to study dynamics in
2 353 photoassimilate transport.

3 354

4 355 X-ray Computed Tomography (X-ray CT) employs X-rays to produce tomographic
5 356 images of specific areas of the scanned object. The process of attenuation of rays
6 357 together with a rotation and axial movement over objects produces 3D images [42]. A
7 358 high throughput phenotyping system based on X-ray CT is ten times more efficient than
8 359 human operators, being capable of detecting a single tiller mutant among thousands of
9 360 rice plants [131]. The remarkable penetration of X-rays, has made this technology a
10 361 great ally of phenotyping carried out below-ground. The study of root systems and their
11 362 quantification has been a field of habitual application of X-ray CT [132–136]. New
12 363 developments address the reduction of penetrability and the increase of the image
13 364 resolution of X-ray CT in plant tissue using phosphotungstate as a contrasting agent,
14 365 due to its capacity of increasing the contrast and penetrability of thick samples [137].
15 366

16 367

17 368 MRI, PET and X-ray imaging techniques are available for screening 3-D objects. MRI
18 369 and PET are two non-destructive and non-invasive scanning technologies that have been
19 370 applied in plant sciences to acquire 3-D structural information [138]. MRI and PET data
20 371 acquisition is time consuming, and software tools need to be further developed to
21 372 analyse data and obtain physiologically interpretable results [107]. High-Resolution X-
22 373 ray computed tomography (HRXCT) promises to be the broadest non-destructive
23 374 imaging method used in plant sciences. HRXCT will provide 3-D data at a resolution
24 375 suited for detailed analysis of morphological traits of *in vivo* plant samples and at a
25 376 cellular resolution for *ex vivo* samples [138]. From of a point of view of the devices the
26 377 trend will be to increase the resolution of images, the size of the fields of view, and
27 378 increase its portability [139].

28 379

29 380 Image analysis

30 381

31 382 Extracting information from images is performed through the process of segmentation.
32 383 The aim of a segmentation procedure is to extract the components of an image that are
33 384 of interest i.e. object or region of interest from the rest of the image i.e. background of
34 385 the image or irrelevant components. Thus, we end up with a partitioned image with

1 385 significant regions. The significant regions may be defined as foreground versus
2 386 background, or by selecting a number of individual components from an image. The
3 387 construction of the selected regions is based on the image characteristics such as colour
4 388 (colour spaces), spectral radiance (vegetation indexes), edge detection, neighbour
5 389 similarity [140] or combinations that are integrated via a machine learning process
6 390 [141]. In some cases, pre-processing is required in order to obtain a meaningful
7 391 segmentation.

8 392

9 393

10 394 1. Image pre-processing

11 395

12 396 Image preprocessing is an important aspect of image analysis. The aim of image
13 397 preprocessing is to improve contrast and eliminate noise in order to enhance the objects
14 398 of interest in a given image [142]. This process can be extremely helpful to enhance the
15 399 feature extraction quality and the downstream image analysis [143]. Preprocessing can
16 400 include simple operations such as image cropping, contrast improvement or others
17 401 significantly more complex such as dimensionality reduction via Principal Component
18 402 Analysis or Clustering [43]. One preprocessing pipeline has been proposed for plant
19 403 phenotyping based on converting the image to grayscale, application of a median filter,
20 404 binarization and edge detection [144]. A similar preprocessing has been developed to
21 405 identify plant species under varying illumination conditions [145]. It comprises
22 406 conversion to grayscale, image binarization, smoothing and application of a filter to
23 407 detect edges. In a comparative study to analyze leaf diseases, histogram equalization
24 408 was found to be the best way to obtain preprocessing of color images converted to
25 409 grayscale [146]. However RGB images have been found to perform better than
26 410 grayscale conversions when identifying leaf pathogens [147].

27 411

28 412 We cannot conclude that a single preprocessing method will outperform other methods.
29 413 The quality and type of image are fundamental to select a type of preprocessing
30 414 procedure. Nevertheless, preprocessing is a basic step that can improve image analysis,
31 415 and sometimes make it possible. It should be described in the materials and methods
32 416 of image procedures to make data comply the new standards -Findability, Accessibility,
33 417 Interoperability, and Reusability (FAIR) [148]

34 418

419 2. Image segmentation

420

421 As we mentioned above, image segmentation is the core of image processing for
422 artificial vision-based plant phenotyping. Segmentation allows the isolation and
423 identification of objects of interest from an image, and it aims to discriminate
424 background or irrelevant objects [149]. The objects of interest are defined by the
425 internal similarity of pixels in parameters such as texture, colour, statistic [143], etc.
426 (See a list of Open software libraries for image segmentation in Table 1).

