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# Cereal crop ear counting in field conditions using zenithal RGB images

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**TITLE:**

# Cereal Crop Ear Counting in Field Conditions Using Zenithal RGB Images

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**KEYWORDS:**

Wheat, barley, ear counting, segmentation, breeding, phenotyping, precision agriculture, Laplacian

**SUMMARY:**

We present a protocol for counting durum wheat and barley ears, using natural color (RGB) digital photographs taken in natural sunlight under field conditions. With minimal adjustments for camera parameters and some environmental condition limitations, the technique provides precise and consistent results across a range of growth stages.

**ABSTRACT:**

Ear density, or the number of ears per square meter (ears/m$^2$), is a central focus in many cereal crop breeding programs, such as wheat and barley, representing an important agronomic yield component for estimating grain yield. Therefore, a quick, efficient, and standardized technique for assessing ear density would aid in improving agricultural management, providing improvements in preharvest yield predictions, or could even be used as a tool for crop breeding when it has been defined as a trait of importance. Not only are the current techniques for manual ear density assessments laborious and time-consuming, but they are also without any official standardized protocol, whether by linear meter, area quadrant, or an extrapolation based on plant ear density and plant counts postharvest. An automatic ear counting algorithm is presented in detail for estimating ear density with only sunlight illumination in field conditions based on zenithal (nadir) natural color (red, green, and blue [RGB]) digital images, allowing for high-throughput standardized measurements. Different field trials of durum wheat and barley distributed geographically across Spain during the 2014/2015 and 2015/2016 crop seasons in irrigated and rainfed trials were used to provide representative results. The three-phase protocol includes crop growth stage and field condition planning, image capture guidelines, and a computer algorithm of three steps: (i) a Laplacian frequency filter to remove low- and high-frequency artifacts, (ii) a median filter to reduce high noise, and (iii) segmentation and counting using local maxima peaks for the final count. Minor adjustments to the algorithm code must be made corresponding to the camera resolution, focal length, and distance between the camera and the crop canopy. The results demonstrate a high success rate (higher than 90%) and $R^2$ values (of 0.62–0.75) between the algorithm counts and the manual image-based ear counts for both durum wheat and barley.

**INTRODUCTION:**

The world cereal utilization in 2017/2018 is reported expand by 1% from the previous year[1]. Based on the latest predictions for cereal production and population utilization, world cereal stocks need to increase yields at a faster rate in order to meet growing demands, while also adapting to increasing effects of climate change[2]. Therefore, there is an important focus on yield improvement in cereal crops through improved crop breeding techniques. Two the most important and harvested cereals in the Mediterranean region are selected as examples for this study, namely, durum wheat (*Triticum aestivum* L. ssp. *durum* [Desf.]) and barley (*Hordeum vulgare* L.*)*. Durum wheat is, by extension, the most cultivated cereal in the south and east margins of the Mediterranean Basin and is the 10th most important crop worldwide, owing to its annual production of 37 million tons annually[3], while barley is the fourth global grain in terms of production, with a global production at 144.6 million tons annually[4].

Remote sensing and proximal image analysis techniques are increasingly key tools in the advancement of field high-throughput plant phenotyping (HTPP) as they not only provide more agile but also, often, more precise and consistent retrievals of target crop biophysiological traits, such as assessments of photosynthetic activity and biomass, preharvest yield estimates, and even improvements in trait heritability, such as efficiency in resource use and uptake[5–9]. Remote sensing has traditionally focused on multispectral, hyperspectral, and thermal imaging sensors from aerial platforms for precision agriculture at the field scale or for plant phenotyping studies at the microplot scale[10]. Common, commercially available digital cameras that measure only visible reflected light were often overlooked, despite their very high spatial resolution, but have recently become popular as new innovative image-processing algorithms are increasingly able to take advantage of the detailed color and spatial information that they provide. Many of the newest innovations in advanced agricultural image analyses increasingly rely on the interpretation of data provided by very high-resolution (VHR) RGB images (for their measurement of red, green, and blue visible light reflectance), including crop monitoring (vigor, phenology, disease assessments, and identification), segmentation and quantification (emergence, ear density, flower and fruit counts), and even full 3D reconstructions based on a new structure from motion workflows[11].

One of the most essential points for improvement in cereal productivity is a more efficient assessment of yield, which is determined by three major components: ear density or the number of ears per square meter (ears/$m^2$), the number of the grains per ear, and the thousand-kernel weight. Ear density can be obtained manually in the field, but this method is laborious, time-consuming, and lacking in a single standardized protocol, which together may result in a significant source of error. Incorporating the automatic counting of ears is a challenging task because of the complex crop structure, close plant spacing, high extent of overlap, background elements, and the presence of awns. Recent work has advanced in this direction by using a black background structure supported by a tripod in order to acquire suitable crop images, demonstrating fairly good results in ear counting[12]. In this way, excessive sunlight and shadow effects were avoided, but such a structure would be cumbersome and a major limitation in an application to field conditions. Another example is an automatic ear counting algorithm developed using a fully automated phenotyping system with a rigid motorized gantry, which was used with good accuracy for counting ear density in a panel composed of five awnless bread wheat (*Triticum aestivum* L.) varieties growing under different nitrogen conditions[13]. Recent work by Fernandez-Gallego[14] has optimized this process for quicker and easier data capture, using VHR RGB color images followed by more advanced, yet still fully automated, image analyses. The efficient and high-quality data collection in field conditions emphasizes a simplified standardized protocol for consistency and high data capture throughput, while the image-processing algorithm employs the novel use of Laplacian and frequency domain filters to remove undesired image components before applying a segmentation for counting based on finding local maxima (as opposed to full delineation as in other previous studies, which may result in more errors with overlapping ears).

This work proposes a simple system for the automatic quantification of ear density in field conditions, using images acquired from commercially available digital cameras. This system takes advantage of natural light in field conditions and, therefore, requires consideration of some related environmental factors, such as time of day and cloud cover, but remains, in effect, simple to implement. The system has been demonstrated on examples for durum wheat and barley but should be extendable in application to bread wheat, which, besides exhibiting ears with similar morphology, are frequently awnless, but further experiments would be required in order to confirm this. In the data capture protocol presented here, zenithal images are taken by simply holding the camera by hand or using a monopod for positioning the digital camera above the crop. Validation data can be acquired by counting the ears manually for subplots in the field or during postprocessing, by counting ears in the image itself. The image-processing algorithm is composed of three processes that, first, effectively remove unwanted components of the image in a manner that, then, allows for the subsequent segmentation and counting of the individual wheat ears in the acquired images. First, a Laplacian frequency filter is used in order to detect changes in the different spatial directions of the image using the default ImageJ filter settings without window kernel size adjustments (https://imagej.nih.gov/ij/plugins/index.html#filters), which reduces the presence of most of the unwanted background elements in the image, including leaves, soil, and some of the awns. Next, a median spatial filter reduces the high-frequency noise around the ears due to the presence of the awns. Then, the **Find Maxima** segmentation technique determines the local peaks after the median spatial filter step, at which stage the pixels related with ears have higher pixel values than soil or leaves. Therefore, Find Maxima is used to segment the high values in the image, and those regions are labeled as ears, which identifies ears while also reducing overlapping ear errors. **Analyze Particles** is then used on the binary images to count and/or measure parameters from the regions created by the contrast between the white and black surface created by the Find Maxima step. The result is then processed to create a binary image segmentation by analyzing the nearest neighbor pixel variance around each local maximum to identify the wheat ear shapes in the filtered image. Finally, the ear density is counted using Analyze Particles, as implemented in Fiji[15]. Both Find Maxima and Analyze Particles are standalone functions and available as plugins in Fiji (https://imagej.nih.gov/ij/plugins/index.html). Though not presented specifically in the protocol here, preliminary results presented as supplementary material suggest that this technique may be adaptable to conducting ear count surveys from unmanned aerial vehicles (UAVs), providing that the resolution remains sufficiently high[14].

**PROTOCOL:**

**1. Prefield crop growth stage and environmental conditions**

1.1. Make sure that the crop growth stage is approximately between grain filling and near crop maturity, with ears that are still green even if the leaves are senescent (which corresponds in the case of wheat to the range 60–87 of Zadoks' scale[16]). Some yellowing of the leaves is acceptable but not necessary.

1.2. Prepare a sampling plan for image capture with various replicates (pictures per plot) in order to capture plot/area variability; the image-processing algorithm will count the number of ears in the image and convert that to ears per square meter (ears/m$^2$) based on the camera specifications.

1.3. Plan the field excursions to capture the images within two hours of solar noon or, alternatively, on an overcast day in diffuse lighting conditions in order to avoid the negative effects of ear shadowing on the ear counting algorithm.

1.4. Once in the field, check the top of the crop canopy to make sure that it is dry in order to avoid specular light reflection from moisture.

NOTE: In considering the objectives of this protocol, it is important to first consider whether the growth stage of the crop is suitable for applying ear counts. Capturing images outside of the recommended growth stage will either result in suboptimal or in meaningless results (if ears are not present or fully emerged). Image quality also has a considerable impact on processing the results, including resolution and sensor size, and some environmental conditions, such as time of day and cloud cover, need to be carefully considered before proceeding with image capture.

**2. Image capture in field conditions with natural light**

2.1. Prepare a “phenopole” as shown in **Figure 1** or a similar acquisition system (even handheld) to capture images quickly and yet in a standardized and consistent manner at each plot or target location.

[Place **Figure 1** here.]

2.2. Position the camera on a suitable monopod or “selfie” pole so that it may be maintained level, either using level bubbles or an in-camera stabilization system, to obtain zenithal images.

2.3. Use a mobile phone, tablet, or another device to connect the camera for both remote control image capture and image visualization for the best results with correctly focused images. Program the camera for autofocus in order to reduce any errors in case the user is not familiar enough with their camera or photography techniques to set a correct manual focus, as demonstrated by the examples of zenithal images with correct focus and exposure in **Figure 2**.