427

428 One of the simplest algorithms used is threshold segmentation, based on creating groups
429 of pixels on a grayscale according to the level of intensity, thus separating the
430 background from targets. Such an approach has been used with Android OS (ApLeaf) in
431 order to identify plant leaves [150].

432

433 The Otsu's method [151] is a segmentation algorithm that searches for a threshold that
434 minimizes the weighted within class variance [142]. This method has been used for
435 background subtraction in a system that records and performs automatic plant
436 recognition [152], and can give high contrast segmented images in an automatic fashion
437 [153]. Under certain circumstances, it can underestimate the signal causing under
438 segmentation, and is significantly slower than other thresholding methods [142].

439

440 The Watershed [154] transformation is a popular algorithm for segmentation. It treats an
441 image as a topological surface that is flooded, and seed regions are included, usually by
442 the user. This generates an image with gradients of magnitudes, where crests appear in
443 places where borders are apparent (strong edges), and causes segmentation to stop at
444 those points [140]. It has been used to identify growth rate [155], recognition of
445 partially occluded leaves [66], individual tree crown delineation [156] or leaf
446 segmentation [157].

447

448 Grabcut [158] is a segmentation algorithm based on graph cut [159]. It is created on
449 graph theory to tackle the problem of separating an object or foreground from the
450 background. The user should mark a rectangle (bounding box) surrounding the object of
451 interest thus defining the outrebound of the box as background [160]. This algorithm
452 has been tested to extract trees from a figure but it has been successful only with very

1 453 simple backgrounds [161]. More recently Grabcut has been deployed as a segmentation
2 454 algorithm in a pipeline for plant recognition with multimodal information i.e. leaf
3 455 contour, flower contour etc [162]. Grabcut loses precision or even fails when pictures
4 456 have complex backgrounds but is highly precise with simple backgrounds [161,163].
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9 458 Snakes are a special type of active contour [164], and are used as methods to fit lines
10 459 (splines) either to open or close edges and lines in an image. These methods have been
11 460 used for face recognition, iris segmentation and medical image analysis. Within the
12 461 field of plant phenotyping, there are procedures where active contours are used inside a
13 462 protocol constructing a vector of features with data of colour intensity, local texture and
14 463 a previous knowledge of the plant incorporated via Gaussian Mixture Models,
15 464 previously segmented [165] . These steps give an initial rough segmentation upon
16 465 which, active contours can operate with a much higher precision.
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25 467 Active contours have used for plant recognition via images of flowers [166], based on a
26 468 combination of the algorithm proposed by Yonggang and Karl [167] and the model of
27 469 active contours without edges [168]. Whilst the work proposed by Minervini et al [165]
28 470 appears to give significantly better results compared to those of Suta et al [166], the
29 471 usage of images with a natural background maybe related to the apparent differences in
30 472 segmentation. Thus, a current problem concerning the comparison of algorithms and
31 473 procedures lies on the different backgrounds used for image acquisition.
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39 474 40 475 3. Features extraction

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42 477 Features extraction constitutes one of the pillars of the identification and classification
43 478 of objects based on computer vision. Beyond the raw image, a feature is information
44 479 which is used to resolve a specific computer vision problem. The features extracted
45 480 from an image are disposed in the so-called “feature vectors”. The construction of
46 481 feature vectors uses a wide set of methods to identify the objects in an image. The main
47 482 features are edges, intensity of image pixels [49], geometries [169], textures [165,170],
48 483 image transformations e.g. Fourier [171], or Wavelet [75,172] or combinations of pixels
49 484 of different colour spaces [141]. The end goal of feature extraction is to feed up a set of
50 485 classifiers and machine learning algorithms (see below).
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487 One system proposed uses a feature vector composed of a combination of RGB and CIE
488 L*a*b* colour spaces to segment the images captured during the day [141]. The night-
489 time image segmentation computed a vector composed of statistical features over two
490 decomposition levels of the wavelet transform using IR images.

491 Iyer-Pascuzzi et al. presented an imaging and analysis platform for automatic
492 phenotyping to identify genes underlying root system architecture. The authors
493 employed a set of 16 statistical, geometrics and shape features obtained from 2,297
494 images from 118 individuals such as median and maximum number of roots, the total
495 root length, perimeter, depth, among others [173].