[Place **Figure 2** here.]

2.4. Take note of the image number prior to image capture in order to match the images correctly with the field plots. Record one image of the general field area at the start and one image of the ground/field between blocks for postprocessing controls.

2.5. Position the camera at approximately 80 cm above the top of the crop canopy, using a ruler or measurement string to periodically check the camera height above the canopy. Ensure that the camera is level and capture the image. This technique may require 1–2 researcher(s).

2.6. If additional field ear count validation is desired apart from a manual image count validation, install an extension arm to the frame (e.g., a small circle) and position it in the middle of the image in order to conduct manual field counts of a precise image subset; this technique may require 2–3 people to implement.

NOTE: Three major considerations in selecting a camera, therefore, include: (1) camera specifications; in this case, the sensor’s physical size; (2) focal length of the image lens; (3) distance between the canopy and the camera: smaller distances or greater zoom lenses will capture a smaller area while images captured from a greater distance will capture a bigger crop area. See **Figure 1** for the details on the relevant camera specifications.

## 3. Algorithm implementation and adjustments

NOTE: Here we present algorithm implementation and adjustments for different camera specifications (sensor size, megapixels, focal length, distance to crop) and crop (durum wheat or barley) for automatic ear counting. An overview of the algorithm is presented graphically in **Figure 3**.

[Place **Figure 3** here.]

3.1. Download and install Fiji, Java 8, and the processing code or the University of Barcelona's proprietary **CerealScanner** plugin (https://fiji.sc/, https://www.java.com/en/download/, and https://integrativecropecophysiology.com/software-development/cerealscanner/ [information] or https://gitlab/sckefauver/CerealScanner [code repository]); contact the corresponding authors for access permissions. The plugin is installed within Fiji by simply copying it into the plugins folder.

3.2. Open the plugin from the top menu through **Plugins** > **CerealScanner** > **Open Cereal Scanner**.

NOTE: Apart from the work presented here, the CerealScanner plugin includes several different RGB-based vegetation indices related to crop vigor, stress, or chlorophyll[17,18]. The specific CerealScanner portion includes specific algorithms for **Early Vigor** (Fernandez-Gallego, In review), **Ear Counting**[14], and **Crop Senescence**[19], as shown in **Figure 4**.

3.3. Enter the adjustments of the camera specifications and image capture details if they are different from the default values (see **Figure 1** and **Figure 4** for details).

[Place **Figure 4** here.]

[Place **Figure 5** here.]

3.3.1. Adjust the algorithm parameter for the camera focal length.

3.3.2. Adjust the algorithm parameter for the distance from the crop canopy.

3.4. Select the center tab **CerealScanner** and the subsequent central tab **Ear Counting** in order to calculate the number of ears in each image of a field data set.

3.4.1. Under **Options**, enter, in **Batch Inputs**, the location of the photos to analyze.

3.4.2. In the **Results Files**, select where to save the results file. The results file will include two columns with the image file name and the ear counting results.

3.4.3. Finally, click on **Process**, and the results file with the ear density in square meters (ears/$m^2$), using a simple ratio using the camera settings and the distance between canopy and camera to convert the image area to an actual canopy area in square meters following **Figure 1**, will be automatically produced in a few minutes, depending on the computer speed.

3.5. Conduct a post-processing validation after the data collection by manually counting the number of wheat or barley ears in the image and then converting this to the number of ears per square meters (ears/$m^2$), as is shown in **Figure 1**, to compare the results with those based on the algorithm values.

3.5.1. Use the simple point placement tool built within Fiji, which provides easy support for this process, and the Fiji **Analyze Particles** function for producing the counts automatically; this is shown graphically in **Figure 6**.

[Place **Figure 6** here.]

3.5.2. Optionally, conduct a validation using a small area circle during field data acquisition as described in step 2.6; manual counts in the field and manual image counts in the laboratory can, then, be used for algorithm validation as shown in **Figure 7**.

[Place **Figure 7** here.]

## REPRESENTATIVE RESULTS:

In **Figure 8**, the results show the determination coefficient between the ear density (number of ears per square meters) using manual counting and the ear counting algorithm for wheat and barley at three different crop growth stages. The first one is durum wheat with a Zadoks' scale between 61 and 65 ($R^2$ = 0.62). The second one is two-row barley with a Zadoks' scale between 71 and 77 ($R^2$ = 0.75), and the last one is durum wheat with a Zadoks' scale between 81 and 87 ($R^2$ = 0.75).

[Place **Figure 8** here.]

## FIGURE AND TABLE LEGENDS:

**Figure 1: Ear counting system.** Ear counting system using the "phenopole" shown in the field on the left, with a remotely controlled natural color (RGB) large sensor and high-resolution digital camera system with camera tilt and height, indicating the necessary parameters for adjusting the image-processing algorithm. The sensor and image resolution are detected automatically by the image properties, while the user should input the specifics for the lens focal length and the distance from the canopy. These are necessary to adjust the algorithm for the estimated number of pixels per ear and also the conversion of the image-based total ear count to ear density (ears/$m^2$). For that reason, it is recommended to use the same camera and lens focal length for all field images.

**Figure 2: Zenithal crop images.** Durum wheat and barley ear zenithal images for ear counting data set examples with an acceptable stage of growth and senescence from approximately 61 to 87 according to Zadoks' scale. (Left) Durum wheat zenithal image data set example. (Right) Barley zenithal image data set example.

**Figure 3: Image-processing pipeline for two-row barley ear counting.** Image-processing pipeline for two-row barley ear counting as implemented using specific computer code or using the **CerealScanner** software, both of which operate within Fiji (ImageJ). Panel 1 shows the original image. Panel 2 shows the results of the applications of the Laplacian filter. Panel 3 shows the application of the median filter, and Panel 4 shows the results of the final Find Maxima and segmentation for producing the final ear count. Then, the calculations are made to convert the image count to ear density, as shown in **Figure 1**. These images are an example taken from the Arazuri field site (Northeastern Spain, 42°48'33.9"N 1°43'37.9"W) in diffuse light conditions.

**Figure 4: The CerealScanner 2.12 Beta central tab on both levels, marking the Ear Counting function within the CerealScanner algorithm collection.** The user must select the **…** button to the right of **Batch Inputs** to select the folder where the image files are stored, change the default values of the H Distance (distance from the camera to the top of the crop canopy) and Focal Length, if different from the default values, and then select the **…** button to the right of **Results File** to choose the name and location of the final results file. The other tabs of the CerealScanner provide algorithms for trait-based phenotyping for **Early Vigor** and onset of **Maturity** as part of the CerealScanner code suite. Under the **Biomass** tab, there are several algorithms for estimations of more general crop vigor and biomass calculations, also for RGB digital images. The example refers to two-row barley, as it was demonstrated in detail in **Figure 3**.

**Figure 5: Algorithm adjustments.** Adjustments required in the image-processing pipeline in order to successfully count both wheat and barley ears using the same algorithm are managed automatically as part of the camera-specific adjustments of H Distance (distance between the camera and the crop canopy) and Focal Length and serve to ensure that the number of pixels per ear remains more or less constant between different applications.

**Figure 6: Algorithm validation using manual in-image ear counts.** Manual in-image ear counts for (left) durum wheat and (right) barley. The small dots were created using the Fiji **Point Tool** and then counted using the **Analyze Particles Function** with a 0.90–1.00 **Circularity constraint** after applying a **Color Threshold** from the **Hue Saturation Intensity** color space for the color specified by the **Point Tool**. This method ensures more accurate image-based manual ear counts.

**Figure 7: Algorithm validation using manual counts in the field and manual in-image ear counts of wheat and barley, using a circle.** (Left) Wheat image count validation example using a circle. (Right) Barley image count validation example using a circle. The subset counts of the ears within the white circle were counted using the same technique as described in **Figure 6** with the **Point Tool**, **Color Threshold**, and then, **Analyze Particles Function** with **Circularity constraints** and color selection using **Hue**.

**Figure 8: The coefficient of determination between ear density (number of ears/m$^2$) using manual image-based counting and the image algorithm for ear counting of durum wheat and two-row barley at different acceptable crop growth stages (at Zadoks' scale 61–87).** Both axes show calculations, including conversions to ear density, rather than image-based results only. The representative results are presented here for two different crops over three different growth stages, as well as under different light conditions, namely direct sunlight images of durum wheat at Zadoks' scale 61–65 on the top ($R^2$ = 0.62, $n$ = 72), diffuse light images of barley at Zadoks' scale 71–77 in the middle ($R^2$ = 0.75, $n$ = 30), and diffuse light conditions for durum wheat at Zadoks' scale 81–87 at the bottom ($R^2$ = 0.75, $n$ = 24). An example image of each is also shown as an inset in the bottom right corner of each graph.

**DISCUSSION:**

Increased agility, consistency, and precision are key to developing useful new phenotyping tools to assist the crop-breeding community in their efforts to increase grain yield despite negative pressures related to global climate change. Efficient and accurate assessments of cereal ear density, as a major agronomic component of yield of important staple crops, will help provide the tools needed for feeding future generations. Focusing on the improvement and support of crop-breeding efforts in field conditions helps keep this research and the techniques presented here more closely tied to real-world climate change scenarios and the needs of the breeding community but also presents technical difficulties. As such, it is important to pay close adherence not only to the data capture and image processing in this protocol but also to the recommendations for optimal environmental conditions and crop growth stages for its successful implementation[11]. As a major agronomical component of yield, ear density is considered one of the most important target traits in the push for increasing cereal crop yields (see the article by Slafer et al.[20] and references therein). The focus in this protocol toward an optimally cost-efficient, agile, and straightforward field phenotyping technique considers that these aspects of the protocol are key to its adoption and implementation by the breeding community at large. In contrast, previous related studies with similar aims at assessing ear density or other yield component quantification have used more involved data capture and environmental control structures, such as enclosing structures and fixed camera supports or even artificial light inputs, that effectively impede practical application under field conditions and implementation in actual breeding programs[12,20,22].

Thus, we have presented here a detailed protocol that is the result of testing various different techniques in an iterative optimization process, resulting in a simple but effective image data capture method requiring only a commercially available, moderately high-resolution RGB digital camera and a rudimentary "phenopole" for holding the camera above the crop canopy. Other image-filtering attempts based on RGB color or alternative color spaces, such as hue-saturation-intensity or CIELAB, were not as effective or consistent as the use of the Laplacian and median frequency domain filters in removing unwanted image elements, especially the awns. We have designed the image capture pipeline system with different elements, some of which can be easily adjusted with minor changes in the image-processing algorithm implementation. In the case studies presented here, we have used two different compact cameras with relatively large sensors and 20.1 megapixels (MP) and 16.0 MP resolutions for capturing images with wide-angle lenses of 16–20 mm from a distance of 80 cm from the crop canopy. This has proven more than sufficient to produce detailed canopy barley and wheat information, with simulations demonstrating that the technique maintains high levels of precision down to approximately 8 MP[14] (with similar lenses and distances from the canopy).

Although the consistency and precision of the presented image-processing techniques depend on the environmental conditions and phenological stage at the time of image capture, the algorithms show promise in providing a robust performance in their application to different small-grain cereals, including durum and bread wheat and two-row and six-row barley varieties. While this algorithm has yet to be fully tested, the image capture would the same, with perhaps some minor adjustments with regard to the relative size and position of the ear in the images in order to provide for optimal ear counting results. In the presented protocol, the image-based ear density estimates achieved maximal accuracy and correlated best with manual image counts and grain yield compared to images captured at later growth stages, when crop senescence resulted in a loss of color and illumination contrast between the rest of the crop canopy and the ears. This may be a result of higher temperatures or lower water availability at the later parts of grain filling, which are especially common in typical Mediterranean conditions, that can cause the leaves and culm to senesce before the ears do[23]; this contrast is essential for the effectiveness of the separation between the ears, leaves, and soil. In overly mature or senescent canopies with the ears already yellow, the contrast between the different image elements is not adequate for ear counting. As such, in other climates, the optimal timing may be slightly different if there is no water stress during senescence onset.

Even though the data collection in field conditions requires close attention to such environmental conditions as sunlight intensity and sunlight illumination angles, the robust image analysis algorithm presented here provides some leeway in the image capture window by using spatial techniques that ignore image albedo effects, given that the correct image exposure was used for the particular light conditions at the moment of image capture; automatic settings have worked well in that respect. In previous work, a fuller range of lighting effects was tested, indicating that the only major source of error with regard to light effects is the production of strong shadows in the image when capturing images in direct sunlight either early or late in the day, due to the angle of the sun[14]. The first two image filter applications help to minimize any apparent effects from excess illumination (although not via camera overexposure) while also reducing any background components of the image; at the same time, these filters also contribute to smoothing and noise reduction, both of which aide in the subsequent Find Maxima process[23,24]. Therefore, while the natural illumination factors must be accounted for, such as the angle of the sun when the images are taken in direct rather than in diffuse light conditions, this is mainly in order to reduce errors related to shadow artifacts.

Furthermore, correlations between algorithm ear counts from the presented protocol and grain yield were greater and more significant than manual (field-based) ear counts of the same experiment[11], which supports the claim that this protocol is not only more precise but also more consistent as a standardized protocol for the assessment of ear density. While not presented here specifically, similar data capture and processing techniques appears to be feasible using mobile phone, aerial or other automated platforms as they performs quite well under simulated reduced image resolutions. Additional tests for greyscale reduction (for faster image processing) and reduced resolution image simulations (from the application of other cameras or UAVs) were conducted by applying the image conversions before the first filter[14] and suggested that, in optimal conditions, processing times may be reduced in these ways without any loss of accuracy. As for possible future directions, the image-processing algorithms presented here only take advantage of the VHR RGB color data as it is captured by the camera (similar to the human eye), but other potential improvements may result from the conversion to hybrid color spaces, such as hue-saturation-intensity, or through data fusion in combination with other more advanced scientific sensors, such as multispectral or thermal, which both have become more affordable recently and offer the potential for improvement in ear density estimations, although perhaps at different growth stages or in different field conditions.

In summary, the critical steps for the implementation of this protocol include first and foremost proper planning for the time of year and environmental conditions of the crop, which are optimal when the crop is in growth stages 60–87 of Zadoks' scale and either at solar noon or in diffuse light conditions. Furthermore, the acquisition of digital images should be conducted in a controlled manner accounting for camera angle, distance from the canopy, and camera focus for each image. Finally, optimized computer-processing options are presented in detail for reproducing the processing code pipeline, or contact the authors for either the original code or the code integrated as a graphical user interface (GUI) in a plug-in for Fiji, namely, the CerealScanner.

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**DISCLOSURES:**
The authors have nothing to disclose.

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Figure1

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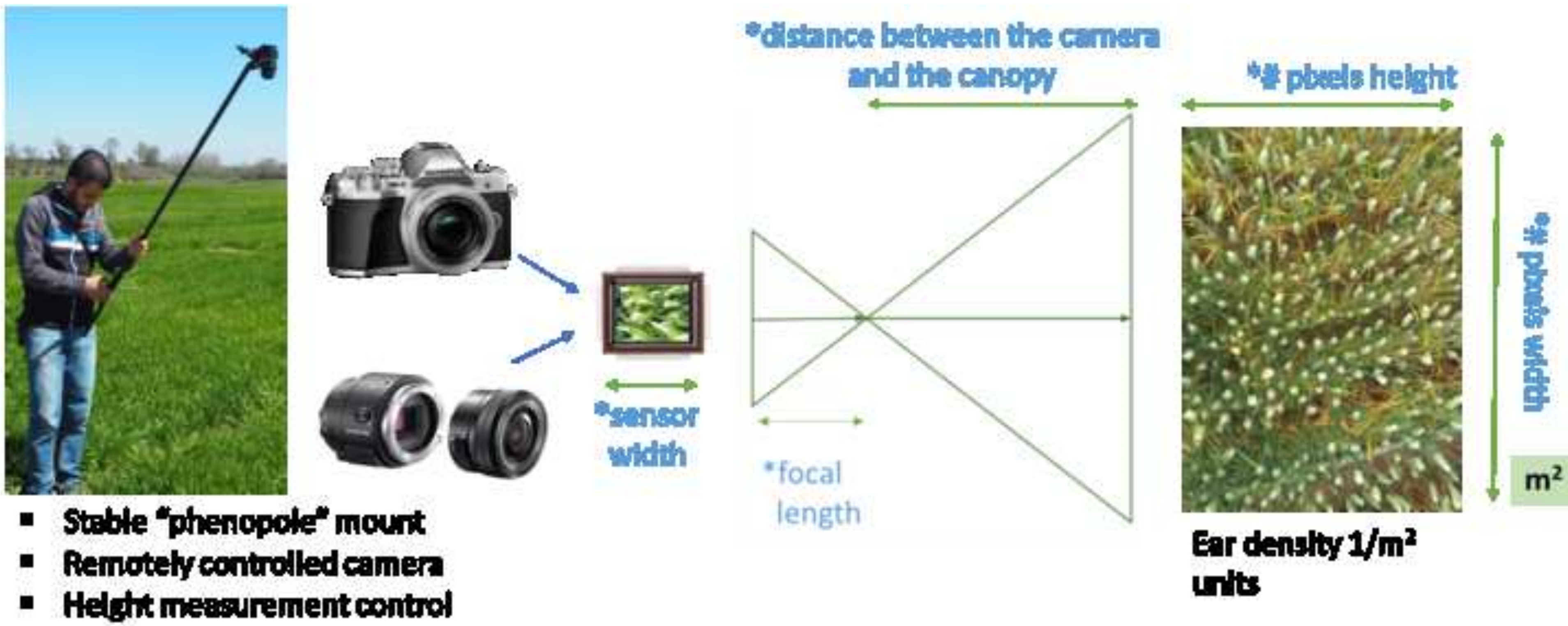

Figure2

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Figure3

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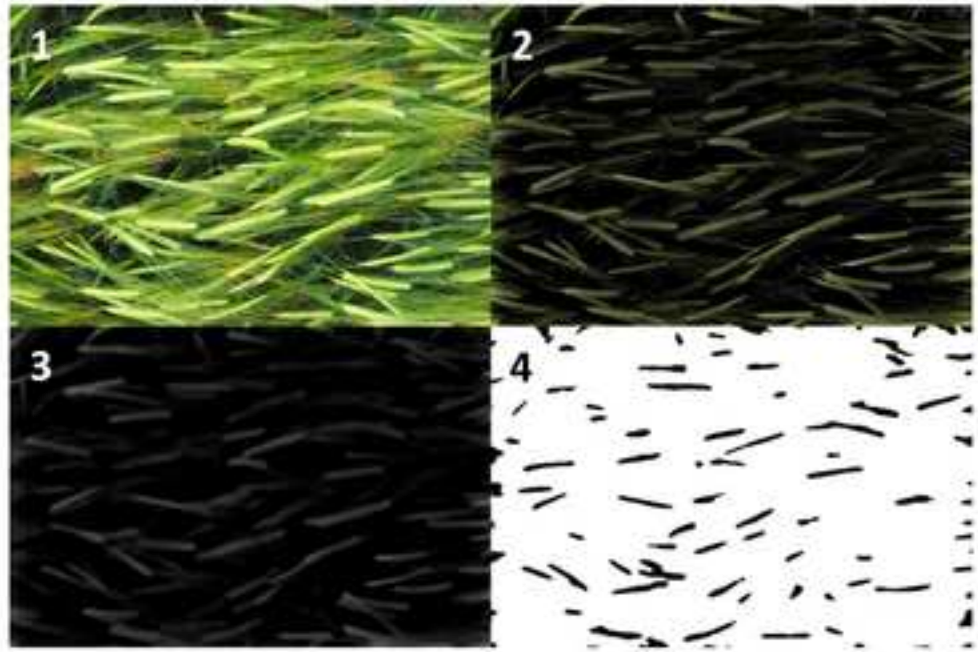
**CerealScanner**