496
497 There are a number of algorithms to identify invariant features detectors and
498 descriptors. This type of image analysis ensures the detection of points of interest in a
499 scale and rotation independent manner. This is crucial for camera calibration and for
500 matching to produce a set of corresponding image points in 3D image reconstruction.
501 Furthermore, it allows the identification of points of interest even when they change
502 scale and/or position or situations of uncontrolled illumination, a common issue when
503 phenotyping plants. The Scale Invariant Features Transforms (SIFT) [174], Speeded-Up
504 Robust Features (SURF) [175] and the Histograms of Oriented Gradients (HoG) [176]
505 are algorithms used to extract characteristics in computer vision and they have been
506 extended to plant phenotyping. Wei et al. [177] presented an image-based method that
507 automatically detects the flowering of paddy rice. The method uses a scale-invariant
508 feature transform descriptor, bag of visual words, and a machine learning method. The
509 SIFT algorithm has been used to combine stereo and ToF images with automatic plant
510 phenotyping. It can create dense depth maps to identify pepper leaf in glasshouses [79].
511 SIFT and SURF algorithms have been tested for detecting local invariant features for
512 obtaining a 3D plant model from a multi-view stereo images [178]. A HoG framework
513 allows the extraction of a reliable quantity of phenotypic data of grapevine berry using a
514 feature vector composed of colour information [179].

515
516 So far, feature extraction is an arduous and difficult task requiring the testing of
517 hundreds of feature extraction algorithms and a greater number of combinations
518 between them. This task demands expert skills in different subjects. The success in the
519 identification does not depend on the robustness of the classification methods, but on
520 the robustness of the data.

521

522 4. Machine Learning in plant image analysis

523

524 The amount of data generated in current and future phenomic setups with high
525 throughput imaging technologies has brought the use of Machine Learning (ML)
526 statistical approaches. Machine Learning is applied in many fields of research [180–
527 182]. As phenotyping can generate Terabytes of information, ML tools provide a good
528 framework for data analysis. A list of ML libraries can be found in Table 3. A major
529 advantage of ML is the possibility to explore large datasets to identify patterns, using
530 combinations of factors instead of performing independent analysis
531 [43].

532

533 Among the ML algorithms a predictive model of regression has been used to phenotype
534 *Arabidopsis* leaves, based on geometric features as training dataset [169]. Three
535 different algorithms were tested, k Nearest Neighbour (kNN), Support Vector Machine
536 (SVM) and Naïve Bayes to segment *Antirrhinum majus* leaves. Colour images have as a
537 characteristic vector intensity in the RGB and CIE L*a*b*, while the NIR vector is
538 obtained with the wavelet transform. The best results were obtained with kNN for
539 colour images and SVM for NIR. This shows that segmentation has several components
540 as mentioned before including the wavelength of image acquisition [141].

541

542 As the specific wavelength used for image acquisition plays a key role in the type of
543 data obtained, hyperspectral cameras are becoming important tools, however, hyper
544 images can be in the order of Gbites of size, making ML a necessity. Examples of
545 coupling hyperspectral and thermal imaging with ML have allowed the early detection
546 of stress caused by *Alternaria* in Brassica [183]. The best image classification was
547 obtained doing a second derivative transformation of the hyperspectral images together
548 with a back propagation of neural networks allowing the identification of fungi on
549 leaves days after infection [183].

550

551 A current concept derived from ML is Deep Learning (DL) comprising a set of
552 algorithms aimed to model with a high level of abstraction. This allows the
553 development of complex concepts starting from simpler ones, thus getting closer to the
554 idea of Artificial Intelligence (AI) (www.deeplearningbook.org). Convolutional Neural

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555 Networks (CNN), are an example of DL derived of Artificial Neural Networks (ANN).
556 These multi-layered networks are formed by a layer of neurons that work in a
557 convolutional way reducing the sampling process and end with a layer of perception
558 neurons for final classification [184]. Recently DL has been implemented using a CNN
559 to automatically classify and identify different plant parts [185], thus obtaining both
560 classification and localization that significantly improve the current methods. A CNN
561 has been used to detect plant pathogen attacks [186]. Although the training period is
562 computationally heavy, requiring several hours of CPU clusters, classification was
563 performed in less than one second [186]. Nevertheless, DL is a step forward in ML and
564 has great potential to allow the management and analysis of the data produced in
565 phenomic experiments.