1. Image acquisition
2. Laplacian Filter
3. Median Filter
4. Ear counting and $m^2$




Dataset: Arazuri and Zamadueñas (R+ R-)
Date: May 2016

Figure4

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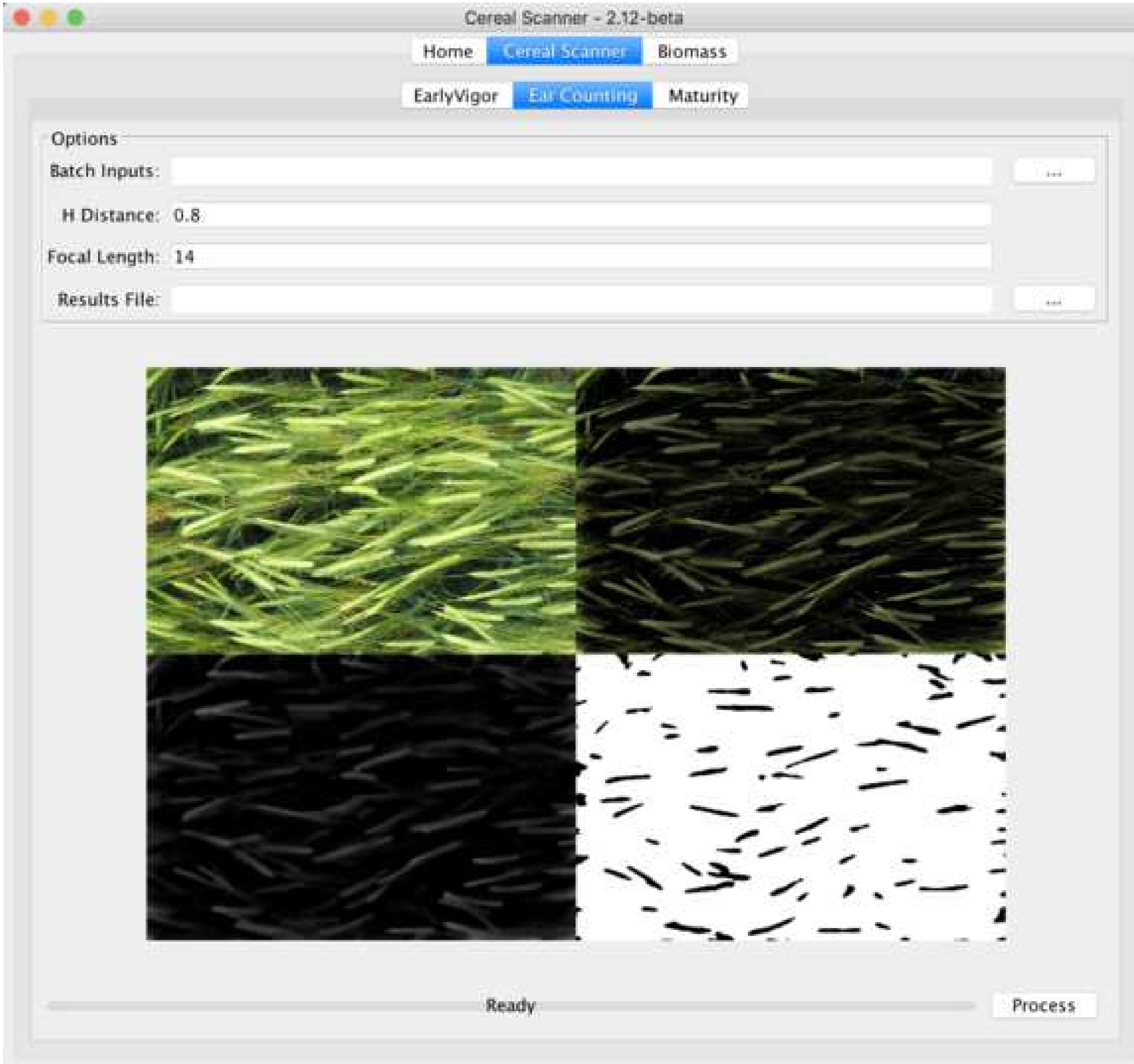

Figure5 Click here to access/download;Figure;Figure 5 JoVE.tif

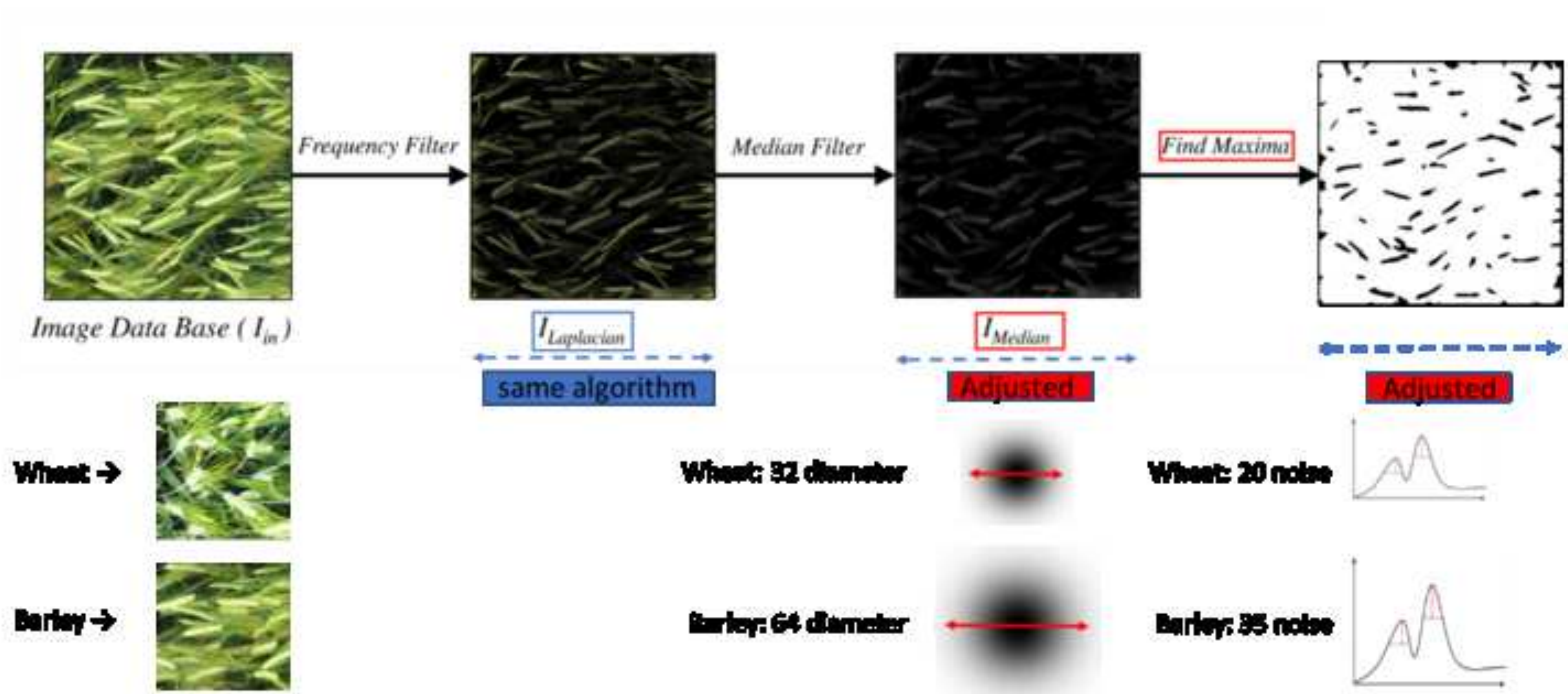

Figure6

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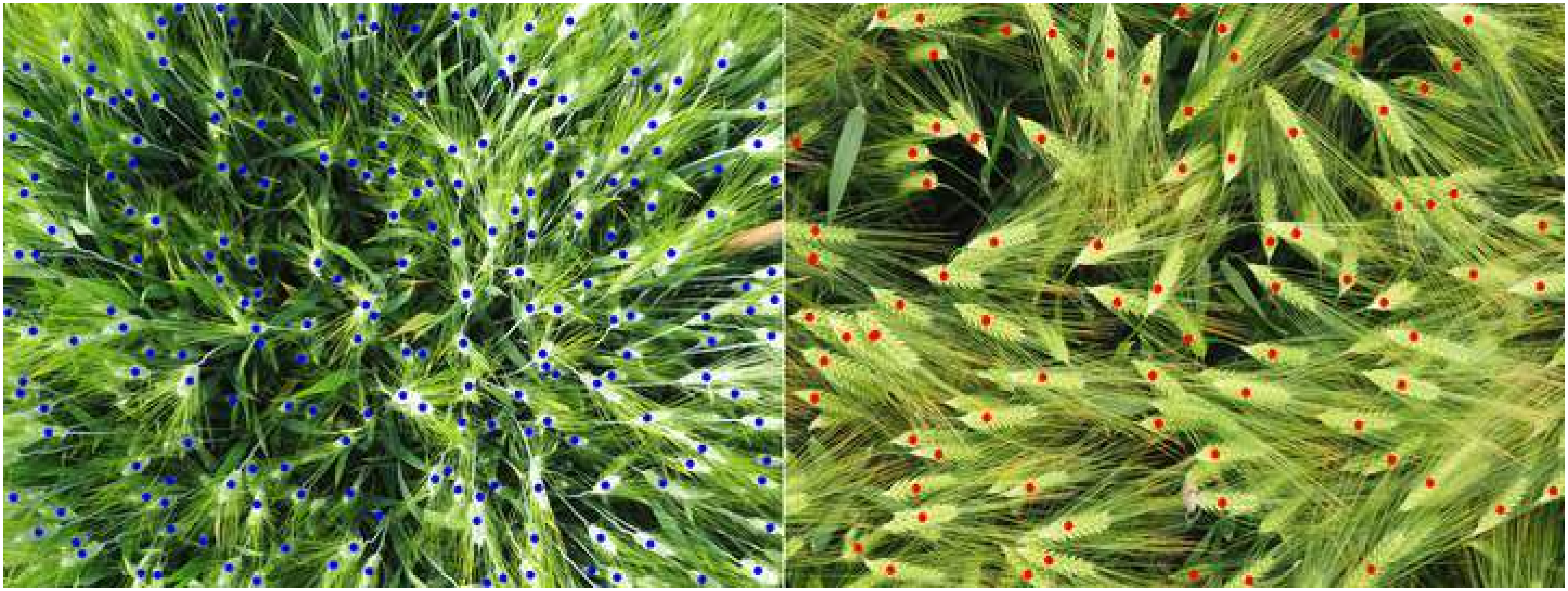

Figure7 Click here to access/download;Figure;Fig7-review(2).jpg

Figure8

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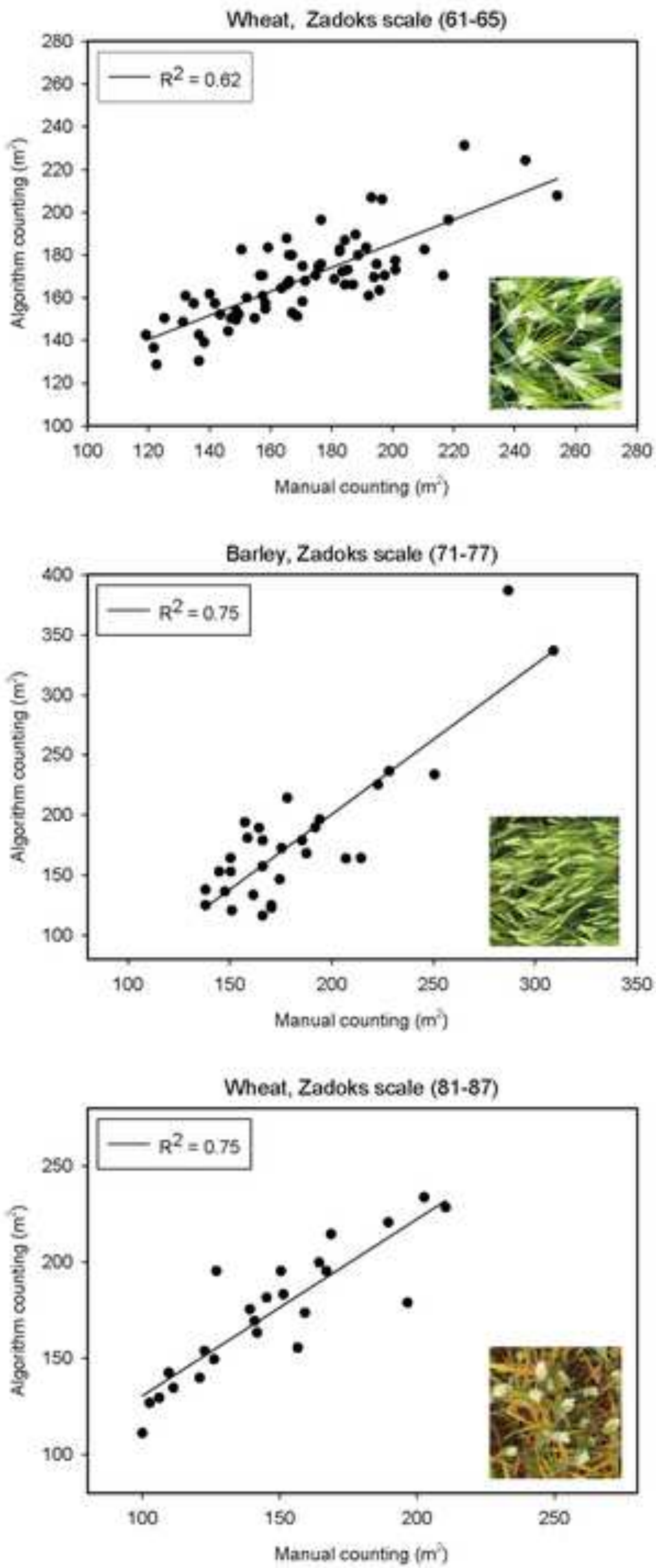

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| **Name of Material/ Equipment** | **Company** | **Catalog Number** |
|---|---|---|
| ILCE-QX1 Camera | Sony | WW024382 |
| E-M10 Camera | Olympus | E-M10 |
| Multipod Monpod | Sony | VCT MP1 |
| Computer | Any PC/Mac/Linux | -- |
| ImageJ/FIJI (FIJI is just Image J) | NIH | http://fiji.sc |
| Circle/Metal Ring | Generic | Generic |
| Crab Pliers Clip | Newer | 90087340 |

| Comments/Description |
| --- |
| Compact large sensor digital camera with 23.2 x 15.4 mm sensor size. |
| Compact large sensor digital camera with 17.3 x 13.0 mm sensor size. |
| "Phenopole" in the JoVE article |
| Data and image analysis |
| Plug-in and algorithms for data and image analysis |
| Metal ring for in-field validation |
| Circle support and extension arm |

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Author(s): osé A. Fernandez-Gallego1, María Luisa Buchaillot1, Adrian Gracia-Romero1, Thomas Vatter1, Omar Ve

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Article Title: Cereal crop ear counting in field conditions using zenithal RGB images

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Dear JoVE Editorial Office,

We are pleased to send you this improved scientific manuscript and video resubmission after going through peer-review. We feel that its novelty and scientific quality in presenting a novel image processing technique of agricultural significance warrant publication in the top video-graphic methodological journal, JoVE.

We have provided below detailed responses in *italics* and within quotes when indicating changes to the original manuscript with the steps taken with regards to each point raised by the editors and the three separate peer reviewer contributions. We have implemented the changes required of the video resubmission, including completely new recordings of the problematic interviews, for which we did have some scheduling conflicts and thus requested a small extension. Finally, we have uploaded the full version of the revised video in the link provided.

Our hope is that you the Editors of JoVE find that our resubmission of this scientific protocol and video-graphic detailed explanation meet your high standards and those of the reviewers that provided comments and criticisms in the standard scientific peer-review process. We remain dedicated to the scientific process of editorial and peer review and look forward to further communication.

Shawn C. Kefauver

Sincerely,

Shawn C. Kefauver, Ph.D.

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You will find Editorial comments and Peer-Review comments listed below. Please read this entire email before making edits to your manuscript.
NOTE: Please include a line-by-line response to each of the editorial and reviewer comments in the form of a letter along with the resubmission.

*We have provided below detailed responses in italics and within quotes when indicating changes to the original manuscript.*

**Editorial Comments:**

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

*In the process of revision, we have taken an extra look for potential spelling and grammatical errors, albeit after completing all of the revisions as part of the general and specific comments provided below. Furthermore, the first author has requested to include his third official institute affiliation, which has been appended to the start of the manuscript accordingly as well as in the online submission forms.*

• **Protocol Language:** The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes). Please re-write your protocol sections 1 and 2 accordingly. Descriptive sections of the protocol can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.

1) E.g, Lines 131-135, 148–160 etc. should be a note.

*The sections starting at Lines 131-135 and Lines 148-160 have been moved to after their respective sections as “Notes.”*

*Moreover, we have moved a large part of the Section 2 Note from lines 149-158 to the Discussion, now at lines 302-310. Lines 186-195 of the paragraph at the start of Section 3 has been moved to the end of the Introduction and Lines 195 to 201 have been moved to the Discussion section and modified according to the recommendations of Reviewer 2 as detailed below.*

2) Steps not in the Imperative voice: entire Section 1, 2.

*The text for all of the steps for the entirety of Sections 1 and 2 have been changed to imperative voice. As this small change often required reorganizing the entire sentence, the authors have not placed it here. In the changing of the voice format for the protocol steps we have tried to minimize the changes to content; however, some changes in voice have resulted in simplification of the steps.*

*In the algorithm adjustments for the CerealScanner, it is not necessary to adjust for image resolution.*

"3.3.1. Adjust algorithm parameter for the camera focal length.

3.3.2. Adjust the algorithm parameter for the distance from crop canopy."

*In the updating of the text of the Protocols to imperative voice, Protocol step 3.5 has also been removed and combined as part of 3.4.3, changing the numbering of the last three validation protocol steps.*

*"3.4.1. In "Options", enter in Batch Inputs the location of the photos to analyze.*

*3.4.2. In the "Results Files", select where to save the results file. The results file will include two columns with the image file name and the ear counting results.*

*3.4.3. Finally, click on "Process" and the results file with ear density in sq. meters using a simple ratio using the camera settings and the distance between canopy and camera to convert the image area to an actual canopy area in square meters following Figure 1 will be automatically produced in a few minutes, depending on the computer speed.*

*3.5. Conduct a post-processing validation after data collection by manually counting the number of wheat or barley ears in the image and then converting this to ears/m2 as in Figure 1 for comparison to the algorithm values.*

*3.5.1. Use the simple point placement tool built within FIJI that provides easy support for this process and the FIJI "Analyze Particles" function for producing the counts automatically; this is shown graphically in Fig. 6."*

• **Protocol Detail:**
1) Please ensure homogeneity between the written protocol and the protocol presented in the video.
2) Please ensure that all specific details from the video are mentioned in the manuscript (the manuscript should be able to serve as a standalone article).