566
567 Although direct testing maybe the best way to determine the superior algorithm in each
568 case, there is a number of examples that may guide initial approaches [43,187,188]. As
569 a general rule discriminating methods such as SVM, ANN, K-NN, give better results in
570 large datasets that are labelled [43]. Generative methods such as Naive Bayes, Gaussian
571 Mixture Models, Hide Markov Models, give better results with smaller datasets, both
572 labelled and unlabelled. The use of unsupervised algorithms i.e. k-means may help
573 identify unexpected characteristics on a dataset. As mentioned above, preprocessing
574 plays a fundamental role in increasing the ML output.

575

576 Conclusions and future prospects

577

578 The implementation of phenomic technologies is a welcome change towards
579 reproducibility and unbiased data acquisition in basic and applied research. A successful
580 approach requires integrating sensors, with wavelength and image acquisitions that will
581 allow the proper identification of the items under analysis. The majority of the work has
582 been made in indoor-setups where reasonable conditions can be created to obtain high
583 quality images, amenable to further processing. The difficulty in outdoor setups
584 increases as a result of limitations in the actual image acquisition devices and the
585 uncontrolled conditions that directly affect image quality. The new technologies such as
586 the high definition LIDAR or the multi-hyperspectral cameras have a great potential to
587 improve in the near future, specially in outdoor environments.

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589 The pre-processing and segmentation data are two aspects of data treatment and
590 acquisition that require careful design in order to avoid distortions and reproducibility
591 [148]. As images are machine-produced data, but image types and processing
592 procedures may be very different, the standardization of image capture, preprocessing
593 and segmentation may play an important role. It is a matter of time that databases with
594 raw image will become part of the standard in phenomics using images very much like
595 NCBI or Uniprot play a key role in genomic and proteomic projects. With the decrease
596 in price of hyperspectral devices, new experiments may be performed that produce even
597 larger data sets, and these data sets will have to go through Artificial Intelligence-based
598 data analysis in order to give the researchers results interpretable by humans. We guess
599 that like in other omic approaches, there will be a confluence to standard procedures
600 that are not currently common ground, making the current literature look intimidatingly
601 diverse. Nevertheless, most of the basic processes described here are shared by the
602 different experimental setups and data analysis pipes.

603

604 Abbreviations

605

606 **AI:** Artificial intelligence

607 **ANN:** Artificial neural networks

608 **CAI:** Cellulose Absorption Index

609 **CAR:** Chlorophyll absorption ratio

610 **CCD:** Charge coupled device

611 **Cig:** Coloration green

612 **Cir:** Coloration Index red

613 **CMOS:** Complementary metal oxide semiconductor

614 **CNN:** Convolutional neural networks

615 **CPU:** Central processing unit

616 **DL:** Deep learning

617 **DLAI:** Difference Leaf Area Index

618 **DSWI:** Disease water stress index

619 **DWT:** Daubechies wavelet transform

620 **EVI:** Enhanced vegetation index

621 **FAIR:** Findability, Accessibility, Interoperability, and Reusability

622 **GI:** Greenness Index

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623 **GMM:** Gaussian mixture model
624 **GNDVI:** Green normalized difference vegetation index
625 **HOG:** Histograms of oriented gradients
626 **KNN:** K nearest neighbour
627 **LAI:** Leaf area index
628 **LCA:** Lignin-Cellulose Absorption Index
629 **LIDAR:** Light detection and ranging
630 **LWVI-1:** Normalized Difference Leaf water VI 1
631 **MCARI:** Modified Chlorophyll Absorption Ratio Index
632 **MCFI:** Multicolour fluorescence imaging
633 **ML:** Machine learning
634 **NDVI:** Normalized Difference Vegetation index
635 **NIR:** Near infrared
636 **NLI:** Nonlinear vegetation index
637 **NTDI:** Normalized Tillage Difference Index
638 **OSAVI:** Optimized Soil Adjusted Vegetation Index
639 **PCA:** Principal component analysis
640 **PWI:** Plant Water Index
641 **QTL:** Quantitative trait locus
642 **RGB:** Red, green, blue
643 **ROI:** Region of interest
644 **SIFT:** Scale invariant features transforms
645 **SURF:** Speeded-up robust features
646 **SVM:** Support vector machine
647 **TDI:** Time delay and integration
648 **ToF:** Time of flight
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661 Authors contributions

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1393 Tables

1394 Table 1. List of software tools for image processing

Vision libraries	Source	Language
OpenCV EmguCV	http://opencv.org http://www.emgu.com/	C++, Python, Java, C#
PlantCV Scikit-image	http://plantcv.danforthcenter.org http://scikit-image.org	Python
Bioimagetools, bayesimages, edci, DRIP, dpmixsim, raster, ...	https://cran.r-project.org/	R
Cimg Simplecv Fastcv Ccv Vxl	http://cimg.eu http://Simplecv.org https://developer.qualcomm.com/software/fastcv- sdk http://libccv.org http://vxl.sourceforge.net	C++
BoofCV OpenIMAJ JavaCV	http://boofcv.org http://openimaj.org https://github.com/bytedeco/javacv	Java

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Table 2. A list of indexes, the corresponding wavelength ranges and their use to analyse plant material.

Index	Range nm	Applications
CAI – Cellulose Absorption Index	2200-2000	Quantification mixed soil–plant litter scenes [189], estimation of non-photosynthetic biomass [190]
LCA – Lignin-Cellulose Absorption Index	2365-2145	Measure the effects of soil composition and mineralogy of crop residue cover [191]
NTDI – Normalized Difference Tillage Index	2359-1150	Used for identifying crop residue cover in conventional and conservation tillage systems [192]
LWVI-1 – Normalized Difference Leaf water VI 2	1094-893	Discrimination of sugarcane varieties, allowed to detect large amounts of non photosynthetically-active constituents within the canopy [193]
DLAI – Difference Leaf Area Index	1725-970	Used for estimating leaf area index based on the radiation measurements in the visible and near-infrared [194]
PWI – Plant Water Index	970-902	Water content estimation and study of the characteristics of canopy spectrum and growth status [195][196]
NLI – Nonlinear vegetation index	1400-780	Measurement of plant leaf water content. In combination with others indexes can detect interaction of biochemicals such as protein, nitrogen, lignin, cellulose, sugar, and starch [197]
DWSI – Disease water stress index	1657-547	To predict larval mosquito presence in wetland [198]and detect sugarcane 'orange rust' disease [199]
NDVI – Normalized Difference Vegetation Index	800-670	Measurement significant variations in photosynthetic activity and growing season length at different latitudes [200]
MCARI – Modified Chlorophyll Absorption Ratio Index	700-670	Study of vegetation biophysical parameters, as well as to external factors affecting canopy reflectance [201]
GI – Greenness Index	670-550	Characterization of corn nitrogen status [202]
CAR – Chlorophyll absorption ratio	700-500	Estimating the concentration of individual photosynthetic pigments within vegetation [203]
GNDVI – Green normalized difference vegetation index	800-550	Providing important information for site-specific agricultural decision making [204] and for identification of chlorophyll content and tissue nitrogen [205]
OSAVI – Optimized Soil Adjusted Vegetation Index	800-670	Measurement with high sensitive of chlorophyll content variations and very resistant to the variations of LAI and solar zenith angle [206]
CI r – Coloration Index red	780-710	Mapping of coastal dune and salt marsh ecosystems [207]
CI g – Coloration Index green	780-550	Characterization of the state of soil degradation by erosion [208]

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1406 Table 3. List of Machine Learning software libraries and their languages

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Libraries ML/DL	Source	Language
MICE, rpart, Party, CARET, randomForest, nnet, e1071, KernLab, igrph, glmnet, ROCR, tree, Rweka, earth, klaR,	https://cran.r-project.org/	R
Scikit-learn Tensorflow Theano Pylearn2, NuPIC Caffe PyBrain	http://scikit-learn.org/stable/ https://www.tensorflow.org/ http://deeplearning.net/software/theano http://deeplearning.net/software/pylearn2 http://numenta.org/ http://caffe.berkeleyvision.org/ http://pybrain.org/	Python
Weka Spark Mallet JSAT ELKI Java-ML	http://www.cs.waikato.ac.nz/ml/weka/ http://spark.apache.org/ http://mallet.cs.umass.edu/ https://github.com/EdwardRaff/JSAT http://elki.dbs.ifi.lmu.de/ http://java-ml.sourceforge.net/	Java
Accord Multiboost Shogun LibSVM mlpack Shark MLC++	http://accord-framework.net/ http://www.multiboost.org/ http://shogun-toolbox.org/ http://www.csie.ntu.edu.tw/~cjlin/libsvm/ http://mlpack.org/ http://image.diku.dk/shark/ http://www.sgi.com/tech/mlc/source.html	C#, C++, C