*In the edition of the video, we have used the revised version of the manuscript as a guide for making any changes, as well as those suggested by the Editors and two Reviewers. Specifically, as the CerealScanner plug-in has been updated with a new informative web site and some new controls, this part of the video will be re-filmed in order to provide the same exact details as described in the protocol.*

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

*With only minor changes to the text, we have changed slightly the organization of the Discussion section in order to ensure that it loosely follows the above format and have added a short final sixth paragraph on the critical steps within the protocol after the ideas for future applications.*

*"In summary, the critical steps for the implementation of this protocol include first and foremost the proper planning for the time of year and environmental conditions of the crop, being between optimally within growth stages of Zadocks 60-87 and either at solar noon or in diffuse light conditions. Furthermore, the acquisition of digital images should be conducted in a controlled manner accounting for camera angle, distance from the canopy and camera focus for each image. Finally, optimized computer processing options are presented in detail for reproducing the processing code pipeline or contacting the authors for either the original code or the code integrated as a graphical user interface (GUI) in a plug-in for FIJI, the CerealScanner."*

• **Figures:**

1) Fig 2/6/7: Please merge panels a and b into 1 page and submit as 1 file.

*We have made the following requested changes and will upload the edited files accordingly.*

• **Figure/Table Legends:** Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

*We have expanded each with an updated and more detailed text as follows:*

*"Figure. 1. Ear counting system using the "phenopole" shown in the field on the left, with a remotely controlled natural color (RGB) large sensor and high-resolution digital camera system with camera tilt and height, indicating the necessary parameters for adjusting the image processing algorithm. The sensor and image resolution are detected automatically by the image properties, while the user should input the specifics for the lens focal length and the distance from the canopy. These are necessary to adjust the algorithm for the estimated number of pixels per ear and also the conversion of the image-based total ear count to ear density (ears/m$^2$) For that reason, it is recommended to use the same camera and lens focal length for all field images.*

*Figure. 2. Durum wheat (a) and barley (b) ear zenithal images for ear counting data set examples with an acceptable stage of growth and senescence from approximately Zadoks 61-87: (a) Durum wheat zenithal image data set example. (b) Barley zenithal image data set example.*

*Figure 3. Image processing pipeline for two row barley ear counting as implemented using specific computer code or using the "CerealScanner" software, both of which operate within FIJI (Fiji is Just ImageJ). Panel 1 shows the original image. Panel 2 shows the results of the applications of the Laplacian filter. Panel 3 shows the application of the Median filter, and Panel 4 shows the results of the final Find Maxima and segmentation for producing the final ear count. Then the calculations are made to convert the image count to ear density as shown in Figure 1. These images are an example taken from the Arazuri field site (NE Spain, 42°48'33.9"N 1°43'37.9"W) in diffuse light conditions.*

*Figure 4. The "CerealScanner" 2.12 Beta central tab on both levels marking the "Ear Counting" function within the "CerealScanner" algorithm collection. The user must select the "…" button to the right of*

*"Batch Inputs" to select the folder where the images files are stored, change the default values of the H Distance (distance from the camera to the top of the crop canopy) and Focal Length, if different from the default values, and then select the "…" button to the right of Results File to choose the name and location of the final results file. The other tabs of the CerealScanner provide algorithms for trait-based phenotyping for Early Vigor and onset of Maturity as part of the CerealScanner code suite. Under the Biomass tab, there are several algorithms for estimations of more general crop vigor and biomass calculations, also for RGB digital images. The example refers to two-row barley as was demonstrated in detail in Figure 3.*

*Figure 5. Adjustments required in the image processing pipeline in order to successfully count both wheat and barley ears using the same algorithm are managed automatically as part of the camera specific adjustments of H Distance (distance between the camera and the crop canopy) and Focal Length and serve to ensure that the number of pixels per ear remains more or less constant between different applications*.

*Figure 6. Algorithm validation using manual in-image ear counts for (a) durum wheat and (b) barley. The small dots were created using the FIJI Point Tool and then counted using the Analyze Particles Function with a 0.90-1.00 Circularity constraint after applying a Color Threshold from the Hue Saturation Intensity color space for the color specified by the Point Tool. This method ensures more accurate image-based manual ear counts.*

*Figure 7. Algorithm validation using manual counts in the field and manual in-image ear counts using a circle (a) wheat and (b) barley. (a) Wheat image count validation example using a circle. (b) Barley image count validation example using a circle. The subset counts of the ears within the white circle were counted using the same technique described in Figure 6 with the Point Tool, Color Threshold, and then Analyze Particles Function with Circularity constraints and color selection using Hue.*

*Figure 8. The coefficient of determination between ear density (number of ears/$m^2$) using manual image-based counting and the image algorithm ear counting for durum wheat and two-row barley at different acceptable crop growth stages (Zadoks scale 61-87). Both of the axes show calculations including conversions to ear density rather than image-based result only. The representative results are presented here for two different crops over three different growth stages as well as different light conditions, direct sunlight images for durum wheat at Zadoks 61-65 on the top (R2=0.62, n=72), diffuse light images for barley at Zadoks 71-77 in the middle (R2=0.75, n=30), and diffuse light conditions for durum wheat at Zadoks 81-87 below (R2=0.75, n=24). An example image of each is also shown as inset in the bottom right corner of each."*

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Lemnatec FieldScanalyzer (Lemnatec Ltd.),
1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

*We have removed the specific reference to the Lemnatec FieldScanalyzer and other commercial products from the text as requested. As this was not part of our actual protocol, but part of a literature reference, the commercial product was not added to the table of materials.*

*The specific text on Lines 102-105 have been edited to read as follows:*

*"Another example is an automatic ear counting algorithm developed using a fully automated phenotyping system with a rigid motorized gantry was used with good accuracy for counting ear density in a panel composed of five awnless bread wheat (Triticum aestivum* L.*) varieties growing under different nitrogen conditions[13]."*

• **Table of Materials:** Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as cameras, software, etc.

*We have updated the table as follows and will provide it separately as an xlsx file.*

| **Name of Material/ Equipment** | **Company** | **Catalog Number** | **Comments/Description** |
|---|---|---|---|
| ILCE-QX1 Camera | Sony | WW024382 | Compact large sensor digital camera with 23.2 x 15.4 mm sensor size. |
| E-M10 Camera | Olympus | E-M10 | Compact large sensor digital camera with 17.3 x 13.0 mm sensor size. |
| Multipod | Sony | VCT MP1 | "Phenopole" in the JoVE article |
| Computer | Generic | -- | Data and image analysis |
| ImageJ/FIJI (FIJI is just Image J) | NIH | http://fiji.sc | Plug-in and algorithms for data and image analysis |
| Circle/Metal Ring | Generic | Generic | Metal ring for in-field validation |
| Crab Pliers Clip | Newer | 90087340 | Circle support and extension arm |

• Please define all abbreviations at first use.

*We have completed an extra check of all of the abbreviations in the manuscript after implementing all of the other requested revisions.*

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

*All of the figures presented here in this manuscript are original. We have even included new data analyses, so additionally the representative results are also new.*

• **Video-related comments:**
Branding concerns
• 0:00-0:09 - The university logos should be removed from the beginning of the video. They can be moved to the end of the video.

*The logos on the start page will be replaced with the textual information regarding the different author affiliations. Logos no only appear at the end of the video.*

• 8:19 - One of the authors (affiliated with Syngenta and wearing a Syngenta shirt) mentions Syngenta in the interview here. Please eliminate/minimize the appearance of commercial bias.

*This video has been re-recorded wearing clothing without any visible logos.*

Audio issues
• 0:25-1:22, 7:47-8:03 - The audio and video appear to be slightly out of synch during this interview. This should be corrected.

*These two interviews have been re-recorded entirely with a more advanced microphone and video setup.*

Frame size/proportions issues
• 0:40 - The white background of this figure should be extended to fill the background

*This will be corrected so that the figure is seen larger and has no background framing.*

**Comments from Peer-Reviewers:**

**Reviewer #1:**
Manuscript Summary:
The manuscript presents a protocol for deriving cereal crop ear counts from zenithal RGB images using a

custom software solution that implements image processing, using Laplacian and frequency domain filters. The video is clearly understandable and professionally produced.

*Thank you, we have worked hard to film with the highest possible quality video at each part of the process. As this is a protocol with necessarily two parts, in the field and the laboratory, we were obliged to undertake the filming by ourselves.*

Minor Concerns:
-Citation 11 on line 90 seems to be wrong. From the text, I expected a review publication summarizing recent agricultural image analysis techniques including the 3d reconstruction based on SfM techniques, but the referenced paper is a publication using the described measurement protocol for ear counting.

*We have checked this error in the listed citations and have changed it to the correct citation with reference to the context of the SfM 3d reconstruction techniques and as they have been used for ear counting.*

-On line 204, the link given to the CerealScanner plugin (htpps://gitlab/sckefauver/CerealScanner) is not working. The installation instructions are a little bit unclear. Is there an option of using the processing code or the CerealScanner plugin instead? Because the webpage is not publically accessible, this can not be verified.

*Section 3.1 has been modified to include a link to an additional new informative website as well as information for how to gain access to the gitlab code and plugin repository. It is not the authors' intention to make the software available without any control, but rather available upon request. For this purpose, we have created a new informative webpage with a description of the software and contact information for acquiring access to either the original code or the graphical user interface (GIU) plugin version. We have also provided copies of both the code for FIJI and the actual plugin as part of the resubmission if the Reviewers wish to personally test the software functionality. The new text for 3.1 reads as follows:*

*"3.1. Download and install FIJI, Java 8 and the processing code or the University of Barcelona proprietary "CerealScanner" plugin at https://fiji.sc/, https://www.java.com/en/download/ and https://integrativecropecophysiology.com/software-development/cerealscanner/ (information) or htpps://gitlab/sckefauver/CerealScanner (code repository); contact corresponding authors for access permissions. The plugin is installed within FIJI by simply copying into the plugins folder."*

-The table of materials on line 252 mentions an "Ordenador", this should probably be "Computer" or "Desktop Computer".

*We have corrected this mistake and apologize for the errata.*

**Reviewer #2:**
Manuscript Summary:
The manuscript describes an automatic method for counting cereal ears based on digital RGB images.