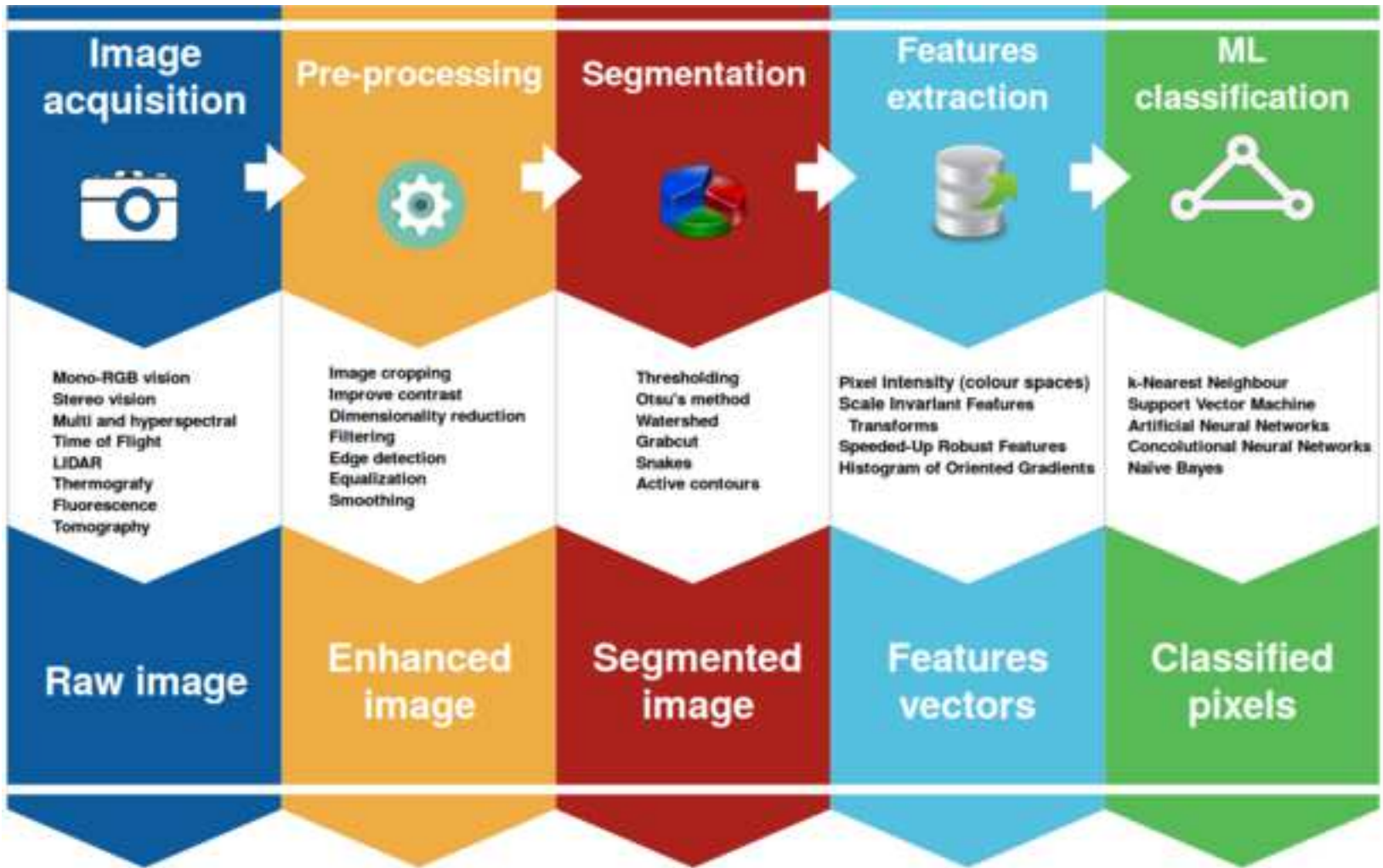
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1412 Figure Legends
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6 1416 **Figure 1.** Basic workflow in computer vision-based plant phenotyping
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8 1417 **Figure 2.** An overview of different spectra used for phenotyping and the associated
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10 1418 cameras. Names of different indexes are found in Table 2.
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Figure