Major Concerns:
While the manuscript shows promise, there are several problems that need to be addressed in terms of text clarity, methodology detailing and results analyses. Some major concerns:
- Line 119: The authors state that "The system may be extended without major problems to bread wheat". Even though morphologies are similar and there is no awn in the latter case, without experimental evidence it is not possible to categorically assert that the system can be easily extended. The authors should state that the system is likely to work with bread wheat, but further experiments are needed to confirm this.

*We have changed the text as listed on line 119 and have changed it to the more conservative language as suggested by Reviewer 2; it now reads as follows:*

*"The system has been demonstrated on examples for durum wheat and barley but should be extendable in application to bread wheat, which, besides exhibiting ears with similar morphology are frequently awnless, but further experiments would be required in order to confirm."*

- Lines 142-144: the authors recommend that images be captured close to the middle of the day or under overcast conditions (diffuse lighting). These are vastly different conditions that produce images with quite different characteristics. Under direct sunlight, shadows become a problem, but the image has much more depth and contrasting features. Under diffuse lighting, the image tends to be "flattened", with many details and small features tending to become indiscernible. Did the authors test both types of conditions? Were the results the same?

*Per the Editors guidance, much of this section has been changed. The relevant part now, in sub-part 1.3 specifies, as the Reviewer notes, that the images should be captured either within two hours of solar noon or in diffuse light conditions, as copied in quotes below. As the Reviewer correctly observes, these conditions are indeed quite different in terms of the qualities of images that they produce, especially with relation to observable depth of field, but both light conditions are quite equally effective in reducing the largest source of error in the presented ear counting protocol, the negative impact of shadows. This is a small point that we have added to this protocol step in order to provide some explanation to why we specify these two particular yet seemingly different light conditions. In a similar related publication, which is cited here, we presented also several results from non-optimal conditions with examples of the errors caused by each. Here we focused on the presentation of the two main optimal type conditions and have added specifics and examples for each below, including early reproductive stage direct sunlight ears for wheat, post anthesis diffuse sunlight ears for barley and late grain filling growth stage diffuse light for wheat..*

*"1.3. Plan the field excursions to capture the images within two hours of solar noon or alternately on an overcast day in diffuse light conditions in order to avoid the negative effects of ear shadowing on the ear counting algorithm."*

- Lines 153-156: the information on the number of MPixels is not nearly as relevant as the number of pixels per ear (or grain) necessary for the system to work properly.

*Reviewer 2 is correct that the number of pixels per ear is more relevant to the quality of the results rather than the full resolution of the images taken by the camera. Given our recommendations here for taking images at a distance of 0.8 m, we have estimated that most modern cameras and standard ranges of lenses would meet the minimal requirements as to the number of resulting pixels per ear. Per the Editors guidance we have moved the description of the megapixels of each camera to the Discussion section where it is covered in more detail with the rest of the relevant camera characteristics and recommendations, with some examples of what was used to produce the Representative Results. Moreover, some literature references where a more complete study of the effect of image resolution reduction has on the quality of the results of the presented algorithm have been added. The text is now present on lines 339 to 344 as follows:*

*"The most important relates to the image pixel resolution compared to the ears, followed by a smoothing factor that must be adjusted depending on whether the target crop is wheat or barley. In the case studies presented here, we have used two different compact cameras with large sensors of 20.1 MP and 16.0 MP capturing images with a wide-angle lens of 16-20 mm from a distance of 80 cm from the crop canopy. This has proven more than sufficient to produce detailed canopy barley and wheat information, with simulations demonstrating that the technique maintains high levels of precision down to 8 MP[11]."*

- Paragraph 186-200: there is important information missing here. It is not possible to reproduce the work without using FIJI and CerealScanner, and even if such software is employed, it is not clear if the information provided here is enough. For example, what are the kernels used in the Laplacian and median filters? How does this "Find Maxima segmentation technique" works? What about "Analyze particles"?

*This is indeed a pertinent request made by Reviewer 2 and we have added in here the additional information of interest to users. As the location of this text has been moved per the instructions of the Editors, we have expanded this relevant section where it now lays in the Introduction and the Discussion, Lines 186-195 of the paragraph at the start of Section 3 has been moved to the end of the Introduction and Lines 195 to 201 have been moved to the Discussion section. The specific part from Paragraph 186-200 referenced by the reviewer has been expanded with details and incorporated into the last paragraph of the Introduction and now reads as follows:*

*"The image processing algorithm is composed of three processes that first effectively remove unwanted components of the image in a manner that then allows for the subsequent segmentation and counting of the individual wheat ears in the acquired images. First, a Laplacian frequency filter has been used in order to detect changes in the different spatial directions of the image using the default ImageJ filter settings without window kernel size adjustments (https://imagej.nih.gov/ij/plugins/index.html#filters), which reduces the presence of most of the unwanted background elements in the image, including leaves, soil and some of the awns. Next, a median spatial filter reduces the high frequency noise around the ears due to the presence of the awns. Then, the Find Maxima segmentation technique determines*

*the local peaks after the median spatial filter step, at which stage the pixels related with ears have higher pixel values that soil or leaves. Therefore, Find Maxima is used to segment the high values in the image and those regions are labeled as ears, which identifies ears while also reducing overlapping ear errors. Analyze particles is then used on the binary images to count and/or measure parameters from the regions created by the contrast between the white and black surface created by the Find Maxima step. The result is then processed to create a binary image segmentation by analyzing the nearest neighbor pixel variance around each local maximum to identify the wheat ear shapes in the filtered image. Finally, the ear density is counted using the Analyze Particles, as implemented in FIJI[15]. Both Find Maxima and Analyze Particles are standalone functions and also available as plugins in FIJI (https://imagej.nih.gov/ij/plugins/index.html)."*

Field Code Changed

- Figure 8: how many images were used to produce the results depicted here? How many of those were taken under direct sunlight? How many were captured under overcast conditions?

*We have added this information as part of the expansion of the figure caption, per the request of the Editors, and have also included the additional information requested by Review 2.*

*"Figure 8. The coefficient of determination between ear density (number of ears/m2) using manual image-based counting and the image algorithm ear counting for durum wheat and two-row barley at different acceptable crop growth stages (Zadoks scale 61-87). Both of the axes show calculations including conversions to ear density rather than image-based result only. The representative results are presented here for two different crops over three different growth stages as well as different light conditions, direct sunlight images for durum wheat at Zadoks 61-65 on the top ($R^2$ 0.62, n=72), direct sunlight images for barley at Zadoks 71-77 in the middle ($R^2$ 0.75, n=30), and indirect or diffuse light conditions for durum wheat again at Zadoks 81-87 below ($R^2$ 0.75, n=24). An example image of each is also shown as inset in the bottom right corner of each."*

- Line 303: the authors state that they tested various different techniques before arriving at the protocols presented in the manuscript. What were the techniques that were tested and discarded? This is important information for the scientific community, especially to support the design of future experiments.

*With respect to providing more details on other preliminary tests that did not produce as reliable results as the presented protocol, we have added in the following sentence to this regard at line 336:*

*"Other image filtering attempts based on RGB color or alternative color spaces, such as Hue-Saturation-Intensity or CIE-Lab, were not as effective or consistent as the use of the Laplacian and Median frequency domain filters in removing unwanted image elements, especially the awns."*

- The authors should discuss the effects of lighting on the accuracy. Did the flattening effect caused by diffuse light influence the results? How did the system deal with images with lesser details?

*Actually, this theme has already been considered as important and is brought up in the third to last paragraph in the Discussion starting on line 367 beginning with the following statement:*

*"Even though the data collection in field conditions requires close attention to such environmental conditions as sunlight intensity and sunlight illumination angles, the robust image analysis algorithm presented here provides some leeway in the image capture window by using spatial techniques that ignore image albedo effects, given that the correct image exposure was used for the particular light conditions at the moment of image capture."*

*In order to better address this issue, we have added the following text at line 371, following the guidelines set forth by Reviewer 2 to better address the potential effects of lighting.*

*"In previous work, a fuller range of lighting effects were tested, indicating that the only major source of error with regards to light effects is the production of strong shadows in the image when capturing images in direct sunlight either early or late in the day due to the angle of the sun."*

*With regards to images with 'lesser details,' we assume that the Reviewer is referring to lower resolution images, either with fewer megapixels or taken from a greater distance. This is discussed in more detail in the second to last paragraph of the discussion, with reference to another previous work in which we simulated lower resolution images and presented its results on the algorithm accuracy, the product of which is the recommended range of spatial resolutions presented in this protocol.*

Minor Concerns:
There are many other problems, most of them related to writing, that also need to be addressed:
- The text needs improvement: there are many typos, grammatical errors and overly long and confusing sentences.

*We have rewritten a number of sections of the manuscript per the indications of the Editors towards improved adherence to the style guidelines, and also as part of the editions suggested by the other reviewers. Furthermore, we have endeavored to conduct a detailed revision for all small errors in the text, which are not copied in detail here.*

- Lines 78-81: sentence is too long and difficult to follow.

*This sentence has been simplified and edited for clarity to a much shorter sentence from:*

*"Although previously applied to precision agriculture and more recently to phenotyping activities, remote sensing has traditionally focused on multispectral, hyperspectral, and thermal imaging sensors from aerial platforms for larger studies, in the case of precision agriculture, or ground-based platforms, for plant phenotyping studies at the small plot canopy scale[10]"*

*And now reads as follows:*

*"Remote sensing has traditionally focused on multispectral, hyperspectral, and thermal imaging sensors from aerial platforms for precision agriculture at the field scale or for plant phenotyping studies at the micro-plot scale[10]."*

- Lines 99-102: sentence is too long and difficult to follow.

*This sentence has been edited into two separate sentences for clarity from the original long sentence:*

*"Recent work has advanced in this direction by using a black background structure supported by a tripod in order to acquire suitable crop images by avoiding excessive sunlight and shadow effects, but is cumbersome in field conditions even though demonstrating fairly good results in ear counting[12]."*

*And now reads as follows:*

*"Recent work has advanced in this direction by using a black background structure supported by a tripod in order to acquire suitable crop images, demonstrating fairly good results in ear counting[12]. In this way, these authors avoided excessive sunlight and shadow effects, but such a structure would be cumbersome and a major limitation in application in field conditions.".*

- Many excerpts are too verbose. For example, the excerpt "More recent work by led Fernandez-Gallego published in 2018…" (line 105) could be replaced with "Recent work…". There are many other instances like this throughout the text.

*We have shortened the text of the manuscript at the indicated location and have edited a number of other locations using more concise language as part of an editorial review of the whole manuscript that is not detailed here as it consisted of a number of small changes.*

- Lines 115-117: convoluted sentence, it should be rewritten for clarity.

*This sentence has been edited from its original version,*

*"This work proposes a simple system for the automatic quantification of ear density, using the examples of durum wheat and barley crops grown in field conditions, based on VHR RGB (natural color) images acquired from commercially available digital cameras."*

*And now reads as follows:*

*"This work proposes a simple system for the automatic quantification of ear density in field conditions using images acquired from commercially available digital cameras."*

- Lines 117-119: This sentence needs to be better explained.

*This sentence has been edited from its original version,*

*"The system takes maximal advantage of natural light in field conditions and therefore needs to take into account various environmental factors but remains in effect simple to implement."*

*And now reads as follows:*

*"This system takes advantage of natural light in field conditions and therefore requires consideration of some related environmental factors, such as time of day and cloud cover, but remains in effect simple to implement."*

- Lines 121-124: confusing. It is not clear how these two sentences relate, nor the reason the authors mention manual counting using images or in the field, since the proposed scheme is supposed to be automatic. This whole paragraph has many problems and should be rewritten for the sake of clarity.

*These sentences have been edited from their original version,*

*"In our protocol, zenithal images were taken by holding an RGB camera by hand above the crop. In real field conditions the easiest way and the cheapest way to acquire information is walking across the plots in the field and counting the ears manually in the image or in the field for validation."*

*And now reads as follows:*

*"In our data capture protocol, zenithal images are taken by simply holding by hand or using a monopod for positioning the digital camera above the crop. Validation data can be acquired by counting the ears manually for sub-plots in the field or during postprocessing by counting ears in the image itself."*

- Line 133: please give examples of factors that affect image quality (shadowing is certainly not the only relevant factor).

*All of Protocol 1 and 2 have been completely re-written at the behest of the Editors. The relevant text is now part of the Note for Protocol 1:*

*"Protocol 1 Note: In considering the objectives of this protocol, it is important to first consider that the growth stage of the crop is suitable for applying ear counts. Capturing images outside of the recommended growth stage will either result in sub-optimal or meaningless results (if ears are not present or fully emerged). Image quality also has a considerable impact on processing results, including resolution and sensor size, and some environmental conditions, such as time of day and cloud cover, need to be carefully considered before proceeding with image capture."*

- Lines 148-149: this needs a better explanation.

*This section has been entirely removed and most of Protocol 2 has been completely re-written. The lines at 148-149 have been redone and reduced to a small Note for Protocol 2.*

*"Protocol 2 Note. Three major considerations in selecting a camera therefore include: (1) camera specifications, in this case the sensor physical size; (2) focal length of the image lens, and (3) distance between the canopy and the camera: closer distances or greater zoom lenses will capture less area while images captured from a greater distance or with lower zoom will capture more crop area. See Figure 1 for the details on the relevant camera specifications."*

- Figure 1: the legend should be more descriptive, so the figure can be understood standing alone.

*All of the Figure legends have been re-written and expanded.*

*"Figure. 1. Ear counting system using the "phenopole" shown in the field on the left, with a remotely controlled natural color (RGB) large sensor and high-resolution digital camera system with camera tilt and height, indicating the necessary parameters for adjusting the image processing algorithm. The sensor and image resolution are detected automatically by the image properties, while the user should*

*input the specifics for the lens focal length and the distance from the canopy. These are necessary to adjust the algorithm for the estimated number of pixels per ear and also the conversion of the image-based total ear count to ear density (ears/m2) For that reason, it is recommended to use the same camera and lens focal length for all field images."*

- Line 162: the way the sentence is written, it looks like the images are captured in motion, which is probably not the case.

*All of Protocol 2 has been redone completely. This sentence was previously:*

*"2.1. Images captured are taken walking between the plots to obtain zenithal images. Using the "phenopole" acquisition system shown in Fig. 1, it is possible to capture images quickly and yet in a controlled and consistent manner."*

*It now reads:*

*"2. Image capture in field conditions with natural light*

*2.1. Prepare a "phenopole" as shown in Fig. 1 or a similar acquisition system (even handheld) to capture images at each plot or target location quickly and yet in a controlled and consistent manner.*

*2.2. Position the camera on a suitable monopod or "selfie" pole such that it may be maintained level either using level bubbles or an in-camera stabilization system to obtain zenithal images."*

- Figure 2: too many examples. One sample from each row in the images would be more than enough. The legend is highly redundant, as the information before and after ":" is exactly the same.

*We have reduced the number of example images here to show only one row of example images for wheat and barley. All of the Figure legends have been completely re-written.*

*"Figure. 2. Durum wheat (a) and barley (b) ear zenithal images for ear counting data set examples with an acceptable stage of growth and senescence from approximately Zadoks 61-87."*

- Lines 174-175: confusing.

*All of Protocol section 2 has been re-written entirely. Section 2.4:*

*"2.4. Note the image number prior to image capture in order to match the images correctly with the field plots. An image of the general field area to start and on image of the ground/field between blocks is recommended for control and counting."*

*Now reads:*

*"2.4. Take note of the image number prior to image capture in order to match the images correctly with the field plots. Record one image of the general field area at start and one image of the ground/field between blocks for post-processing controls."*

- Lines 186-188: sentence is too long.

*This whole section has been deleted, with some relevant parts incorporated into either the Introduction or Discussion sections accordingly.*

- Figure 5: legend should be more descriptive, as is it is a bit ambiguous.

*All of the Figure legends have been re-written and expanded.*

*"Figure 5. Adjustments required in the image processing pipeline in order to successfully count both wheat and barley ears using the same algorithm are managed automatically as part of the camera specific adjustments of H Distance (distance between the camera and the crop canopy) and Focal Length and serve to ensure that the number of pixels per ear remains more or less constant between different applications."*

- Figures 6 and 7 should be better explained. Are the points marked manually or automatically? The way text and legends are written, it is very difficult to understand the process.

*All of the Figure legends have been re-written and expanded.*

*The captions for Figures 6 and 7 were previously:*

*"Figure 5. Adjustments required in the image processing pipeline in order to successfully count both wheat and barley ears using approximately the same algorithm.*

*Figure 6. Algorithm validation using manual in-image ear counts for (a) durum wheat and (b) barley."*

*They now read:*

*"Figure 6. Algorithm validation using manual in-image ear counts for (a) durum wheat and (b) barley. The small dots were created using the FIJI Point Tool and then counted using the Analyze Particles Function, with a 0.90-1.00 Circularity constraint, after applying a Color Threshold from the Hue Saturation Intensity color space for the color specified by the Point Tool. This method ensures more accurate image-based manual ear counts.*

*Figure 7. Algorithm validation using manual counts in the field and manual in-image ear counts using a circle. (a) Wheat image count validation example using a circle. (b) Barley image count validation example using a circle. The subset counts of the ears within the white circle were counted using the same technique described in Figure 6 with the Point Tool, Color Threshold, and then the Analyze Particles Function with Circularity constraints and color selection using Hue."*

- Figure 3 (legend): what do you mean by "Fiji is Just ImageJ"?

*All of the Figure legends have been re-written and expanded. The Figure 3 legend now reads:*

*"Figure 3. Image processing pipeline for two row barley ear counting as implemented using specific computer code or using the "CerealScanner" software, both of which operate within FIJI (Fiji is Just*

*ImageJ). Panel 1 shows the original image. Panel 2 shows the results of the applications of the Laplacian filter. Panel 3 shows the application of the Median filter, and Panel 4 shows the results of the final Find Maxima and segmentation for producing the final ear count. Then the calculations are made to convert the image count to ear density as shown in Figure 1. These images are an example taken from the Arazuri field site in diffuse light conditions."*

*With regards to this specific text, the acronym **FIJI** is **F**IJI **i**s **j**ust **I**mageJ.*

**Reviewer #3:**
Manuscript Summary:
This manuscript describes a protocol for collecting top down images of wheat and barley fields, as well as a software approach to identifying and counting wheat heads within the resulting images. The methodology is clearly written and the technique is likely to be of interest to a significant number of breeders and quantitative geneticists working in these two species. I have only a couple of minor points about the manuscript as currently written.

Minor Concerns:
Lines 119-121: "The system may be extended without major problems to bread wheat, which besides exhibiting ears with similar morphology than bread wheat, the ears frequently are awnless, which would make ear counting easier."

*We have changed the text as listed on line 119 and have changed it to the more conservative language as suggested by Reviewer 2 and Reviewer 3; it now reads as follows:*

*"The system has been demonstrated on examples for durum wheat and barley but should be extendable in application to bread wheat, which, besides exhibiting ears with similar morphology are frequently awnless, but further experiments would be required in order to confirm."*

Line 136: Zadoks is a sufficiently narrowly used scale that it would make sense to describe it a bit more here as some of the target audience is presumably researchers working in other crops who may want to adapt this technique to their target species.

*We have re-written all of the Protocol 1 section and have added additional text and also language in the video to indicate that the optimal image data acquisition should be as follows:*

*"1.1. Make sure that the crop growth stage is approximately in the range of Zadoks 60-87, between grain filling and near crop maturity, with ears that are still green even if the leaves are senescent[14]. Some yellowing of the leaves is acceptable, but not necessary.*